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In vitro release of Tacrolimus from Tacrolimus ointment and its speculated mechanism

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Abstract

The in vitro release profiles and the bleeding phenomenon of Tacrolimus and propylene carbonate (PC) as a dispersing solvent for Tacrolimus drug substance in Tacrolimus ointment were investigated when changing concentrations of Tacrolimus and PC in the ointment were used, respectively. The bleeding test result indicated that Tacrolimus was in equilibrium between inside and outside of PC droplets in intact ointment base. A cumulative release amount of Tacrolimus from ointment, plotted against the square root of time, showed a straight line initially with a slope of $q1$ followed to change a slope to be $q2$ at a certain time, where the relation of these slopes being $q1 < q2$. The $q1$ values increased with the concentration of Tacrolimus but decreased with PC concentration in Tacrolimus ointment. And the $q2$ values increased with Tacrolimus concentration but were independent of PC concentration. These profiles indicated that there were two phases for Tacrolimus release from ointment, namely, first phase was related with the period during PC release and the second phase was related with the state of ointment after PC release. When the PC release was applied to the Higuchi's release equation, the above slope $q1$ was found to be correlated to the parameter of A/ϕ_0, where A was a parameter of release rate of PC and ϕ_0 was an initial volume fraction of PC droplets. It should be indicated that more rapid release rate of PC rather than that of Tacrolimus resulted in the generation of amorphous phase of Tacrolimus outside of remaining PC droplets. During PC release, the slope $q1$ could be influenced by the thermodynamic activity of Tacrolimus dissolved in PC droplets. After PC release, it would be reasonable to speculate that the amorphous cluster of Tacrolimus with a constant thermodynamic activity would give constant $q2$ values regardless of PC contents in Tacrolimus ointment.

Keywords: In vitro release; Higuchi's equation; Franz diffusion cell; Bleeding; Ointment; Thermodynamic activity

1. Introduction

Tacrolimus is a novel immuno-suppressant with a macrolide-type structure fermented by *Streptomyces tsukubaensis*, which was discovered by Research Laboratory of Fujisawa Pharmaceuticals Co., Ltd, Japan, in 1984. This immuno-suppressive activity is 50–100 times higher than that of cyclosporine in vitro, and 10–20 times in vivo, respectively (Honbo et al., 1987). The oral and/or parenteral products of Tacrolimus have already been launched worldwide as to prevent organ rejection after the transplantation surgeries. Then, after an assessment of physicochemical characteristics of Tacrolimus, a formulation development of the novel topical product of Tacrolimus was carried out to produce Tacrolimus ointment (Protopic®) for an atopic dermatitis (Tanaka et al., 2000). This formulation has been a white petrolatum-based ointment in which droplets of propylene carbonate (PC) as solvent

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for dissolving Tacrolimus drug substance were uniformly dispersed. This Tacrolimus ointment has also been launched worldwide. In this paper, in vitro releasing profiles of drug and/or solvent from Tacrolimus ointment were investigated. And the concentrations of Tacrolimus in bleeding liquid (BL) were also measured to discuss the equilibrium state of Tacrolimus in the initial state of ointment formulation. From thermodynamic point of view, the activities of drug and solvent in BL could be related to their releasing phenomena. Coupled with Higuchi's equation (Higuchi, 1961) regarding their releasing phenomena, a possible mechanism of drug release was discussed.

2. Materials and method

2.1. Materials

Tacrolimus drug substance manufactured by Fujisawa Pharmaceuticals Co., Ltd. was used. PC was purchased from Huntsman Co. Ltd and white petrolatum was from Penreco Co. Ltd. All other components formulated in ointment, which were paraffin, mineral oil, and white wax, were pharmaceutical grade.

2.2. Preparation method of ointment

Tacrolimus drug substance was dissolved in an appropriate amount of PC. When the concentration of Tacrolimus in ointment was changed, a corresponding amount of Tacrolimus drug substance was dissolved in 5% (w/w) of PC. The droplets of PC solution were dispersed in the ointment vehicle with a homogenizer operated by an appropriate manufacturing procedure of typical oleaginous ointment. When Tacrolimus ointment with 1, 2.5, 5, and 10% (w/w) of PC compositions were prepared, the concentration of Tacrolimus in ointment was kept constant at 0.1% (w/w), respectively.

2.3. In vitro releasing test

Franz diffusion cell system (Shah et al., 1999) as shown in Fig. 1 was used to monitor the cumulative release amounts of Tacrolimus (Q) and PC (Q_S) in receptor medium with following experimental conditions:

(1) *Receptor medium*: A degassed solution of polyethylene glycol 400/water = 50/50 with 25 ml volume was selected to satisfy sink conditions for Tacrolimus and PC release amount in receptor medium. Stirrer bar was spinned at 600 rpm. Temperature was controlled at 34 °C by jacketed circulating water. Integrity of ointment that there was no penetration of this medium into ointment by forming channels was confirmed before release experiment.

(2) *Synthetic membrane*: Vinylidene polyfluorinate membrane with a pore size of 0.45 μm, a thickness of 125 μm, and a diameter of 25 mm was selected to avoid the adsorption of Tacrolimus and PC and/or the impediment of the diffusion of Tacrolimus and PC, where the diffusion area (F) through this synthetic membrane was calculated as 4.91 cm^2.

(3) *Ointment sample*: 50 mg of ointment, for which volume (V_0) was, for example, 57.5 μl in case of 5% PC–0.1% Tacrolimus ointment calculated by its ointment density 0.87 g/ml, was spread in approximately 150 μm thickness on the synthetic membrane by an appropriate tool.

(4) *Sampling*: Each 200 μl volume of receptor medium was sampled with replacement of fresh medium.

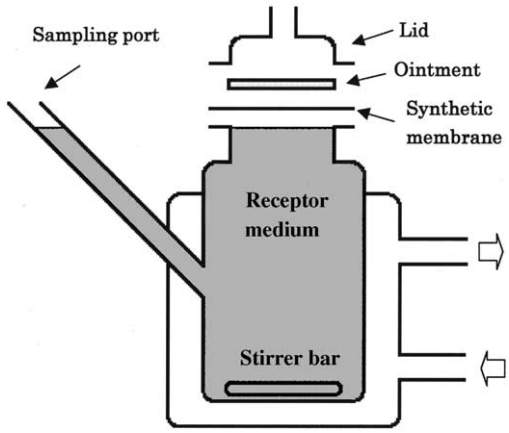

Fig. 1. Franz diffusion cell system.

2.4. Drug and solvent concentration in bleeding liquid

Cone mesh filtration method (Saeki and Yasumori, 1972) as shown in Fig. 2 was used to collect BL from a

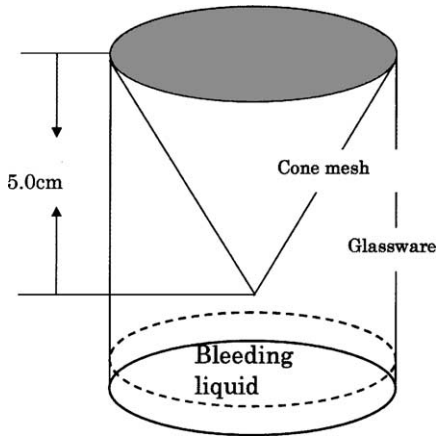

Fig. 2. Cone mesh filtration method.

prepared ointment in order to measure concentrations of Tacrolimus and PC in BL. Experimental conditions were as follows:

(1) *Stainless steel cone mesh*: Cone mesh with 40 mm diameter and 50 mm length having 180 μm mesh opening was set on a glass beaker.
(2) *Ointment sample*: Approximately 15 g ointment was placed on cone mesh, which was stored at 34 °C for 2 weeks. BL from ointment was collected in the bottom of glass vessel.

2.5. Microscopic evaluation

PC droplets in ointment were monitored microscopically at appropriate time during in vitro release experiment. At these timings, the intactness of ointment nature was also checked whether such a long time releasing study would be conducted well through the experiments.

In the bleeding test, no PC droplets in BL were confirmed by microscope.

2.6. Measurement of Tacrolimus and PC concentrations

All samples from in vitro release test and the BL were analyzed to measure Tacrolimus and PC concentration by High Performance Liquid Chromatography (HPLC).

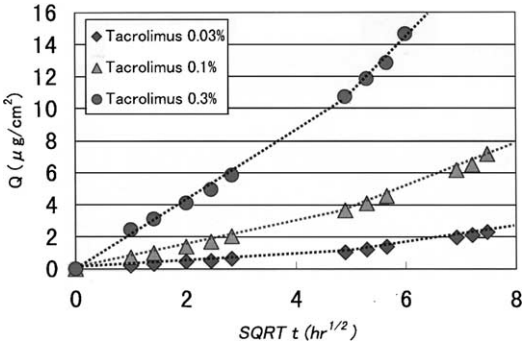

Fig. 3. Concentration dependence of Tacrolimus release from ointment with 5% PC.

3. Results

3.1. In vitro Tacrolimus release from ointment

3.1.1. Tacrolimus concentration dependence of 5% PC ointment formulations

As shown in Fig. 3, the cumulative release amount of Tacrolimus drug substance in receptor medium, Q (μg/cm^2), was monitored and plotted against square root of time ($\sqrt{t}$, h$^{1/2}$) in the case that Tacrolimus concentration in ointment formulation was changed from 0.03 to 0.3%. An individual Q was apparently composed of two straight lines, which meant that the release profile of Tacrolimus had two phases. The first straight line with a slope ($q1$) was changed to be turned at the point of ($\sqrt{t^*}$, Q^*) following to the next straight line with a slope ($q2$) for which value was larger than that of $q1$. These $q1$ and $q2$ values were calculated by the statistical least square method from experimental data and listed in Table 1. The $q1$ and $q2$ values were increased in parallel with Tacrolimus concentration in ointment. And t^* was almost constant being around 24 h, and Q^* was increased in proportion to Tacrolimus concentration in ointment correspondingly.

3.1.2. PC concentration dependence of 0.1% Tacrolimus ointment formulation

As shown in Fig. 4, when PC concentration in ointment was changed from 1 to 10%, the cumulative release amount of Tacrolimus was also measured and plotted against square root of time. A first straight line was changed to be turned at ($\sqrt{t^*}$, Q^*) to a next straight line in a similar manner to that in Fig. 3, where

Table 1
Parameters of in vitro drug release from Tacrolimus ointment

	$q1$ ($\mu g/cm^2/h^{1/2}$)	$q2$ ($\mu g/cm^2/h^{1/2}$)	$\sqrt{t^*}$ ($h^{1/2}$)	Q^* ($\mu g/cm^2$)
Tacrolimus (−0.03%) with 5% PC	0.21	0.48	5.0	1.1
Tacrolimus (−0.1%) with 5% PC	0.74	1.37	5.2	3.9
Tacrolimus (−0.3%) with 5% PC	2.14	3.47	4.8	10.4
Tacrolimus (−0.1%) with 1% PC	1.07	1.84	3.8	4.1
Tacrolimus (−0.1%) with 2.5% PC	0.90	1.70	4.4	4.0
Tacrolimus (−0.1%) with 10% PC	0.58	1.33	6.7	3.9

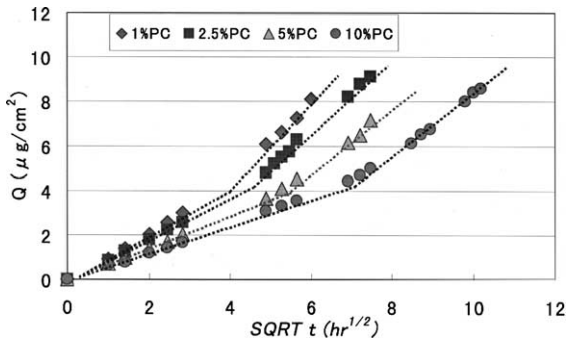

Fig. 4. The release of Tacrolimus from ointment with various PC contents.

each value of Q^* was almost constant value around 4 $\mu g/cm^2$ and t^* was increased with PC concentration formulated in ointment. The $q1$ values were decreased by about half times when PC contents in ointment increased from 1 to 10%, but $q2$ values were almost constant independently on PC contents which were always larger than $q1$ values. It was also observed that the smaller the PC contents the smaller the difference between $q1$ and $q2$. These values were also listed in Table 1.

3.2. In vitro PC release from ointment

As shown in Fig. 5, the release profiles of PC (Q_S expressed as % values) plotted against $\sqrt{t}$ were determined for the four kinds of 0.1% Tacrolimus ointment formulations when PC concentrations were changed from 1 to 10%, respectively. The profiles had straight lines initially and deviations would be seen in course of time. It was apparent that the endpoint of PC release from ointment was almost in accordance with above turning point, t^* of Tacrolimus release from ointment in Fig. 4.

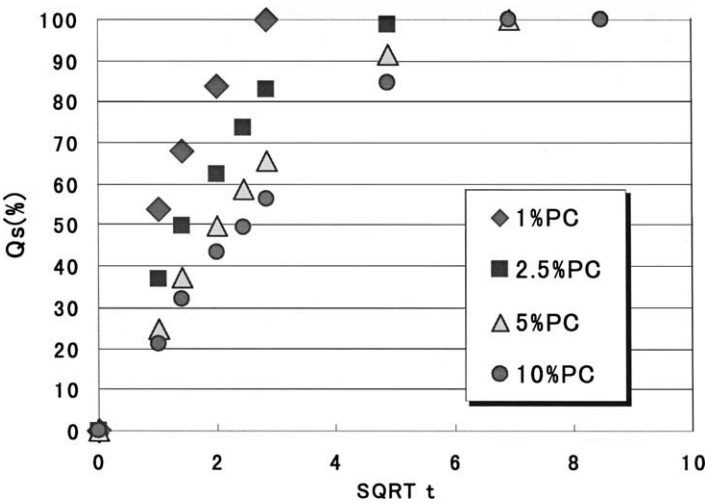

Fig. 5. PC release from 0.1% Tacrolimus ointment with various PC contents.

Table 2
Tacrolimus and PC concentration in BL from 0.1% Tacrolimus ointment with four kinds of PC concentrations

	PC composition in ointment			
	1% PC	2.5% PC	5% PC	10% PC
S_0 (mg/ml)	8.62	21.6	43.5	88.0
S_L^0 (mg/ml)	0.046	0.035	0.037	0.043
C_0 (μg/ml)	862	865	870	880
C_L^0 (μg/ml)	10.1	5.15	2.40	1.19

3.3. Amount of Tacrolimus and PC in bleeding liquid of ointment

About 5% of BL from applied amount of ointment were obtained by cone mesh filtration method for the four kinds of 0.1% Tacrolimus ointment with PC concentration from 1 to 10%, respectively. The concentrations of Tacrolimus (C_L^0) and PC (S_L^0) in BL are shown in Table 2, respectively. According to the result of the solubility measurement for the drug in the ointment by cone mesh filtration method (Kobayashi and Saitoh, 1998), it was interpreted that the drug concentration in BL was equal to the drug content, which was dissolved in the ointment vehicle.

The formulation of Tacrolimus ointment was defined as the droplet dispersion-type ointment (Fig. 6). Then, the amount of Tacrolimus and PC dissolved in the ointment vehicle outside of PC droplets could be expressed as C_L^0 and S_L^0, respectively. It was found that S_L^0 values did not depend on the total PC concentration, S_0, namely, those were almost constant values

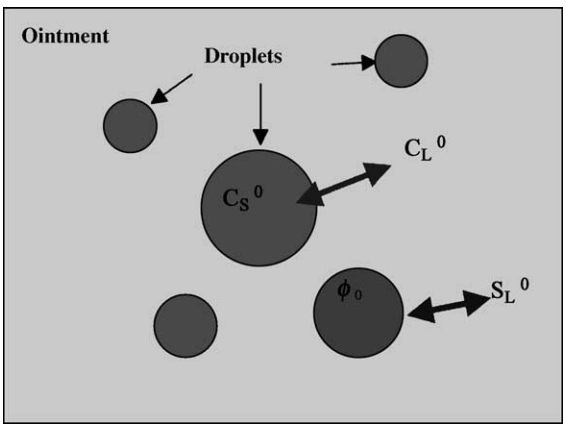

Fig. 6. The structure of Tacrolimus/PC ointment.

(Kobayashi and Saitoh, 1998). On the other hand, C_L^0 values were inversely related with S_0.

3.4. Microscopic observations

As shown in Plate 1, uniformly dispersed PC droplets in ointment vehicle were observed. Mean diameter of droplet was around 3 μm. During in vitro release test, a donor ointment was checked microscopically. It was observed that the droplets gradually disappeared from initial state of ointment. After the end of PC release, there was no trace of droplet observed in ointment.

In bleeding experiment, no droplets were observed in BL by microscope.

4. Discussion

4.1. Physicochemical state of intact ointment

The structure of Tacrolimus ointment formulation could be schematically shown as in Fig. 6, in which PC droplets were dispersed in ointment vehicle. It was generally said that the ointment vehicle prepared by petrolatum base was the mixture of liquid paraffin with relatively low molecular weight and microcrystalline fraction with relatively high molecular weight. The microcrystalline component would make a kind of three-dimensional network structures (Barry and Grace, 1971), and the oily liquid composition would lie to be immobilized between this network which could be related to the BL that might be separated by cone mesh filtration method (Saeki and Yasumori, 1972). It was also said that these components of the mixture had an equilibrium state that depended on temperature and pressure (Kato and Saito, 1967). According to Kobayashi and Saitoh (1998), it must be considered that Tacrolimus and PC concentrations in BL would be those in the vehicle outside of PC droplets. Then, as shown in Table 2, PC dissolved and saturated in the ointment base and the remaining PC dispersed as droplets. And some amounts of Tacrolimus also dissolved outside of PC droplets and most of Tacrolimus dissolved inside of such droplets.

Regarding a mass balance of Tacrolimus and PC between the amount within the PC droplets and the

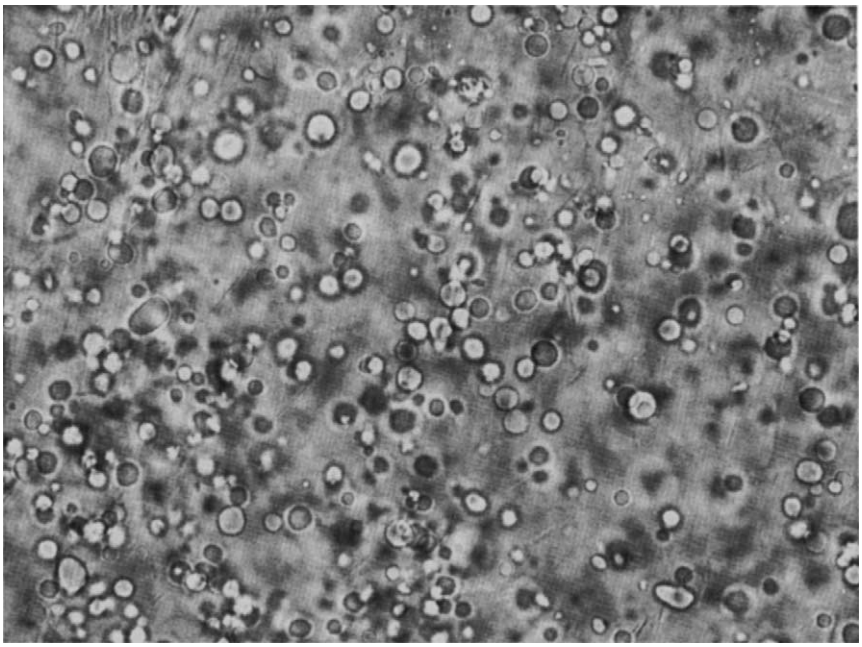

Plate 1. Microscopic observation of Tacrolimus ointment.

amount in the ointment vehicle (outside of the PC droplets) when introducing a volume fraction of PC droplets, ϕ_0, the concentrations of Tacrolimus outside and inside of the droplets, C_L^0 and C_S^0, respectively, and the concentration of PC outside of the droplet, S_L^0, following equations could be expressed,

$$S_0 = d_S\,\phi_0 + S_L^0(1-\phi_0) \tag{1}$$

then,

$$\phi_0 = \frac{(S_0 - S_L^0)}{(d_S - S_L^0)} \tag{2}$$

where d_S was a density of droplet which could be postulated equal as a density of pure PC (1.207 g/ml). Since S_L^0 values are shown in Table 2, ϕ_0 could be estimated as shown in Table 3. Regarding the drug substance, the total concentration of Tacrolimus, C_0, should be also expressed as,

$$C_0 = C_S^0\,\phi_0 + C_L^0(1-\phi_0) \tag{3}$$

As shown in Table 3, most of Tacrolimus drug substance was confirmed to localize inside of PC droplets, and in the case of the ointment with 1% PC, the value of C_S^0 was near the solubility of Tacrolimus in PC

Table 3
Calculated parameters for 0.1% Tacrolimus ointment with different PC contents

	PC composition in ointment			
	1% PC	2.5% PC	5% PC	10% PC
ϕ_0	0.0072	0.018	0.036	0.072
C_S^0 (μg/ml)	1.20×10^5	4.81×10^4	2.41×10^4	1.21×10^4
K_{SL}^0	11830	9330	10050	10120
A ($h^{-1/2}$)	0.00346	0.00587	0.00903	0.01554
D_S (cm^2/h)	0.0030	0.0046	0.0051	0.0065

(unpublished data). When we considered a partition coefficient of Tacrolimus between inside and outside of PC droplets, K^0_{SL} could be defined as,

$$K^0_{SL} = \frac{C^0_S}{C^0_L} \tag{4}$$

Eq. (3) was rewritten as Eq. (5).

$$\frac{C_0}{C^0_L} = K^0_{SL}\,\phi_0 + (1 - \phi_0) \tag{5}$$

From Table 2 and above equations, the calculated K^0_{SL} values are also listed in Table 3. K^0_{SL} values were almost constant, which indicated that Tacrolimus must be equilibrated between inside and outside of PC droplets in the intact ointment.

4.2. PC release from Tacrolimus ointment

From the PC releasing amount, Q_S of Fig. 5, the corresponding volume fraction of PC droplets in ointment, ϕ, was obtained as Eq. (6) and plotted in Fig. 7.

$$S_0 = d_S\phi + S^0_L(1 - \phi) + Q_S\left(\frac{F}{V_0}\right) \tag{6}$$

where F was the diffusion area through the membrane and V_0 was the ointment volume.

In this figure, each ϕ had a straight line at beginning of its release and deviated from the line in course of time. It could be reasonable to take application of Higuchi's release equation (Martin, 1993) regarding the substance release from suspension-type formulation to this releasing phenomenon of PC from ointment, because PC should be saturated in ointment base and the remaining PC was dispersed in the vehicle. In this sense,

$$Q_S\left(\frac{F}{V_0}\right) = [D_S(2S_0 - S^0_L)S^0_L]^{1/2}\sqrt{t} = k_S\sqrt{t} \tag{7}$$

where D_S was diffusion constant. Then, combination with Eq. (6) gave,

$$\phi = \phi_0 - A\sqrt{t} \tag{8}$$

where

$$A = \frac{k_S}{(d_S - S^0_L)} \tag{9}$$

As shown in Fig. 7, ϕ was changed according to Eq. (8) approximately at the initial releasing phenomenon of PC. Then, each A value was determined by application of Eq. (8) with the beginning of few data points of Fig. 7 and listed in Table 3. From Eq. (7), D_S were also calculated to give values from 3×10^{-3} to 6.5×10^{-3} cm^2/h, even though PC concentrations in ointment varied from 1 to 10% as shown in Table 3.

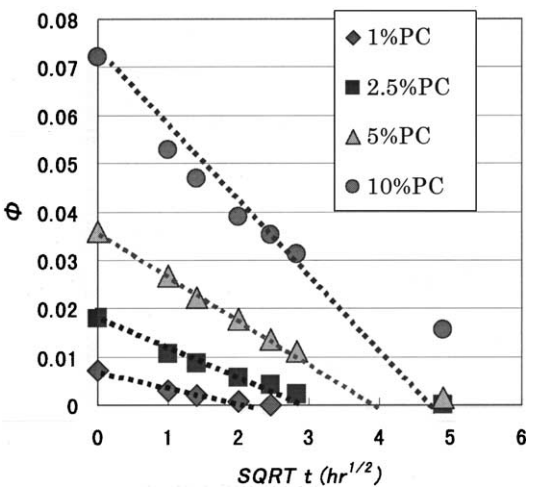

Fig. 7. The time course of volume fraction ϕ of PC droplets in ointment.

4.3. Theoretical consideration of first phase release of Tacrolimus

As mentioned above, there were two phases regarding the release phenomenon of Tacrolimus drug substance, which were combined at the turning time, t^*. Until t^*, it was considered that the release of PC might influence the first phase release of Tacrolimus because Tacrolimus was dissolved in PC droplets. During the period of PC release from ointment, the first phase of in vitro release of Tacrolimus ($Q1$) should be defined by the mass balance consideration as follows,

$$C_0 = C_S\phi + C_L(1 - \phi) + Q1\left(\frac{F}{V_0}\right) \tag{10}$$

where C_S and C_L were the concentrations of drug substance inside and outside droplets at the first release phase, respectively. If K_{SL} would be also defined as follows,

$$K_{SL} = \frac{C_S}{C_L} \tag{11}$$

then, $Q1$ was modified as Eq. (12) by using Eqs. (8) and (11),

$$Q1\left(\frac{F}{V_0}\right) = [C_0 - C_L\{1 + (K_{SL} - 1)\phi_0\}] + C_L(K_{SL} - 1)A\sqrt{t} \quad (12)$$

Based on the concept of Higuchi's equation (Martin, 1993), at initial short period of release time, namely, at $t \fallingdotseq 0$, C_L and K_{SL} could be regarded as C_L^0 and K_{SL}^0, respectively, and the first term of Eq. (12) became zero according to Eq. (5). Therefore, Eq. (12) was modified as,

$$Q1 = k\left(\frac{A}{\phi_0}\right)C_0\sqrt{t} \quad (13)$$

where k was constant.

Eq. (13) meant that the first phase release of Tacrolimus drug substance was expressed as a function of $\sqrt{t}$ with slope of $q1$ which was correlated with the terms of C_0 and A/ϕ_0, respectively. By using parameter values of Tables 1 and 3 regarding all these experiments of Tacrolimus ointment, including 0.03 and 0.3% concentrations, $q1/C_0$, were plotted against A/ϕ_0. As shown in Fig. 8, the correlation became a straight line. Therefore, it was interpreted that the release rate of Tacrolimus drug substance was controlled by the release rate of PC normalized by its initial volume fraction A/ϕ_0. Since the release rate of Tacrolimus would be influenced by its thermodynamic activity in PC which could be expressed as the ratio of the drug concentration and the solubility in PC (Shahi and Zats, 1968), it was interpreted that $q1$ decreased when the Tacrolimus concentration in PC decreased with increase of the initial PC volume. This releasing rate of drug could be influenced by the balance between the PC volume dispersed in ointment base and the release rate of PC.

4.4. Speculated consideration for generation of amorphous cluster

From the results of in vitro release experiment, it was found that PC had released faster than Tacrolimus from the ointment. Therefore, it would be interesting to speculate how the physicochemical state of the remaining Tacrolimus would change, which had dissolved in the released PC.

As shown in Fig. 4, it was observed that the slopes of $q2$ at the second phase release were constant and the values of turning point, Q^* were also constant among four kinds of 0.1% ointment. In the second release phase, there existed no more PC and no crystalline form of Tacrolimus were observed microscopically. Therefore, it was speculated that Tacrolimus should exist as the amorphous state in this phase.

If we would like to prepare a saturated PC solution of Tacrolimus and manufacture 0.1% Tacrolimus ointment with this PC solution and then investigate a releasing behavior of this ointment, what kind of result could be seen? Probably, the slope of $q1$ might have a maximum value and reach to the value of $q2$. In this

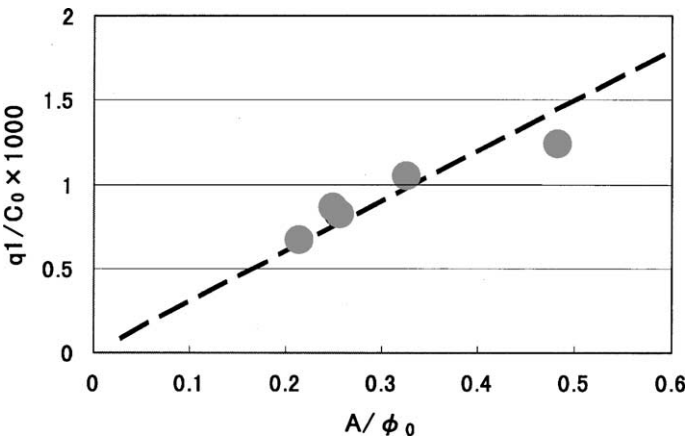

Fig. 8. Correlation between A/ϕ_0 and $q1/C_0$.

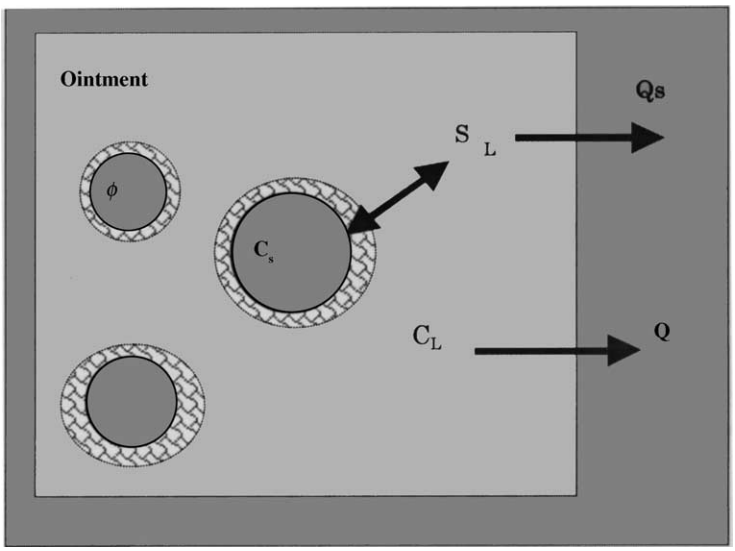

Fig. 9. Precipitation of amorphous Tacrolimus from saturated PC solution in m ointment (speculation).

case, it was anticipated that the dissolved Tacrolimus should be changed to an amorphous state precipitated around PC droplets simultaneously when PC released faster than Tacrolimus because the dissolving place of Tacrolimus should be decreased, which was schematically shown in Fig. 9. In this sense, Fig. 9 could be the representation of the state around the turning point.

In the case of the ointment with larger PC content, it was considered that the PC droplets became smaller as the releasing behavior progressed, where the equilibrium of Tacrolimus between inside (C_S) and outside (C_L) of PC droplets could be maintained. As PC released faster than Tacrolimus, the remaining Tacrolimus would still dissolve in the PC droplets, that is, the concentration of Tacrolimus in PC droplets would increase until the state of Tacrolimus would become saturated. When saturated, the amorphous could be generated afterward as stated above. Irrespective of PC content between those ointments, all PC droplets would reach to the saturated state with Tacrolimus, followed to precipitate amorphous Tacrolimus. Since the thermodynamic activity at the state of this turning point could be constant among those ointments, it was speculated that the release amount would reach to constant value, namely, Q^*.

4.5. Speculated consideration of second phase release for Tacrolimus

It would be anticipated that after PC released completely from ointment, all remaining Tacrolimus might become amorphous cluster being dispersed in ointment vehicle, for which concentration would reach a constant value, regardless of original PC content among 0.1% ointment, namely, $C_0 - Q^*(F/V_0)$ at each t^*. The release phenomenon of Tacrolimus from such kind of ointment should also obey Higuchi's equation based on constant activity of Tacrolimus.

$$Q2\left(\frac{F}{V_0}\right) = \left[D\left(C_0 - Q^*\left(\frac{F}{V_0}\right) - C_L^*\right)C_L^*\right]^{1/2} \times \sqrt{t} = q2\sqrt{t} \quad (14)$$

where C_L^* would be a saturated concentration of Tacrolimus when the amorphous drug substance would be dispersed in ointment vehicle. Then, the slope of release after t^*, $q2$ should be constant among 0.1% ointment. In fact, as shown in Fig. 3 and Table 1, $q2$ values were almost constant. If C_L^* could be monitored by such BL prepared from ointment without PC, the true value of D could be determined.

In order to support the speculated release mechanism of amorphous drug substance from ointment

base, it would be appropriate to obtain the release data of Tacrolimus from the ointment prepared without PC. In this case, as there was no PC, the releasing behavior would show just one straight line without turning point. But unfortunately by the normal manufacturing procedure without PC, Tacrolimus drug substance could not be solubilized in the ointment base and most of the Tacrolimus remained in crystalline state, in which the release rate was shown to be very small. And also it was difficult to prepare this ointment by using volatile solvent currently. Then, it would be interesting if a novel preparation method of ointment with amorphous drug without solvent would be explored.

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References

Barry, B.W., Grace, A.J., 1971. Structural rheological textual properties of soft paraffin. J. Texture Stud. 2, 259–279.

Higuchi, T., 1961. Rate of release of medicaments from ointment bases containing drugs in suspension. J. Pharm. Sci. 50, 874–875.

Honbo, T., Kobayashi, M., Hane, K., Hata, T., Ueda, Y., 1987. Oral dosage form of FK506. Transplant. Proc. XIX, 17–22.

Kato, T., Saito, T., 1967. Physicochemical studies on ointment 11. Arch. Pract. Pharm. 27., 127–141.

Kobayashi, N., Saitoh, I., 1998. A method to measure the solubility of drugs in ointment bases. Chem. Pharm. Bull. 46, 1833–1835.

Martin, A., 1993. Physical Chemical Principles in the Pharmaceutical Sciences. “Diffusion and Dissolution”, Physical Pharmacy, 4th ed. Lea & Febiger, Philadelphia, London (Chapter 13).

Saeki, H., Yasumori, S., 1972. Procedure for bleeding test of ointment. Yakuzaigaku 32, 182–189.

Shah, V.P., Elkins, J.S., Williams, R.L., 1999. Evaluation of the test system used for in vitro release of drugs for topical dermatological drug products. Pharm. Dev. Tech. 4, 377–385.

Shahi, V., Zats, J.L., 1968. Effect of formulation factors on penetration of hydrocortisone on the in vitro release of fluocinolone acetonide and its acetate ester. J. Pharm. Sci. 57, 928–933.

Tanaka, N., Kado, K., Murakami, Y., Yoshida, H., Ohnishi, N., Ibuki, R., 2000. Formulation development of protopic ointment. In: Abstract of Millennium World Congress of Pharmaceutical Sciences in San Francisco, April.