Development of a Test Method for *in Vitro* Drug Release from Soluble and Crystal Dispersion Type Ointments

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We established an *in vitro* drug release test for ointments, using oxybenzone as a model drug. At first, we concentrated upon the reproducibility of the results, the effects of the receiver solution composition, and the effects of the sample loading weight to fix the conditions for the *in vitro* drug release test.

Once the conditions for the test were fixed, we examined the validity of the test by evaluating ointments containing oxybenzone at concentrations of 0.11—15.1%. In this study, we applied T. Higuchi's equation directly to the soluble-type ointment to maintain continuity.

Then, for crystal-dispersion type ointments, the diffusion coefficient was calculated by applying the solubility determined by cone mesh filtration method to T. Higuchi's equation. For soluble-type ointments, the diffusion coefficient was calculated by applying a modification of T. Higuchi's equation. Consequently, apparent diffusion coefficient (D_{app}) showed constant values, irrespective of the state of the drug (soluble or crystal dispersion). Thus, a validity of D_{app} was suggested.

Moreover, the theoretical curve of slope fitted well to the observed values in practical drug concentration levels up to 10%. It was suggested that this *in vitro* drug release test for ointments is a useful and practical method for quality assurance specifications.

Key words ointment; oxybenzone; *in vitro* drug release test; topical drug product

In vitro drug release tests for topical drug can be useful for determining biological equivalence between pioneer and generic products, checking equivalence after change in batch size, detecting changes in components or composition, and checking equivalence after modification of manufacturing equipment or processes.[1,2] The test method has also been suggested as a possible standard for quality control of topical drug products.[3,4] We thus established an *in vitro* drug release test for ointments.

The theory of the drug release profile from ointments was established by T. Higuchi[5] and W. I. Higuchi[6] and has been used in various methods.[7—9] However, there has been no *in vitro* drug release test for topical drug products aimed at topical action validated like that for oral dosage forms. In the 90's a test method was described for evaluating transdermal delivery systems (TTS's) in the Drug Release section in the USP 22 revision.[10] However, this method was not for the purpose of evaluating topical drug products aimed at topical action, but for the purpose of evaluating TTS.

The *in vitro* drug release profile for practical formulation aimed at topical action was reported to be suitable as an evaluation method for bioequivalence.[2] The study used the slope obtained from plotting the cumulative amount of drug released against the square root of time as an index for release, but further analysis was not performed. With respect to practical formulation, there have been few studies analyzing drug release profiles from hydrocarbon bases, which display inferior release results. In the present study, using oxybenzone as a model drug, we performed intensive parameter analysis on *in vitro* drug release profiles from hydrocarbon bases by applying T. Higuchi's equation.

For analysis of the cumulative amount of drug released, T. Higuchi's equation[5]: $q=\{(2C_o-C_s)C_s\cdot D\cdot t\}^{1/2}$ and an equation modified for soluble-type ointments: $q=C_o(2\cdot D\cdot t)^{1/2}$ were used. The latter equation is the same form as that of W. I. Higuchi, except for the constant terms. The above-mentioned are hereafter referred to as Higuchi's equation. It was pointed out that, practically speaking, there is no serious errors between T. Higuchi's equation modified for soluble-type ointments and W. I. Higuchi's equation.[11]

The "slope" obtained from plotting the amount of drug released per unit area against the square root of time was designated as an index for the release profile, with q: amount of drug released per unit area, C_o: drug concentration in base, C_s: solubility of drug in base, D: diffusion coefficient of drug in base, and t: time.

Experimental

Materials and Reagents The ointment base consisted of white petrolatum (JP) and liquid paraffin (JP). Other reagents used were of special or HPLC grade.

Preparation of Ointments The ointments were prepared as described in our previous report.[12]

***In Vitro* Drug Release Test from Ointments** The *in vitro* drug release test was performed according to the method of Saitoh *et al.*[13] using a Franz type diffusion cell; a liquid phase-separating filter, 1PS (Whatman, U.K.) as a support membrane; and a methanol–water mixture as the receiver solution at the experimental temperature of 37 °C.

The amount of drug (oxybenzone as a model drug) released was determined by measuring drug concentration in the receiver solution at precise time intervals. Drug concentrations in a 0.5 ml receiver solution collected from sampling ports were measured under the HPLC conditions described.[12] The cells were refilled with methanol–water solution each time to keep the receiver solution at a constant volume.

Measurement of Drug Concentration in the Ointment Bases The drug concentration in the ointment bases was measured as described.[12]

Measurement of Ointment Density To measure the density of the ointment, a 10 cm^3 portion was weighed at 37±1 °C.

Results and Discussion

Confirmation of *In Vitro* Drug Release Test Method The test method was set based on the results of the reproducibility, composition of the receiver solution and sample loading weight.

Reproducibility To confirm reproducibility, six consecutive measurements were performed with a sample at the

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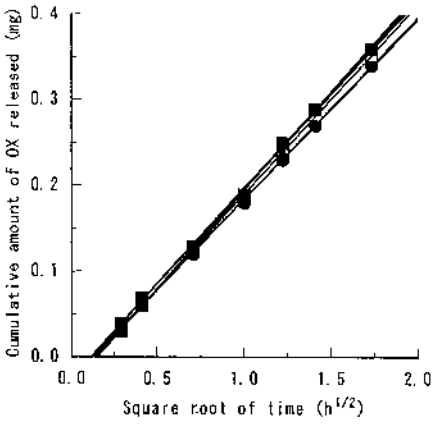

Fig. 1. Fitting on the Release Profile to Higuchi's Equation
OX: Oxybenzone.

Table 1. Reproducibility of *In Vitro* Drug Release Test

Sample No.	Slope ($mg/h^{1/2}$)	Correlation coefficient
1	0.231	0.9989
2	0.216	0.9993
3	0.230	0.9980
4	0.217	0.9994
5	0.231	0.9988
6	0.243	0.9989
Mean	0.228	
S.D.	0.009	
C.V.	3.98	

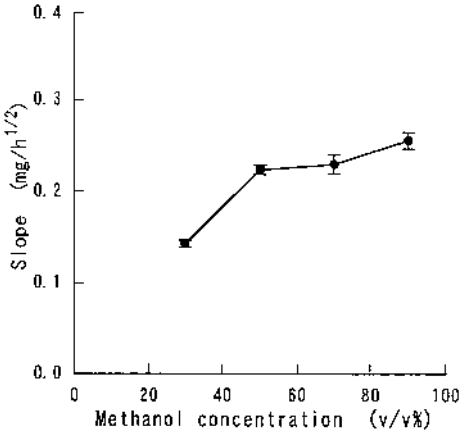

Fig. 2. Relationship between Methanol Concentration in Receiver Solution and Slope

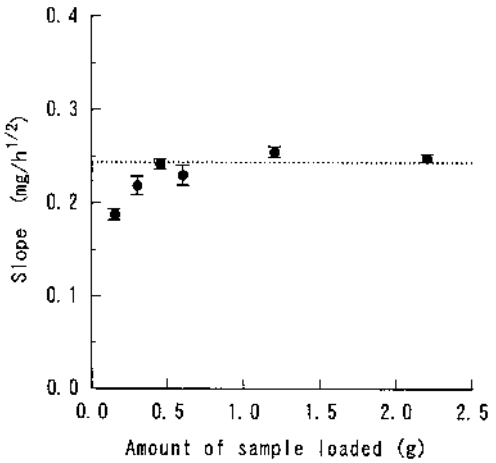

Fig. 3. Relationship between Amount of Sample Loaded on Donor Cell and Slope

weight of 0.6 g and a 70% methanol receiver solution. The amount of drug released (Q) against the square root of time ($\sqrt{t}$) is shown in Fig. 1. While Higuchi's equation indicates that Q is proportional to $\sqrt{t}$, it has been pointed out that a lag time exists in the actual release process due to the time it takes for the drug to infiltrate the membrane.[14] As shown in Fig. 1, the plots of Q against $\sqrt{t}$ give a straight line with a lag time. Slopes and the correlation coefficients of these straight lines are shown in Table 1. Good linearity was obtained with correlation coefficients >0.998. The reproducibility was confirmed by C.V. value of the slopes $<4\%$.

Relationship between Receiver Solution Composition and Release Profile *In vitro* drug release test using a Franz type diffusion cell, it was suggested that the membrane permeability affects the *in vitro* drug release profile.[15] Consequently, the receiver solution composition would affect the membrane permeability because of the difference in the solubility of the drug to receiver solution that fills the pores of the membrane.

We therefore examined the relationship between the receiver solution composition and release profile. As shown in Fig. 2, when the methanol concentration in the receiver solution was 50 to 90%, almost no effect on the slope was noted. At 30% methanol concentration, a decrease of the slope was noted.

From these results, we chose the 70% methanol solution as the receiver solution. At this level, there would also be no marked effect in the receiver solution composition due to evaporation.

Relationship between Sample Loading Weight and Release Profile Several conditions must be satisfied prior to applying Higuchi's equation, which was used for analysis of the release profile. Especially, infinite dose conditions should be kept over the course of the study, finite dose conditions would result in different release profiles even for the same samples. To determine the optimal sample loading weight, we examined the relationship between the sample loading weight and the release profile. As shown in Fig. 3, when the sample loading weight was above 0.45 g, the slopes were almost the same. On the other hand, at below 0.3 g, the slope increased with the sample loading weight. Thus, the optimal weight was fixed as 0.6 g, so that diffusion occurred under infinite dose conditions.

It was pointed out that under usual experimental conditions, where the amount of formulation loaded is 100—300 mg, the amount available for diffusion may be considered to be infinite.[3] The release area of our diffusion cell was about 3 times as large as the area of that report. Therefore, we regarded a sample loading weight of 0.6 g as reasonable.

Establishing the *In Vitro* Drug Release Condition In establishing the *in vitro* drug release conditions for topical drug products, three major factors needed to be considered.

The first was the choice of the support membrane. One report suggested the use of isolated human skin,[16] but it is very difficult to obtain human skin with consistent perme-

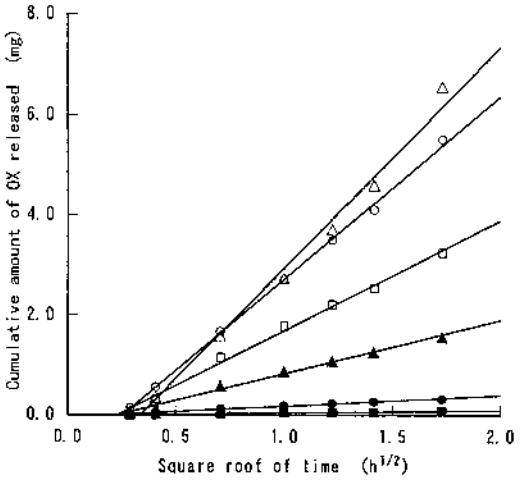

Fig. 4. Fitting on the Release Profile to Higuchi's Equation

OX: Oxybenzone. OX concentration in ointment: ■, 0.11%; ●, 0.47%; ▲, 1.81%; □, 4.54%; ○, 8.81%; △, 15.1%.

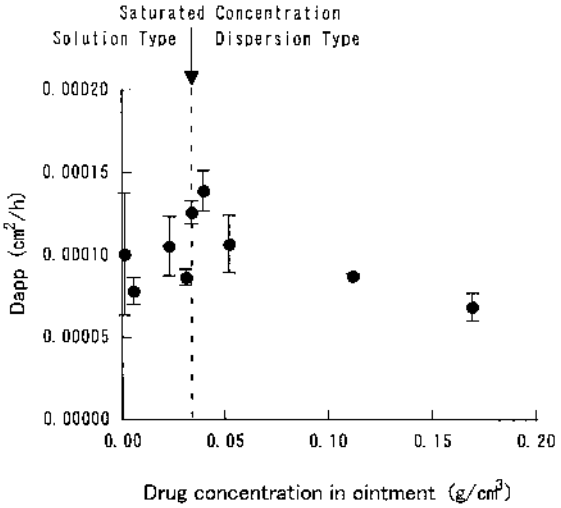

Fig. 5. Apparent Diffusion Coefficient (D_{app}) from Higuchi's Equation

The units of drug concentrations were changed from percent to g/cm^3 based on the density of each ointment.

ability. The purpose of that study was primarily to predict *in vivo* percutaneous absorption. In the present study, we wanted to establish an *in vitro* drug release test as checking biological equivalence between pioneer and generic products, checking equivalence after change in batch size, determining changes in components or composition, or checking equivalence after modification of the manufacturing equipment or processes. For these purposes, a synthetic membrane was considered suitable.

The second important factor was the choice of the receiver solution. The use of buffers of aqueous solutions as the receiver solution has been suggested.[8] However, such buffers are not generally considered to be good solvents for major active ingredients like corticosteroids for topical drug products, even after addition of detergents. Therefore, appropriate organic solvents should be selected to maintain high drug solubility. Many studies have used receiver solutions containing organic solvents.[3,7,9]

The final important point was the choice of instrument. Nearly all published studies on *in vitro* drug release tests from topical drug products used Franz type diffusion cell.

Based on the above considerations, we settled on the following conditions for our *in vitro* drug release test:

Instrument: Franz type cell (volume about 30 ml)

The receiver solution is stirred with a magnetic stirrer to maintain the sink condition.

Membrane: Phase separator 1PS (Whatman, U.K.)

Receiver solution: Methanol/water (70/30 v/v)

Sample loading weight: 0.6 g.

Confirmation of Validity of *In Vitro* Drug Release Test. Drug Concentration-Dependence on Release Profile To confirm the validity of the test, the drug concentration-dependence on the release profile was examined. Ointments containing oxybenzone at concentrations of 0.11—15.1% were prepared and the *in vitro* drug release test was performed. Q against $\sqrt{t}$ is shown in Fig. 4. The plotting exhibited a straight line, indicating the validity of applying Higuchi's equation.

Calculation of Apparent Diffusion Coefficient (D_{app}) To measure drug solubility in ointment bases, the drug concentrations in the bleeding liquid collected by the cone mesh filtration method have been useful.[12] The drug solubility in ointment bases are a very important parameter for T. Higuchi's equation. If the drug solubility is unknown, the diffusion coefficient could not be determined. The drug solubility of 3.1% obtained by this method was applied to Higuchi's equation to give D_{app}. In addition, in order to calculate D_{app}, the unit of concentrations were changed from percent to g/cm^3 based on the density of each ointment. Therefore, the diffusion coefficient in crystal dispersion ointment was determined directly.

The relationship between the drug concentration in the ointment and D_{app} is shown in Fig. 5. D_{app} showed constant values at about 0.0001 cm^2/h, irrespective of the state of the drug (dissolved or dispersed). It was suggested that the drug crystals dispersed in ointment hardly hinder the diffusion of the drug molecules.

The diffusion coefficients of steroids in hydrocarbon bases are generally in the range of 10^{-10}—10^{-8} cm^2/s.[17] They are theoretically in inverse relation to the cubic root of the molar weight of the diffusing substance.[18] Molar weights for oxybenzone and hydrocortisone of 170 ml[19] and 290 ml,[20] respectively, have been reported. Theoretically, the diffusion coefficient of oxybenzone is about 1.2 times that of hydrocortisone. The *in vitro* release tests were performed at 37 °C. Applying Arrhenius' equation to the diffusion coefficient, the diffusion coefficient at 37 °C would be about 3 (= exp(310/298)) times as large as that at 25 °C. Taking these facts into consideration, the D_{app} value of 3×10^{-8} cm^2/s obtained in the present study seems to valid.

Application to Theoretical Equations The relationship between the theoretical curve obtained from applying the solubility to the ointment base and D_{app} to Higuchi's equation and the observed values is shown in Fig. 6. At a point of the highest drug concentration in the ointment, the observed value was found to be less than the theoretical curve. The most likely explanation for this is that the dissolution rate of the drug crystals dispersed in the ointment reaches a level that cannot be overlooked when compared to the release rate of the drug from the ointment to the receiver solution, or the amount of drug crystal dispersed in the ointment is too much

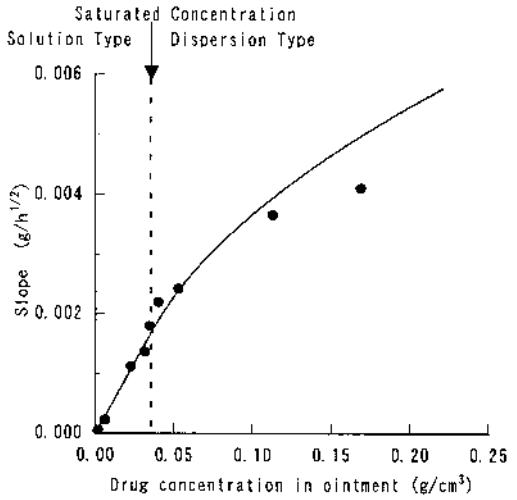

Fig. 6. Relationship between Observed Slope and a Theoretical Curve from Higuchi's Equation

to avoid its interference with the diffusion of drug molecules in the base. In any case, this point of the drug concentration of 15.1% (0.17 g/cm^3) is beyond the practical concentration range. For practical drug concentration levels up to 10% (0.11 g/cm^3), the theoretical curve fitted well to the observed values. These results suggest that the solubility of the drug to the ointment base and D_{app} obtained in the present study are valid.

Few studies have examined the parameters of the *in vitro* drug release test in detail, especially for the crystal dispersion-type. There has been a report on *in vitro* drug release from practical formulations aimed at topical effects focusing mainly on the difference in release profiles between samples with respect to the bioequivalence of pioneer and generic products.[3] Another study reported analysis of parameters such as drug solubility in vehicles and diffusion coefficients from the standpoint of drug delivery system.[21] The reason for the lack of studies on parameters of this type of test is that they require measurement of drug solubility to bases, which is difficult. To resolve this issue, the comparison of slopes alone is adequate. This removes the need for detailed scrutiny of parameters to detect differences between pioneer and generic products or detecting batch-to-batch differences. Moreover, W. I. Higuchi's equation on the soluble-type is not continuously linked to T. Higuchi's equation on the crystal dispersion-type because both equations were induced by approximation. Thus, there was a report which treated the theoretical aspects of the release profiles of the soluble and crystal dispersion-type ointments comprehensively,[22] but there have been no studies which have comprehensively examined the release profiles of the two ointment types. In the present studies, we applied T. Higuchi's equation directly to the soluble-type ointment to maintain continuity at the expense of the strictness. As a result, the theoretical curve fitted well to observed values at practical drug concentration levels up to 10% (0.11 g/cm^3).

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