CASE 9

Optimization of Model Ointment Prescriptions for In Vitro Percutaneous Permeation

Abstract: Evaluation of percutaneous permeation of a transdermal drug delivery system usually requires expensive and repeated animal experimentation, which also requires confirmation of a species difference between animals and human beings. In this study, considering these issues, we applied the quality engineering method to technological development in prescription optimization of an effective transdermal drug delivery system.

1. Introduction

In recent days, transdermal drug delivery that supplies medical essence through the skin has attracted a great deal of attention because this causes few side effects, such as gastrointestinal disorder, compared with a conventional drug delivery system via the digestive tract. The new method can be used with patients or aged people who have a feeble swallowing movement (movement of the muscles of the throat while swallowing food).

However, in general cases it is not easy for medicine to permeate the skin. Since normal skin is equipped with a barrier ability to block alien substances from the outside, penetration by medicine is also hampered. Therefore, to obtain sufficient permeation for medical treatment, several additives, including a drug absorption enhancer, need to be compounded. As a result, the prescription becomes more complex and a longer period of research on prescription optimization is required. Moreover, for the evaluation of percutaneous permeation of a transdermal drug delivery system (e.g., an ointment), animal experimentation is needed. This animal experimentation not only is economically inferior due to the recommendation that four or more repeated experiments be performed to eliminate individual differences but also requires confirmation of a species difference between animals and human beings. In this study, considering these issues, we used the quality engineering method for the technological development of an effective transdermal drug delivery system.

2. Experimental Procedure

Among several existing evaluation methods of percutaneous permeation of a transdermal drug delivery system, we selected an in vitro percutaneous permeation experiment, the most inexpensive and handiest. Figure 1 shows the structure of a diffusion cell used in the in vitro percutaneous permeation experiment.

Between a donor cell (supplying medicine) and a receptor cell (receiving medicine), we place a horny layer of skin extracted from an animal on the side of the donor cell. Skin extracted from various types of animals was used for the horny layer. We started with an application of ointment on the skin extracted from the side of the donor cell. Since medical essence (a principal agent in medicine) that penetrates the skin is dissolved in the receptor solution, by measuring the amount of the principal agent in solution at each point in time, we calculated the cumulative amount of skin permeation per unit area.

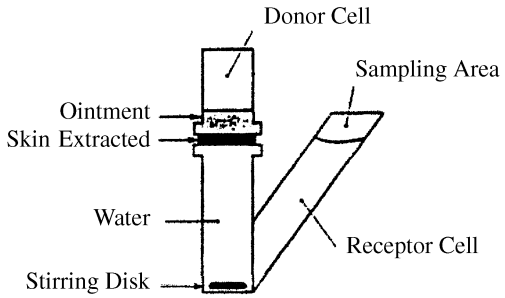


Figure 1
In vitro skin permeation experiment

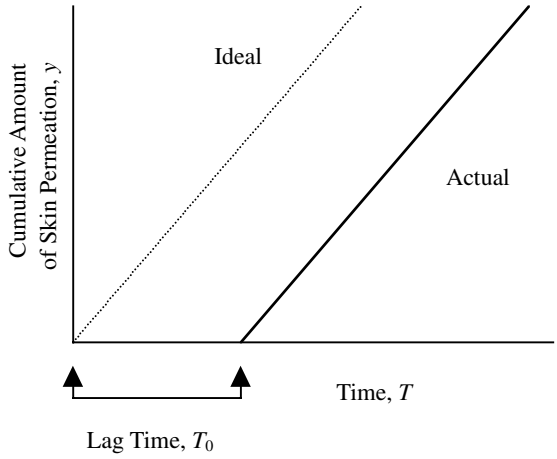


Figure 2
Relationship between cumulative amount of skin permeation (y) and time (T)

When optimal configuration is determined from the result of animal experimentation, the animal species difference comes into question, as is often the case with other experiments. If we could use human tissues, this problem would be solved easily. Yet for ethical reasons, it is difficult to do this in Japan. To solve this problem, we used an animal species difference as a noise factor. By doing so, we attempted to obtain an optimal prescription that could achieve skin permeation that is not only independent of the animal species difference but also superior for a human being with respect to the effects of barrier ability or absorption enhancer in skin permeation.

3. Signal and Noise Factors

Assuming that concentration of the principal agent included in the ointment that is applied to the donor cell is constant, the cumulative amount of skin permeation, y, normally changes with time, T (Figure 2). That is, while there occurs a lag time, T_0, because the rate of skin permeation is small due to the gentle slope of concentration of principal agents contained in the skin and receptor solution in the initial stage, as time elapses, the slope of a line corresponding to skin permeation speed becomes constant. The ideal is an ointment with a short lag time and high skin permeation speed.

Owing to this lag time, we can express the scientific phenomenon by $y = \beta(T - T_0)$. If this equation were selected as the generic function, we would spend most of our time pursuing this phenomenon. Therefore, although it was unrealistic that lag time was equal to zero at this point in time, by assuming an ideal prescription where a principal agent permeates at a high and constant speed with no lag time shortly after being applied to the skin, we adopted a zero-point proportional equation, $y = \beta T$, as the generic function. Following this idea, we evaluated deviation from the ideal function using the SN ratio.

As levels of the noise factor, we selected an abdominal skin extracted from a rat (hairless rat, 8 weeks old, male) (N_1), which is considered normally to have higher skin permeation than that of a human and is highly subject to an absorption enhancer, and the back skin of a minipig (Yucatan micropig, 5 weeks old, female) (N_2), which is regarded to be analogous to a human's skin structure, less susceptible to an absorption enhancer, and has low permeation.

For N_1 (rat), after killing a rat in an ether atmosphere, we extracted the skin in its abdomen and used it for the in vitro skin permeation experiment. On the other hand, for N_2 (minipig), we obtained a commercial skin set and removed its under-skin fat after thawing it.

As a signal factor, we chose the sampling time of the receptor solution in the in vitro skin permeation experiment. Because of a great difference in skin permeation speed between N_1 and N_2, we adjusted the signal ranges of N_1 and N_2 to approximately equalize each amount of permeation (Table 1).

4. SN Ratio

Table 2 shows part of the data regarding the principal agent's cumulative amount of skin permeation per skin area for N_1 and N_2 as the output. Based on this result, we performed the following analysis (analysis 1).

Below is an example of calculation for experiment 2.

Total variation:

$$S_T = 26.5^2 + 152.8^2 + \cdots + 1138.7^2 = 2{,}461{,}900 \qquad (f = 9) \tag{1}$$

Effective divider:

$$\gamma_1 = 2^2 + 4^2 + 6^2 + 8^2 + 10^2 = 220 \tag{2}$$

$$\gamma_2 = 4^2 + 8^2 + 24^2 + 48^2 = 2960 \tag{3}$$

Linear equations:

$$L_1 = (2)(26.5) + (4)(152.8) + \cdots + (10)(748.3) = 142{,}892.2 \tag{4}$$

$$L_2 = (4)(1.0) + (8)(13.4) + \cdots + (48)(1138.7) = 65{,}503.9 \tag{5}$$

Table 1
Signal factors and levels (sampling point) (h)

Noise Factor	T_1	T_2	T_3	T_4	T_5
N_1	2	4	6	8	10
N_2	4	8	24	48	—

Variation of proportional term:

$$S_\beta = \frac{(L_1 + L_2)^2}{\gamma_1 + \gamma_2} = 2{,}002{,}184 \qquad (f = 1) \tag{6}$$

Variation of differences between proportional terms:

$$S_{N\beta} = \frac{(L_1 - L_2)^2}{\gamma_1 + \gamma_2} = 375{,}496 \qquad (f = 1) \tag{7}$$

Error variation:

$$S_e = S_T - S_\beta - S_{N\beta} = 84{,}220 \ (f = 7) \tag{8}$$

Error variance:

$$V_e = \frac{S_e}{7} = \frac{84{,}220}{7} = 12{,}031 \tag{9}$$

SN ratio:

$$\eta = 10 \log \frac{[1/(\gamma_1 + \gamma_2)]\ (S_\beta - V_e)}{V_e} = -12.84 \text{ dB} \tag{10}$$

Sensitivity:

$$S = 10 \log \left[\frac{1}{\gamma_1 + \gamma_2} (S_\beta - V_e) \right] = 27.97 \text{ dB} \tag{11}$$

In this calculation we computed the SN ratio without including $N \times \beta$, corresponding to an animal species difference in the error variance. This is because the objective of our study was to obtain not a prescription with a small difference in permeation between a rat and minipig but an optimal prescription with superior permeation for a human being as well as for a rat and minipig. If an animal species difference was included in the error variance, we might select good levels for a rat at the sacrifice of a minipig, or vice versa. Furthermore, the effort of finding good levels for a human being might also be affected. By dealing with an animal species difference as an indicative factor without including $N \times \beta$ in the error variation, we could obtain an optimal prescription with excellent permeation for both a rat and a minipig and adjust the amount of principal agent and application time for humans. Therefore, we considered that a difference in per-

Table 2
Principal agent's cumulative amount of skin permeation per skin area (μg/cm²)

Noise Factor	No.	T_1	T_2	T_3	T_4	T_5
N_1	1	0.0	0.0	2.3	4.6	7.7
	2	26.5	152.8	319.3	528.3	748.3
	⋮	⋮	⋮	⋮	⋮	⋮
	18	15.0	142.8	378.5	545.7	718.8
N_2	1	0.2	1.0	25.4	123.8	—
	2	1.0	13.4	447.4	1138.7	—
	⋮	⋮	⋮	⋮	⋮	⋮
	18	2.3	20.1	335.7	1059.7	—

meation due to an animal species difference was not a noise factor.

What is important in permeation is a large amount of permeation in a short time (i.e., a high SN ratio). According to Table 2, it is obvious that there is a significant difference in permeation per unit time between N_1 and N_2, that is, a considerable difference in sensitivity. In this case we should conduct an analysis using time as the output under the same amount of permeation. In addition, since a certain medical effect need was determined by the amount of medicine given, we attempted an analysis based on the cumulative amount of skin permeation as the signal, and its corresponding time as the output, that is, an analysis that swaps the input signal and the output characteristic in analysis 1 (analysis 2). The calculation procedure of analysis 2 was basically the same as that for analysis 1. However, since the amount of permeation differed largely from experiment to experiment, the range of a signal factor was determined by picking up an overlapping range between N_1 and N_2 for each experimental run. Thus, while the range of a signal factor for each column was different in analysis 2, the effective divider for each row, r, was common for N_1 and N_2.

5. Results of Experiment

The control factors are allocated as shown in Table 3. To determine an ointment prescription with

Table 3
Control factors and levels

Control Factor	Level 1	Level 2	Level 3
A: amount of sampled sollution (mL)	Mid	Large	—
B: amount of applied ointment (mL)	Small	Mid	Large
C: amount of principal agent (%)	Low	Mid	High
D: amount of absorption enhancer 1 (%)	None	Mid	High
F: amount of absorption enhancer 3 (%)	None	Mid	High
G: amount of additive agent 1 (%)	Low	Mid	High
H: amount of additive agent 2 (%)	Low	Mid	High

excellent permeation, we assigned each prescription ingredient to control factors *C* to *H*. Considering that the amounts of applied ointment or receptor solution affect the amount of skin permeation as well as the variability in an in vitro skip permeation experiment, we also allocated them to factors *A* and *B*.

Table 4 shows SN ratios and sensitivities. Now, for rows 5, 10, and 15, since we could not arrange a uniform prescription because suspension occurred in the concentrate of ointment, no experiment was implemented. Then, because we could not judge whether or not the absorption was good or poor, the process average was substituted into these rows. Figure 3 shows the response graphs of the SN ratio, η, and sensitivity, *S*, for analysis 1. In analysis 2, the sensitivity shown in Figure 4 is the more desirable the smaller it is, since the output was time. This is contrary to analysis 1.

Based on the idea that the sensitivity is more important than the SN ratio to obtain an optimal prescription enabling a principal agent to be quickly absorbed, we determined the optimal configuration considering sensitivity a higher priority. In this case, since factor *C*, amount of principal agent, was an adjusting factor in the case of dealing with a human being, we selected the second level: the same as the initial configuration. According to analysis 1, the optimal configuration turned out to be $A_1B_2C_2D_2E_3F_2G_1H_2$, whereas $A_2B_3C_2D_2E_3F_2G_1H_2$ was chosen from

Table 4
SN ratios and sensitivities (dB)

No.	*A* 1	*B* 2	*C* 3	*D* 4	*E* 5	*F* 6	*G* 7	*H* 8	SN Ratio	Sensitivity
1	1	1	1	1	1	1	1	1	−16.74	6.79
2	1	1	2	2	2	2	2	2	−12.84	27.96
3	1	1	3	3	3	3	3	3	−20.84	20.26
4	1	2	1	1	2	2	3	3	−20.59	3.75
5	1	2	2	2	3	3	1	1	−19.58[a]	17.64[a]
6	1	2	3	3	1	1	2	2	−20.04	10.38
7	1	3	1	2	1	3	2	3	−21.32	9.37
8	1	3	2	3	2	1	3	1	−22.12	25.67
9	1	3	3	1	3	2	1	2	−13.56	38.46
10	2	1	1	3	3	2	2	1	−19.58[a]	17.64[a]
11	2	1	2	1	1	3	3	2	−14.87	22.73
12	2	1	3	2	2	1	1	3	−23.29	24.52
13	2	2	1	2	3	1	3	2	−24.89	6.36
14	2	2	2	3	1	2	1	3	−19.65	17.59
15	2	2	3	1	2	3	2	1	−19.58[a]	17.64[a]
16	2	3	1	3	2	3	1	2	−16.78	17.33
17	2	3	2	1	3	1	2	3	−32.45	6.64
18	2	3	3	2	1	2	3	1	−13.79	27.25

[a] Process average was used.

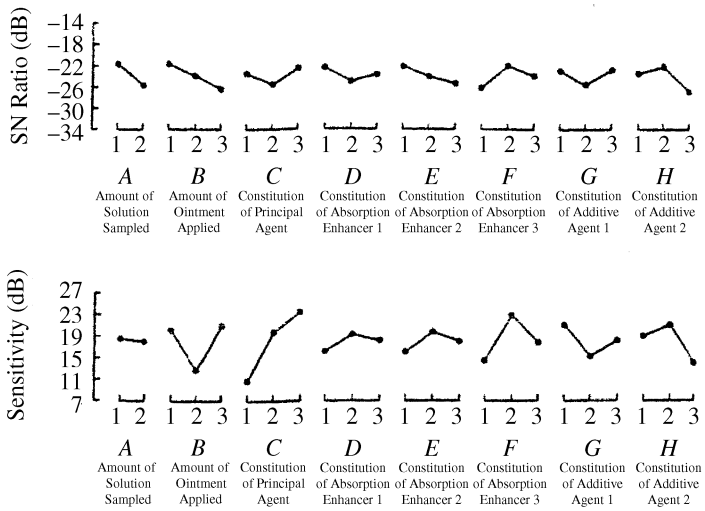


Figure 3
Analysis 1 of in vitro skin permeation experiment (signal: time)

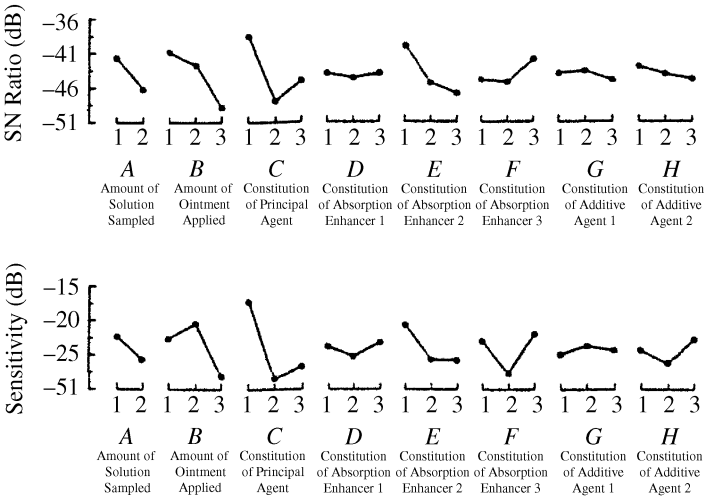
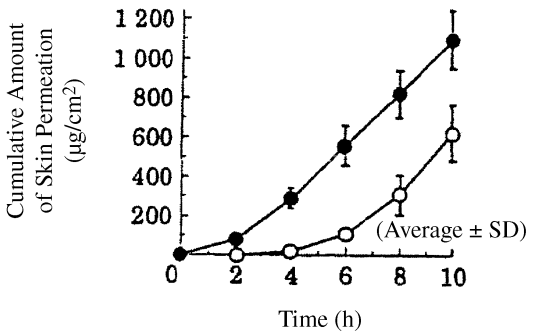
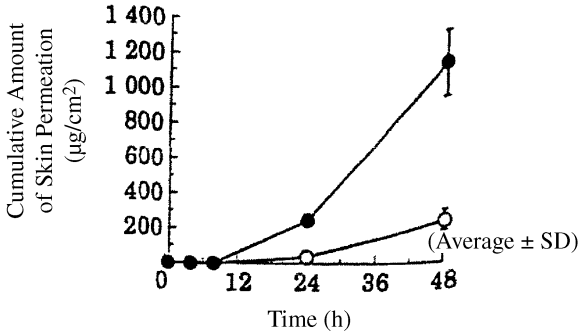


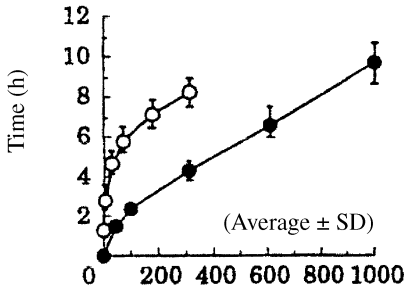
Figure 4
Analysis 2 of in vitro skin permeation experiment



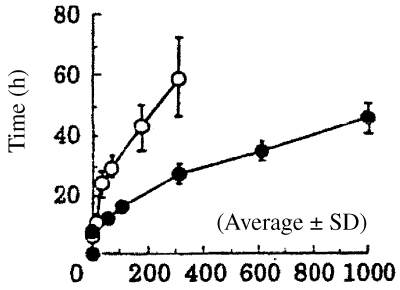
(a) Analysis 1 (Signal: Time)
Permeation Profile for N_1 (Rat)
under Initial (○) and Optimal (●) Configurations



(b) Analysis 1 (Signal: Time)
Permeation Profile for N_2 (Minipig)
under Initial (○) and Optimal (●) Configurations



(c) Analysis 2 (Signal: Time)
Permeation Profile for N_1 (Rat)
under Initial (○) and Optimal (●) Configurations



(d) Analysis 2 (Signal: Time)
Permeation Profile for N_2 (Minipig)
under Initial (○) and Optimal (●) Configurations

Figure 5
Graph for confirmatory experiment

analysis 2. Comparing both of the optimal configurations, we could see differences at A and E. Since factor A was an experimental condition in an in vitro skin permeation experiment, and also because E_1 and E_2 were nearly equivalent, we concluded that the optimal configurations obtained from both of the experiments were almost consistent as a result.

As for the confirmatory experiment, we adopted the optimal configuration obtained for analysis 1 because analysis 2 had been performed after all other experiments, including the confirmatory experiment, were completed.

Initial configuration: $A_1B_3C_2D_2E_1F_1G_1H_1$

Optimal configuration: $A_1B_3C_2D_2E_2F_2G_2H_2$

6. Results and Discussion of Confirmatory Experiment

In the confirmatory experiment, considering the differences between each individual animal, we repeated each experiment six times. Figure 5*a* and *b* show profiles of skin permeation for lapsed time un-

Table 5
Estimation and confirmation of SN ratio and sensitivity (dB)

	Analysis 1				Analysis 2			
	SN Ratio		Sensitivity		SN Ratio		Sensitivity	
Configuration	Estimation	Confirmation	Estimation	Confirmation	Estimation	Confirmation	Estimation	Confirmation
Initial	−18.9	−25.8	21.6	15.5	−45.9	−36.5	−28.4	−18.1
Optimal	−12.4	−15.5	36.0	27.9	−53.9	−46.1	−40.5	−30.2
Gain	6.5	10.3	14.5	12.4	−8.0	−9.6	−12.1	−12.1

der both initial and optimal configurations for N_1 and N_2.

Under the optimal configuration, we confirmed excellent skin permeation for both a rat and a minipig, which led to remarkable improvement compared with those under the initial configuration. Additionally, combining this result with that of analysis 2 and plotting each data point by setting the amount of permeation as the signal and time as the output characteristic, we obtained Figure 5*c* and *d*. These results reveal that a considerable amount of time was reduced when a certain amount of permeation is achieved.

Table 5 shows each pair of estimations and confirmations of the SN ratio, η, and sensitivity, S, for analyses 1 and 2. Now all factors are used for estimation. In analysis 2, since shorter permeation time was a characteristic (i.e., a smaller sensitivity was preferable), the estimated gain turns out negative. The reason that the estimated gain of the SN ratio becomes negative is that if an improvement in σ is relatively smaller than that of β because a decrease in β becomes larger than an improvement in σ, eventually the SN ratio (defined as β^2/σ^2) diminishes.

Comparing the estimated and measured values of the SN ratio and sensitivity, we found that both were nearly consistent in terms of the gain of sensitivity for both analyses 1 and 2; and for analysis 2, the reproducibility of the SN ratio also improved. In analysis 2 the gain in the SN ratio resulted in negative values for both estimation and confirmation. However, as the standard deviations in Figure 5*c* and *d* show, although the variability under the optimal configuration was less than or equal to that under the current, the improvement in sensitivity, which was larger than that of the variability, turned the gain of the SN ratio into negatives.

Although animal testing usually requires four or more repetitions because of individual differences, we confirmed that a single experiment allowed us to obtain good reproducibility, thereby streamlining the experimental process.

7. Conclusions

Using a characteristic of in vitro skin permeation, we have optimized prescription of ointment. Setting a rat and minipig to noise factor levels and assuming that an animal species difference was an indicative factor, we calculated an SN ratio using V_e. As a result, we obtained an ideal, optimal prescription with a high skin permeation speed and short lag time.

Although we have shown two different types of analyses in this study, considering the quality engineering concept that a signal factor is a customer's demand in nature, we have realized that analysis 2 is more appropriate. In fact, reproducibility of the SN ratio is more improved in analysis 2 than in analysis 1, where time was selected as a signal factor.

In addition, despite omuitting the details in our research, response graphs based on a conventional SN ratio calculation method that uses V_N as an error variance have almost agreed with those calculated by V_e (Figure 5). This implies that even if there are significant noise conditions, such as a rat and a minipig, the resulting optimal configurations are not so different for the two types, and these optimal configurations are not susceptible to species differences. Therefore, we can predict that only a simple adjustment such as of principal agent, amount of applied medicine, or application time is needed to apply a transdermal drug delivery system effectively.

When optimizing an ointment prescription, not only skin permeation but also the problems of skin stimulation and stable storage need to be taken into consideration. Our research succeeded in reducing development cycle time drastically. In addition, for the characteristics obtained from the animal experiment, we confirmed that only one experiment is necessary for optimization, and consequently, achieved a considerable economic benefit. Therefore, we believe that the quality engineering method is applicable to prescription optimization of other types of drugs or other animal experiments.

Reference

Hatsuki Asahara, Kouya Yano, Yuichiro Ota, Shuichi Takeda, and Takashi Nakagawa, 1998. Optimization of model ointment prescription for the improvement of in vitro skin permeability *Quality Engineering*, Vol. 6, No. 2, pp. 65–73.

This case study is contributed by Hatsuki Asahara and Kouya Yano.