Pharmaceutical Research, Vol. 24, No. 2, February 2007 (© 2006)
DOI: 10.1007/s11095-006-9165-4

Research Paper

Skin Irritation in Transdermal Drug Delivery Systems: A Strategy for its Reduction

Koji Kawahara[1,2,3] and Kakuji Tojo[1]

Received July 13, 2006; accepted September 11, 2006; published online December 27, 2006

Purpose. Active pharmaceutical ingredients (API) in transdermal drug delivery systems (TDS) often causes skin irritation such as erythema and edema. We have studied a possible approach for the reduction of skin irritation by patch formulations that control the rates of skin permeation and elimination of API.
Methods. Loxoprofen (LX-base) was used to induce the skin irritation. The redness value (Δa) was evaluated as a measure of erythema by Chroma Meter. The *in vitro* skin permeation and release profiles were also investigated by using a side-by-side diffusion cell.
Results. The redness values were not correlated either with the cumulative amount of API permeated or the concentration of LX-base in the skin, but well correlated with the elimination rate of LX-base from the skin after the removal of the formulation. The formulation with gradual decrease of permeation rate during application accelerated the elimination rate after application, and resulted in the reduction of the skin irritation.
Conclusions. The skin pharmacokinetics of API, not only permeation during application but also release after the patch removal, was found to be a significant factor for skin irritation. To minimize the skin irritation, it's also important to eliminate the residual API in the skin promptly after application.

KEY WORDS: reduction of skin irritation; skin pharmacokinetics; transdermal drug delivery systems.

INTRODUCTION

Skin irritation is a clinical response of the skin to a variety of external stimuli which induce skin inflammation without production of specific antibodies (1). Acute irritation which is caused by the contact of external substances is characterized by the direct production of cytokines and adhesion molecules by epidermal cells, due to permeation of substances such as API through the skin (2,3). This reaction involves the accumulation of activated T-cells and leads to dermatosis-like erythema and edema.

In designing patch formulations such as Transdermal Drug Delivery Systems (TDS), the focus has widely been on achieving the target flux. Many investigators found that a number of prototypes produced severe skin irritation which may be caused by API (4).

In such occasions, the relationships between skin irritation and the concentration (5) or exposure time (6,7) of model irritants have been reported. However, API accumulated in the skin is also possible to act as a skin irritation substance if stayed for a long duration after the end of application. It is therefore necessary to investigate the skin pharmacokinetics not only during the application but also after the application as well. In this paper, we have evaluated the skin pharmacokinetics of API during and after application of formulations.

We used Loxoprofen (LX-base) as a model irritant to evaluate the *in vivo* irritation and the *in vitro* permeation and release study in guinea pigs. The patch formulations including 10% LX-base were applied to the guinea pig skin for 24 h and then erythema (redness value) was measured by Chroma Meter (8) after peeling off. Both the *in vitro* skin permeation and release experiments were also performed by using a side-by-side diffusion cell. We have investigated the effect of various *in vitro* factors, such as cumulative amount of LX-base permeated, LX-base concentration in the skin after application and elimination rate of LX-base from the skin after application on the redness value measured by Chroma Meter.

MATERIALS AND METHODS

Materials

Loxoprofen sodium was obtained from Koron Chemical Co., Ltd. (Korea), and isopropyl palmitate (IPP) was from

[1] Department of Bioscience and Bioinformatics, Kyushu Institute of Technology, 680-4, Kawazu, Iizuka, Fukuoka, 820-8502, Japan.
[2] TDS research laboratory, Nichiban Co., Ltd., 100, Nishihara, Oyazawa, Hidaka, Saitama 350-1293, Japan.
[3] To whom correspondence should be addressed. (e-mail: koji-kawahara@nichiban.co.jp)

ABBREVIATIONS: API, active pharmaceutical ingredients; BHT, butylated hydroxytoluene; DSC, differential scanning caloriometry; IPP, isopropyl palmitate; LX-base, loxoprofen-base; PSA, pressure sensitive adhesive; SIS, styrene isoprene styrene block copolymer; TDS, transdermal drug delivery system.

Nikko Chemicals Co., Ltd. Styrene isoprene styrene block copolymer (SIS) was obtained from JSR Co., Ltd., and hydrogenated rosin ester (KE-311) was from Arakawa Chemical Co., Ltd. All other chemicals and solvents used were of reagent grade or HPLC quality.

Animals

Male guinea pigs aged 6 weeks (SPF/VAF) supplied by Charles River Japan (Tokyo), Inc. were allowed to freely access standard food and water for the duration of the study.

Preparation of Loxoprofen Base

LX-base was prepared from Loxoprofen sodium (LX-Na) in distilled water and 1 N HCl. Following extraction of the solution with chloroform, $MgSO_4$ was mixed with the organic phase. They were filtrated and the solution was evaporated to dryness under reduced pressure. The residues were then dissolved in toluene at 5°C, and recrystallized (yield: 78.6%).

Analysis of Loxoprofen Base

Melting point was measured using the differential scanning caloriometry (DSC-50, Shimadzu Corp., Japan). The scan range of temperature was 40°C to 300°C, at the scan rate of 5°C/min and the sample mass was 10 mg. The purity was confirmed using a HPLC system (TOSO SC-8020 system, Japan) consisting of a UV detector (UV-8020), a pump (CCPM-II), an automatic sampler (AS-8020) and a system controller (SC-8020). The wavelength of the UV detector was set at 222 nm and a reversed phase column (CLC-ODS, 4.6×150 mm, Shimadzu Corp., Japan) was used for HPLC assay. The flow rate was adjusted so that the retention time of LX-base was 6.6 min.

Preparation of Adhesive Tapes

Details of formulations for selecting the enhancer is listed in Table I. The composition of adhesive tapes to investigate the influence of IPP concentration is listed in Table II.

SIS, KE-311 (rosin ester), butylated hydroxytoluene (BHT) and enhancers were dissolved in toluene using a propeller-type agitator with a stainless-steel blade (solid content: 40%). Fifty percent LX-base in a small amount of methanol (less than tenth part of toluene) was added to the toluene solution and the two solutions were then well mixed. This adhesive solution was spread onto a polyethylene terephthalate release liner using an Adjustable Baker Film Applicator (Tester Sangyo Co. Ltd., Tokyo, Japan). The thickness was controlled to be 50 μm-thick or 100 μm-thick and dried at 90°C for 1 min. The pressure sensitive adhesive (PSA) tape was covered with a 25 μm-thick polyethylene terephthalate film.

Table I. Composition of Loxoprofen Patches Containing Several Enhancers (%)

No.	LX-base	Enhancer		SIS	KE-311	BHT
101	10.0	–	–	44.5	44.5	1.0
102	10.0	PGMC	10.0	39.5	39.5	1.0
103	10.0	PGML	10.0	39.5	39.5	1.0
104	10.0	IPM	10.0	39.5	39.5	1.0
105	10.0	IPP	10.0	39.5	39.5	1.0

PGMC: Propylene glycol monocaprylate.
PGML: Propylene glycol monolaurate.
IPM: Isopropyl myristate.
IPP: Isopropyl palmitate.

Table II. Composition of Loxoprofen Patches Containing IPP (%)

No.	LX-base	IPP	SIS	KE-311	BHT
130	–	–	59.4	39.6	1.0
131		10	53.4	35.6	1.0
132		20	47.4	31.6	1.0
133		30	41.4	27.6	1.0
134	10.0	–	53.4	35.6	1.0
135		10	47.4	31.6	1.0
136		20	41.4	27.6	1.0
137		30	35.4	23.6	1.0

In Vitro Skin Permeation and Release Study

The abdominal hair of guinea pigs was removed with an electric clipper and an electric razor, 24 h and immediately before the experiments, respectively. Anesthetized with pentobarbital sodium (30 mg/body, i.p.), the abdominal skin was carefully excised. The PSA tape (1.77 cm^2; 1.5 cmϕ) was then applied to the stratum corneum side of the skin. The dermal side of the skin was mounted on a horizontal diffusion cell (effective area: 3.14 cm^2; 2 cmϕ) as shown in Fig. 1. The receiver compartment of the diffusion cell was filled with 8.0 ml saline. The diffusion cells were thermoregulated with a water jacket at 32°C during the experiment.

Samples (1.0 ml) were withdrawn from the receiver compartment every 2 h and replaced with fresh saline. The 1.0 ml aliquots of receiver fluid were deproteinized with 1.0 ml of methanol, and centrifuged at 10,000 rpm for 5 min. The supernatants were then injected onto a HPLC column.

The release study was carried out after the end of the 24 h permeation experiment. PSA tapes were rapidly peeled off from the surface of the skin after sampling at 24 h. After the skin was detached from the diffusion cell, the surface of the dermal side rinsed in distilled water, and the water was gently blotted away using a Kimwipe without rubbing. The dermal side of the skin was then mounted on the *in vitro* diffusion cells again and the release experiment was performed under the same condition and methodology employed for the permeation experiment.

Skin Irritation Study

Guinea pig skin with the hair removed was used for the skin irritation study, as well as for skin permeation and release studies. PSA tapes with 3.14 cm^2 (2 cmϕ) were applied to four sites on the abdomen of each guinea pig. The surface of the application site was covered with absorbent gauze (JP gauze, Hakujyuji, Tokyo, Japan) and immobilized

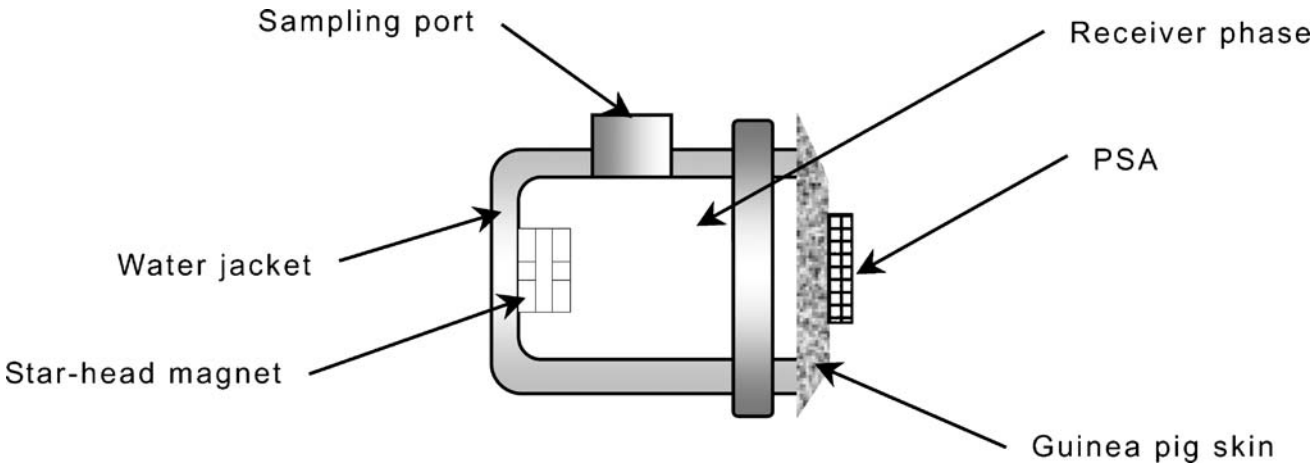

Fig. 1. Schematic diagram of the *in vitro* diffusion cell.

by strapping an adhesive bandage (Elastpore, Nichiban Co., Ltd. Tokyo, Japan) around the body.

Skin irritation was evaluated by the Draize method (9) without using the abraded site. After 24 h of patch application, the wrappings were removed and the test sites were evaluated for erythema (redness value) and edema by the visual score described in Table III. Test sites were investigated 1 h, 24 h and 48 h after the patch removal. The primary irritation index, in conformity with the Draize method, was calculated by averaging the erythema and edema scores. In addition, we used Chroma Meter to measure the redness value for evaluation of edema to compare with the Draize method. Chroma Meter CR-300 (Minolta, Osaka, Japan) was used as a colorimetry for measuring redness value of the skin. The probe of Chroma Meter was lightly pressed against the skin not to decrease blood stream, and took off the skin surface every time each measurement (n=3). In this method, a color is expressed in a three-dimensional coordinate system with an a^*-axis (green–red), ab^*-axis (yellow–blue), and an L^*-axis (white–black). In normal skin, the a^* value showed a linear correlation with the erythema index obtained spectrophotometrically (10). The degree of erythema (Δa) was calculated from the difference between a^* (n=3) of applied skin and that of surrounding normal skin as described by Eq. (1).

$$\Delta a = a^*[\text{redness value of applied site}] - a^*[\text{redness value of normal skin}] \quad (1)$$

Table III. Grading Scale Typically Used in the Draize Method

Description	
Erythema and eschar formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formations (injuries in depth)	4
Edema	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edge of area well defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond the area of exposure)	4

RESULTS

Characterization of LX-base

The DSC thermograms of LX-Na confirmed the endothermic peaks at 81.5, 128.0 and 197.0°C. On the other hand, LX-base was recognized by a single peak at the expected melting point (111.90°C).

The purities of LX-Na and LX-base were confirmed using HPLC. Both peaks showed the same retention time and there was no evidence of impurity.

Solubility of LX-base in the Solvents

The solubility of LX-base in the solvents used is shown in Table IV. LX-base revealed adequate lipophilicity (log-Kow 2.25) for transdermal absorption (11).

Table IV. Physicochemical Parameters of LX-base at 32°C

Solvent	Solubility (%)
Distilled water	0.058±0.0002
Saline	0.060±0.0015
40% PEG#400 in water	0.664±0.0005
Octanol	10.25±0.24

n=3, mean±SD.

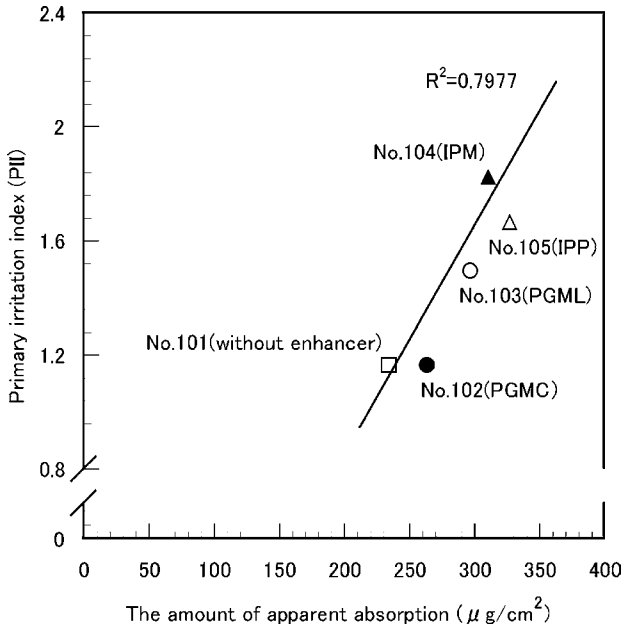

Fig. 2. Relationship between primary irritation index and the amount of apparent absorption in guinea pig skin (n=3, mean±SEM).

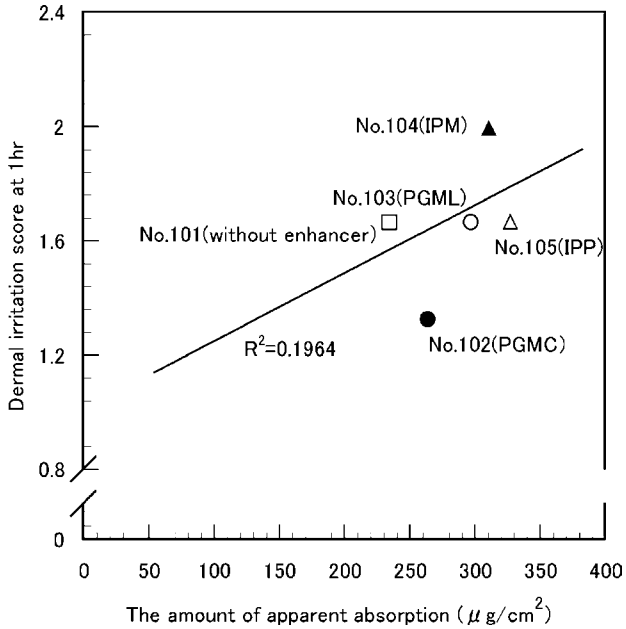

Fig. 3. Relationship between dermal irritation score at 1 h and the amount of apparent absorption in guinea pig skin (n=3, mean±SEM).

Measurement Time of Skin Irritation

Figure 2 shows the relationship between Primary Irritation Index (PII) calculated by the Draize method and the amount of apparent absorption Eq. (2).

$$\begin{aligned}&[\text{The amount of apparent absorption}]\\ &\quad = [\text{Initial content in the patch}]\\ &\qquad - [\text{Residual content in the patch after}\\ &\qquad\quad 24\text{ hr of the patch application}]\end{aligned} \tag{2}$$

where the amount of apparent absorption is calculated on the basis of the residual in the patch extracted by acetone.

The irritation score at 1 h and 24 h after peeling off by the Draize method were also compared with the amount of apparent absorption by using Eq. (2), (Figs. 3 and 4).

It can be seen from Fig. 2 that PII of every formulations using enhancers (isopropyl palmitate, isopropyl myristate, propylene glycol monolaurate and propylene glycol monocaprylate) were classified into weak irritant (PII≦2.0) in accordance with Draize method (9). While PII was not correlated with the amount of apparent absorption of LX-base. In contrast, the average dermal irritation score at 24 h after peeling off was well correlated with the amount of apparent absorption by using Eq. (2), (Fig. 4). The irritation score after 1 h is almost independent of the amount of apparent absorption (Fig. 3). The irritation score after 48 h could not been determined because the dermal irritation had completely been disappeared. We therefore adopted the irritation score (redness value) at 24 h after peeling off as an indication of the present chemical irritation.

Isopropyl palmitate (IPP) was selected as an enhancer in subsequent experiments due to its high degree of enhancement and relative safety.

Comparison Between Placebo and Active Patches

The redness values of placebo patches (No. 130–133) were compared with that of active patches (No. 134–137) in Figs. 5 and 6. In the placebo group, the redness values were clearly reduced within 24 h after peeling off. In the active group, however, the redness values of every formulation at 1 h showed the equivalent values at 24 h after application.

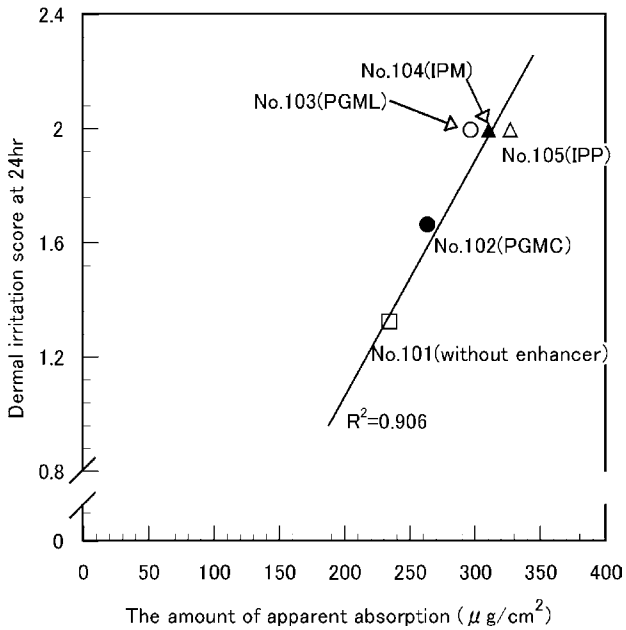

Fig. 4. Relationship between dermal irritation score at 24 h and the amount of apparent absorption in guinea pig skin (n=3, mean±SEM).

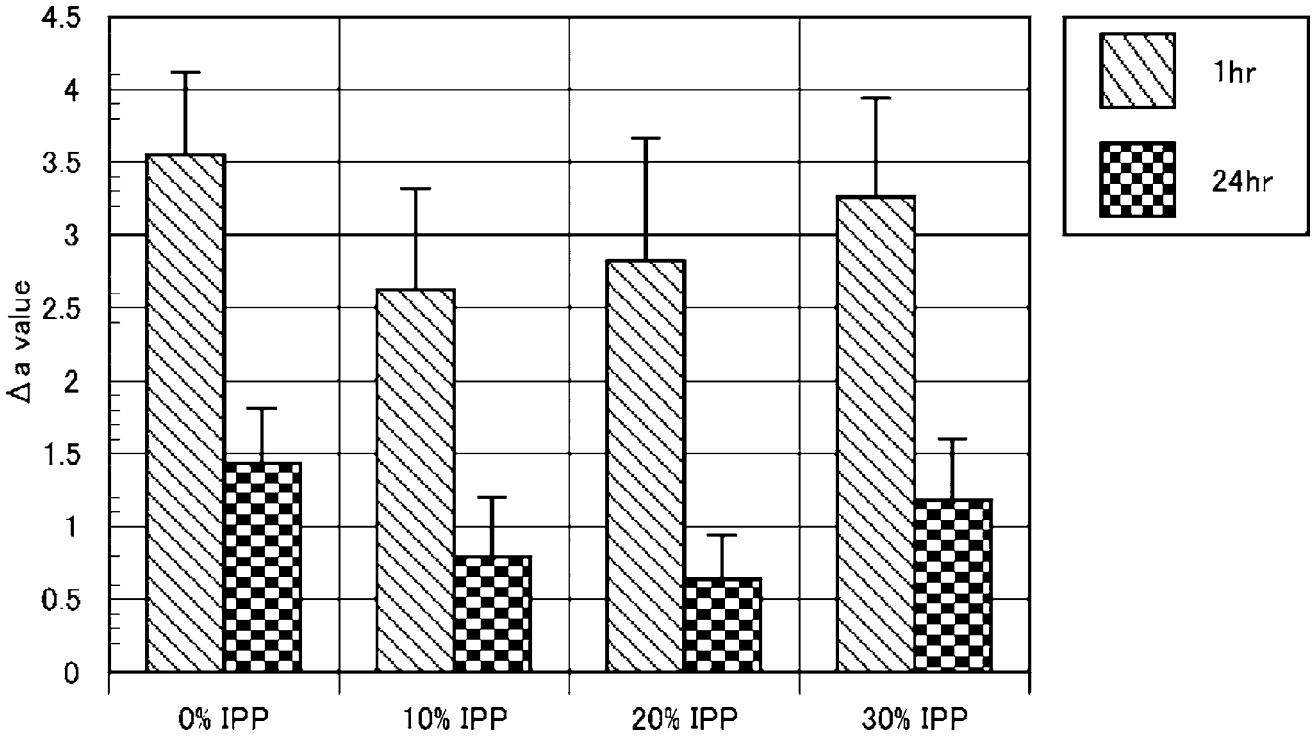

Fig. 5. Change of redness (Δa value) at 1 h and 24 h after peeling off using placebo patches of 100 μm in guinea pig skin (n=5, mean±SEM).

Relationship Between Skin Irritation and Skin Permeability of LX-base I (An Adhesive Thickness of 50 μm)

The influence of IPP content on skin irritation and skin permeability of LX-base was investigated. An instance of the surface of application site at 24 h after peeling off the patches from guinea pig skin is shown in Fig. 7. The IPP content was set at 30% or lower in the PSA with 50 μm-thick to control the level of creep consistent with maintaining properties of a PSA.

The correlation of skin irritation with the IPP content is shown in Fig. 10. The highest redness value (Δa value) was

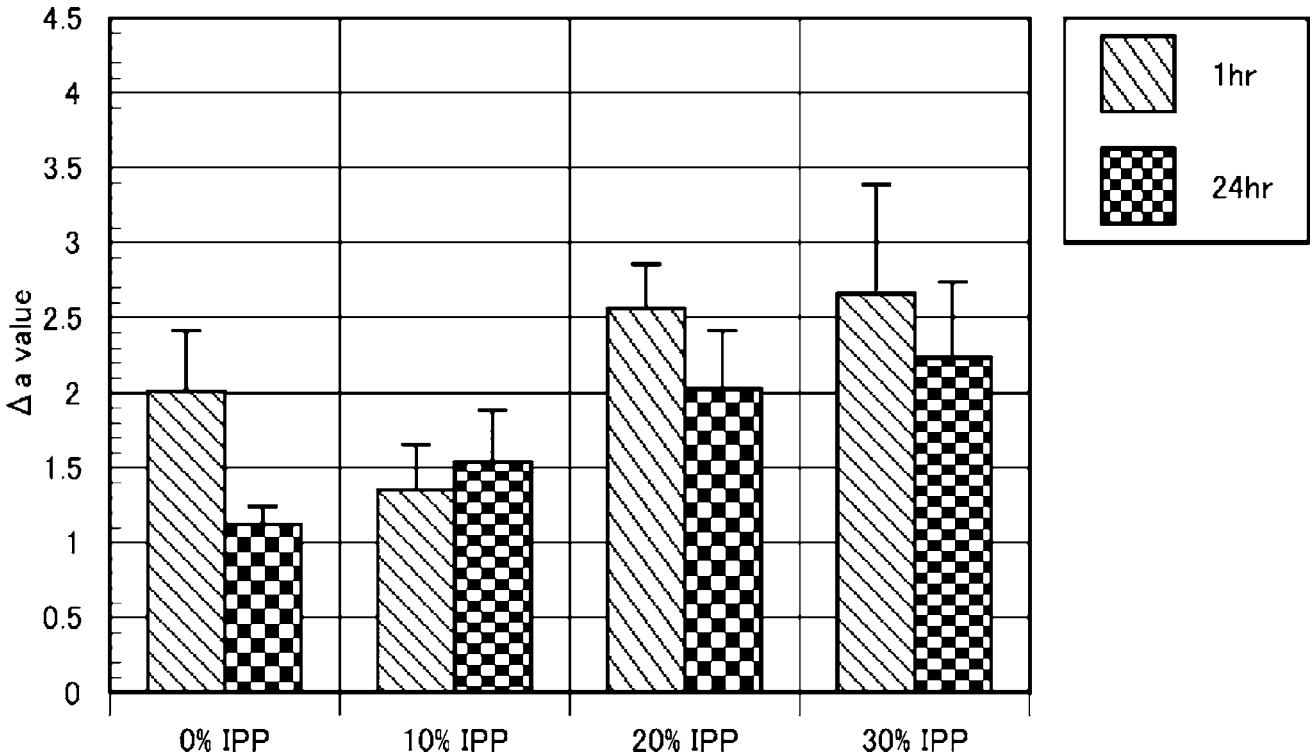

Fig. 6. Change of redness (Δa value) at 1 h and 24 h after peeling off using active patches of 100 μm in guinea pig skin (n=5, mean±SEM).

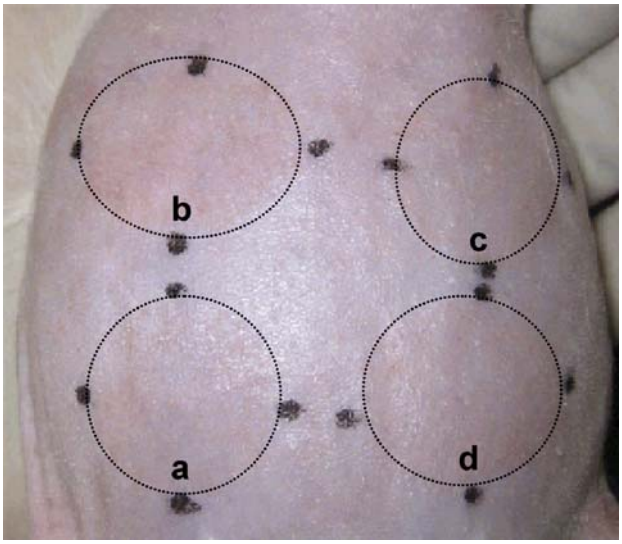

Fig. 7. The surface of application site at 24 h after peeling off from guinea pig skin. The IPP concentration of applied patches at each site were (a) 0% IPP [*a*: 6.54] (b) 10% IPP [*a*: 7.50] (c) 20% IPP [*a*: 6.39] (d) 30% IPP [*a*: 7.16].

observed for the patch formulation containing 10% IPP, while the lowest values were obtained for the 20% and 30% IPP formulations; it is interesting to see that both 20% and 30% IPP formulations exhibited similar redness value and skin permeation levels (Figs. 8 and 9). To clarify the reason for the high irritation caused by the 10% IPP formulation, the relationship between the cumulative amount of LX-base permeated for 24 h and the amount of apparent absorption by using Eq. (2) was also examined (Fig. 10). As can be seen, both of the amount of apparent absorption and the cumulative amount of LX-base indicated a similar tendency, and there was no correlation between these factors and the redness values.

The distribution ratio of LX-base in guinea pig skin after the end of the release study is shown in Fig. 11. Each rate calculated as the distribution ratio to the initial amount in the patch formulation. The cumulative amount of LX-base permeated and released, used the results of *in vitro* permeation and release study for 24 h, respectively. And the

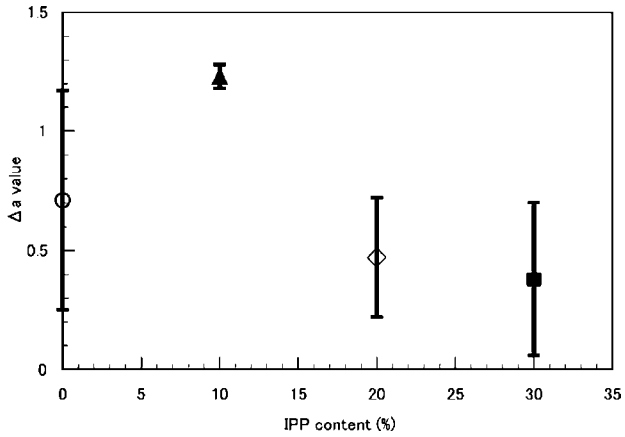

Fig. 8. The effect of IPP content on redness at 24 h after application of various patches of 50 μm in guinea pig skin (*n*=3~4, mean±SEM).

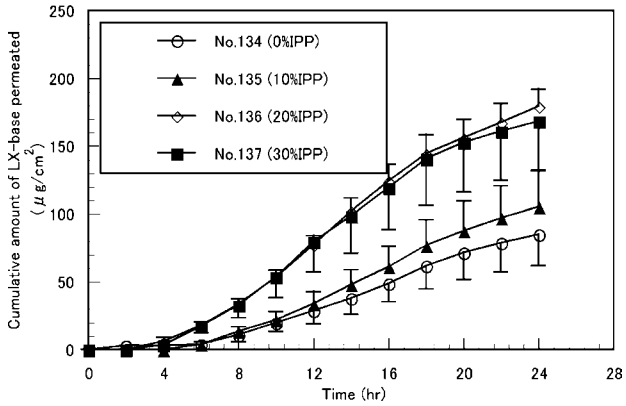

Fig. 9. Permeation profiles of LX-base from various patches of 50 μm through the guinea pig skin (*n*=3, mean±SEM).

residual amount in the skin can be determined by using methanol extract of the skin after the end of release study. And uncollectible amount of LX-base described as the metabolized amount. The formulation containing 10% IPP showed the highest residual skin content among those tested.

The cumulative amount of LX-base released from the dermal side of intact skin after the permeation study was also examined (Fig. 12). The formulation containing 10% IPP showed the highest cumulative amount of LX-base released after 24 h (this amount tended to increase up to 24 h). While the IPP formulation of 30% indicated a minimal increase in the amount released after 12 h, and this showed the same trend as the 20% IPP formulation after 18 h.

Relationship Between Skin Irritation and Skin Permeability of LX-base II (An Adhesive Thickness of 100 μm)

The skin irritation was observed for the adhesive thickness of 100 μm-thick both in the *in vitro* permeation

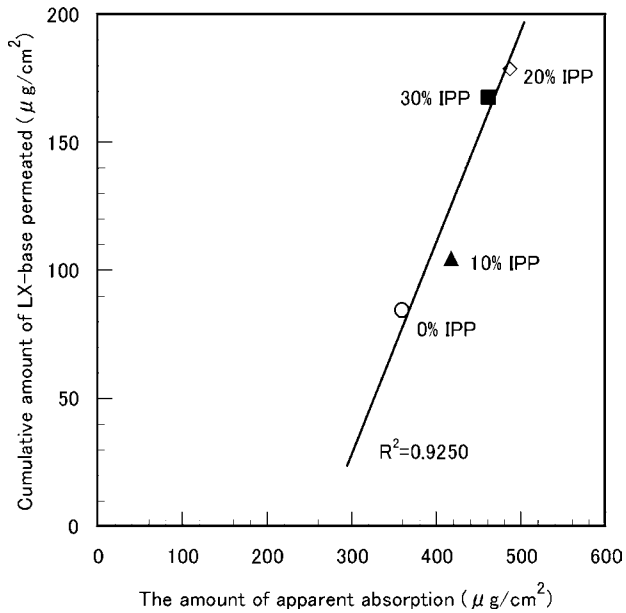

Fig. 10. Relationship between cumulative amount of LX-base permeated and the amount of apparent absorption after 24 h of application (50 μm patches) in guinea pig skin (*n*=3~4).

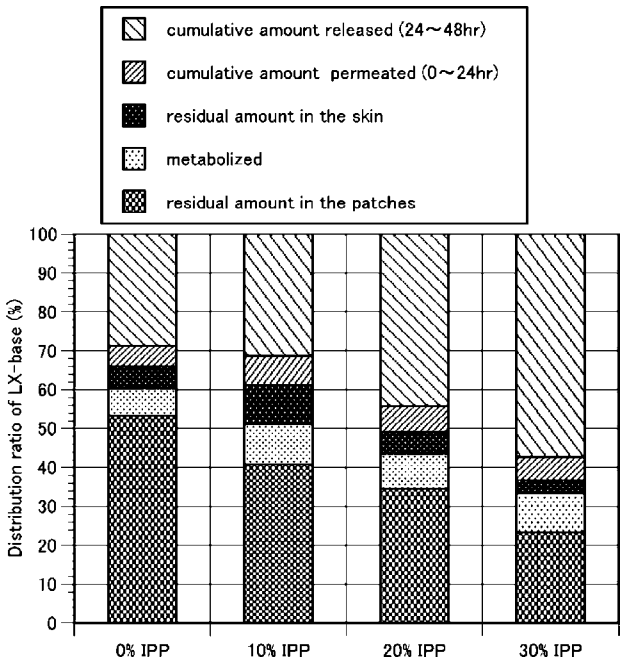

Fig. 11. Distribution ratio of LX-base in guinea pig skin: application for 24 h and 24 h after peeling off of various patches of 50 μm (n=3).

and release experiments (Figs. 13 and 14). The relationship between the redness values and skin penetration or release profiles was then evaluated.

The thermodynamic activity of LX-base in the adhesive was maintained at the high level by doubling the adhesive thickness from 50 to 100 μm-thick. The permeation profiles indicated skin permeation reached a steady state after 14 h. Both formulations with and without enhancer showed similar permeation profiles (Fig. 13). However, the release profile from the formulation without enhancer depended significantly on the IPP content; the amount released from the formulation with 30% IPP was much lower than that with 20% IPP (Fig. 14).

DISCUSSION

Factors which may contribute to skin irritation (acute or delayed acute irritant dermatitis) in TDS include 1) physical

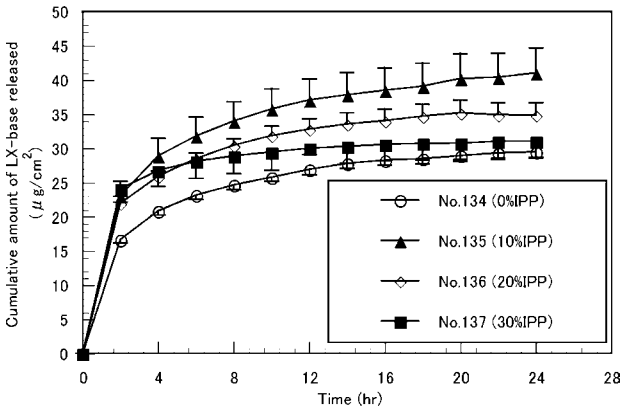

Fig. 12. Release profiles of LX-base from guinea pig skin after peeling off of various patches of 50 μm (n=3, mean±SEM).

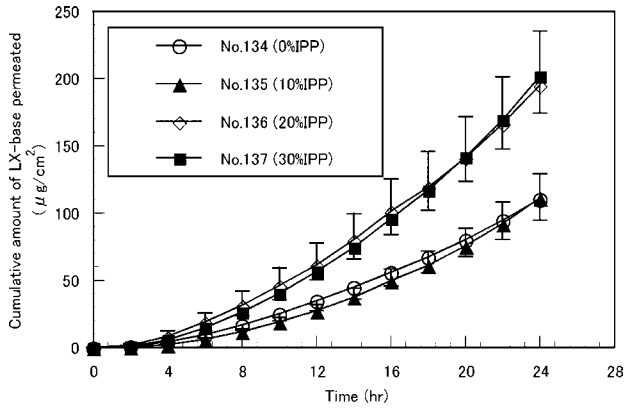

Fig. 13. Permeation profiles of LX-base from various patches of 100 μm through the guinea pig skin (n=3, mean±SEM).

irritation gradually occurred due to lack of skin flexibility(12), 2) physical irritation to the skin occurred immediately after the removal of the adhesive device(13) and 3) chemical irritation by API as a side-effect (14,15) and excipient including enhancers (16,17). Other factors include bacterial infection produced by sweating (18,19) and chemical or allergic irritation produced by the adhesive itself (20,21). However the difference in physical irritation could be excluded in this study because the same backing layer and basic adhesive composition were used in the devices. It was, therefore, suggested that the different levels of skin irritation generated by the samples were attributable mainly to 2) physical irritation to the skin occurred immediately after the removal of the adhesive device and 3) chemical irritation. Skin irritation may be caused by peeling off due to transient erythema; this is not the case in this study, since peeling forces are very low to guinea pig skin. Furthermore the number of corneocytes attached to the adhesive surface of tapes removed was negligible and physical irritation to the skin occurred immediately after the removal of the adhesive device would have little produced or rapid disappeared. In addition, this tape form is suitable for pharmacokinetic evaluations in the skin, because of easy removal from the skin surface and negligible amount of drug residue compared to an ointment.

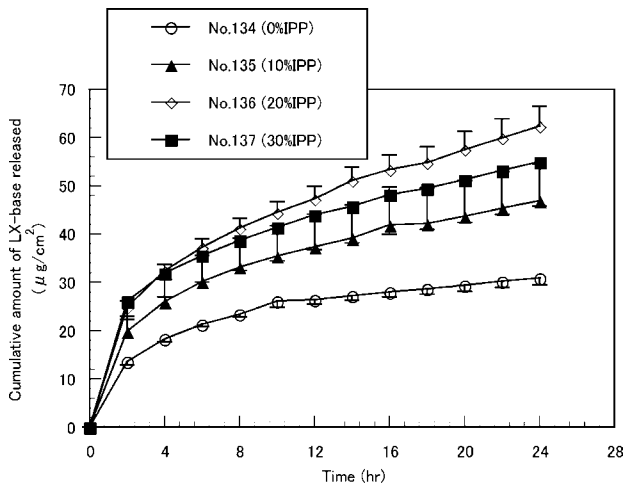

Fig. 14. Release profiles of LX-base from guinea pig skin after application of various patches of 100 μm (n=3, mean±SEM).

In order to quantitatively evaluate skin irritation in TDS, the redness value (Δa) was measured using Chroma Meter CR-300, and this value was adopted as the irritation index. Chroma Meter was effective for slight differences in redness value (22).

For evaluating chemical irritation caused by API, the skin irritation score at 1 h and 24 h, respectively, after peeling off was compared with the amount of apparent absorption by using Eq. (2), (Figs. 3 and 4). Skin irritation derived from API may be typically present in sustainable erythema; thus it is important to determine the redness values at 24 h after peeling off rather than at 1 h (or PII by the Draize method) because a measurement after 1 h would have been directly affected by the physical irritation of the peeling event.

The placebo patch showed the redness values stronger than that of the active patch (Figs. 5 and 6). The higher peeling force shown by the placebo patch (containing no LX-base) had a direct effect on the physical irritation of peeling off. The redness values caused by physical irritation declined 24 h after removal of the patch. On the other hand, chemical irritation due to the active patch remained almost constant after 24 h, and LX-base obviously confirmed to be a substance.

As shown in Fig. 9, the least cumulative amount of LX-base permeated was observed for the 50 μm-thick active patch containing 10% IPP for 24 h. The trend of the cumulative amount of LX-base permeated and time profile was similar to that of the formulation containing no IPP. Since there was no difference in the permeation profiles between formulations containing 20% and 30% IPP, it may suggest that the enhancement effect of IPP reaches the maximal with 20% IPP.

The patch formulation containing 10% IPP (50 μm) showed the highest residual skin content from the result of distribution ratio of LX-base in guinea pig skin (Fig. 11). This was attributed to the increased apparent solubility of LX-base due to the extensive distribution of the enhancer IPP into the stratum corneum, without changing the diffusion coefficient. In fact, the kinetics of LX-base permeation into the skin calculated from the release experiment and from measurement of the residual content in the skin indicated that the formulation containing 10% IPP exhibited the highest residual content among all formulations studied (Fig. 15).

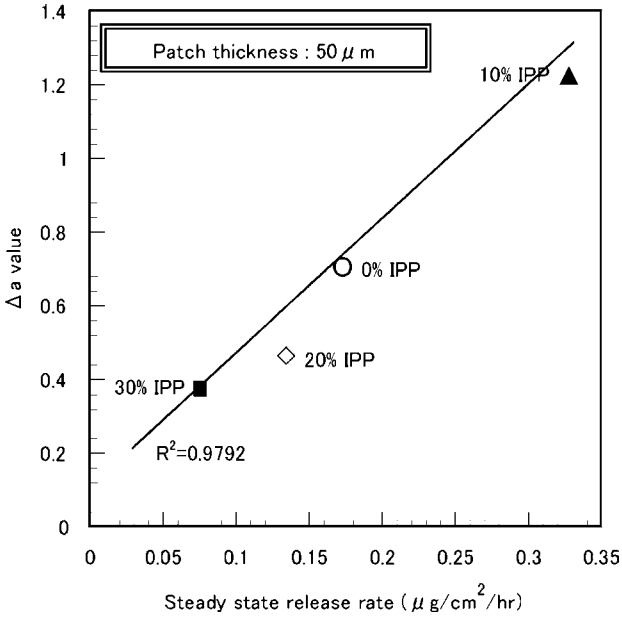

Fig. 16. Relationship between steady state release rate among 12 h and 24 h and redness at 24 h after peeling off in guinea pig skin (n=3~4).

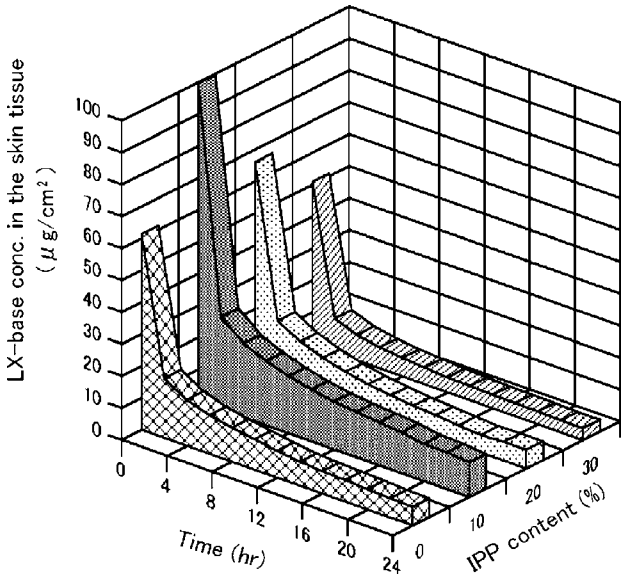

Fig. 15. Comparative elimination profiles of LX-base from guinea pig skin after peeling off various patches containing different IPP content at 50 μm.

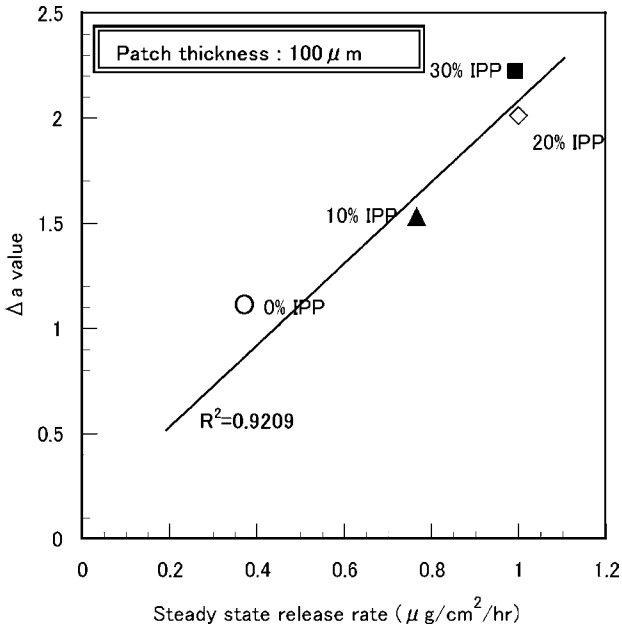

Fig. 17. Relationship between steady state release rate among 12 h and 24 h and redness at 24 h after peeling off in guinea pig skin (n=3~5).

The effects of various skin pharmacokinetic parameters including cumulative amount of API permeated and released, API concentration in the skin after application and elimination rate of API from the skin after application upon skin irritation are evaluated. It was found that a large amount of release of LX-base did not necessarily imply high accumulation of LX-base in the skin; the formulation containing 10% IPP (50 μm-thick) showed the release amount smaller than that for 20% or 30% devices (Fig. 9). The cumulative amounts of LX-base permeated and released were found to be directly independent of the incident of skin irritation.

The effect of IPP on cumulative amount of LX-base released is shown in Figs. 12 and 14. The release rate, evaluated among 12 h and 24 h, was well correlated with the redness values as an indicator of skin irritation (Figs. 16 and 17). This trend was similar when the adhesive thickness was changed from 50 to 100 μm-thick. This finding may suggest that the skin irritation caused by LX-base can be reduced by changing the elimination rate of LX-base from the skin after application.

Even though both 50 and 100 μm-thick formulations containing 10% IPP as an enhancer showed similar permeation profiles to that without enhancer, all the formulations with enhancer showed skin irritation (indicated by redness values) more than those without enhancer. This release experiment suggests that the increase in skin irritation is caused by the increased solubility in the stratum corneum due to the accumulation of the enhancer. Thus, in the presence of API-derived skin irritation, there is the possibility of further increase in skin irritation if threshold quantities of enhancer are used. We may therefore need to care with not only the API but inactive ingredients as well for improving the topical products such as TDS, ointments, and solutions of pharmaceutical and cosmetic products.

For formulations with 20% IPP or 30% IPP of the adhesive thickness of 100 μm-thick, the redness value response was more significant than that with 0% or 10% IPP (Fig. 17). In the 50 μm-thick, however, the trend was completely reversed (Fig. 16). In the initial process of skin permeation for the 50 μm-thick device with 20% IPP, LX-base concentration in the skin decreased gradually (Fig. 9). The rapid disappearance of LX-base in the skin after peeling off was caused by the high concentration of enhancer in the stratum corneum. Inadequate amount of enhancer may retard the elimination of LX-base from the skin after peeling off and rather induce severe skin irritation ultimately appeared.

Skin irritation is clinically classified as acute irritant dermatitis, irritant reaction, delayed acute irritant dermatitis, cumulative irritant contact dermatitis and so on. When the exposure time is sufficient and the offending agent is potent, classic symptoms of acute skin irritation are seen (23). It was widely thought that skin irritation frequently occurred due to a prolonged application period or high API concentration in the formulation. Both epidermis and dermis, in which sites the irritation is expressed, retain API after application; therefore, at these sites may give rise to severe irritation. The concentration–time profiles of API in the skin not only permeation but also release after application is therefore one of the most important factors for investigating skin irritation.

This study clearly indicated that the enhancer concentration in the TDS influences the skin irritation as well as skin permeability of API. It is, therefore, necessary to understand the pharmacokinetics of both API and inactive ingredient such as enhancer in the skin for reducing side effects of formulations. We found that the formulation with a gradual decrease in permeation rate of API during application is effective in reducing API concentration in the skin after application. Rapid disappearing of residual API from the skin following the removal of the patch formulation is also important to minimize the skin irritation.

REFERENCES

1. E. Berardesca and F. Distante. The modulation of skin irritation. *Contact Dermatitis* **31**:281–287 (1994).
2. T. Hunziker, C. U. Brand, A. Kapp, E. R. Waelti, and L. R. Braathen. Increased levels of inflammatory cytokines in human skin lymph derived from sodium lauryl sulphate-induced contact dermatitis. *BR. J. Dermatol.* **127**:254–257 (1992).
3. J. L. Wilmer, F. G. Burleson, F. Kayama, J. Kanno, and M. I. Luster. Cytokine induction in human epidermal keratinocytes exposed to contact irritants and its relation to chemical-induced inflammation in mouse skin. *J. Invest. Dermatol.* **102**:915–922 (1994).
4. M. R. Holdiness. A review of contact dermatitis associated with transdermal therapeutic systems. *Contact Dermatitis* **20**:3–9 (1989).
5. K. Sugibayashi, T. Watanabe, T. Hasegawa, H. Takahashi, and T. Ishibashi. Kinetic analysis on the *in vitro* cytotoxicity using living skin equivalent for ranking the toxic potential of dermal irritants. *Toxicol. in Vitro* **16**:759–763 (2002).
6. J. F. G. M. Hurkmans, H. E. Boddé, L. M. J.Van Driel, H.Van Doorne, and H. E. Junginger. Skin irritation caused by transdermal drug delivery systems during long-term (5 days) application. *Brit. J. Dermatol.* **112**:461–467 (1985).
7. R. J. Babu, A. Chatterjee, E. Ahaghotu, and M. Singh. Percutaneous absorption and skin irritation upon low-level prolonged dermal exposure to nonane, dodecane and tetradecane in hairless rats. *Toxicol. Ind. Health* **20**:109–118 (2004).
8. J. C. Seitz and C. G. Whitmore. Measurement of erythema and tanning responses in human skin using a tri-stimulus colorimeter. *Dermatologica* **177**:70–75 (1988).
9. J. H. Draize, G. Woodard, and H. O. Calvery. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. *J. Pharmacol. Exp. Ther.* **82**:377–390 (1944).
10. S. I. Ale, J. K. Laugier, and H. I. Maiback. Spacial variability of basal skin chromametry on the ventral forearm of healthy volunteers. *Arch. Dermatol. Res.* **288**:774–777 (1996).
11. T. Yano, A. Nakagawa, M. Tsuji, and K. Noda. Skin permeability of various non-steroidal anti-inflammatory drugs in man. *Life Sci.* **39**:1043–1050 (1986).
12. F. Tokumura, K. Ohyama, H. Fujisawa, T. Matsuda, and Y. Kitazaki. Conformability and irritancy of adhesive tapes on the skin. *Contact Dermatitis* **37**:173–178 (1997).
13. B. Russell and N. A. Thorne. Skin reactions beneath adhesive plasters. *The Lancet* **8**:67–70 (1955).
14. J. R. Horning, E. T. Zawada, J. L. Simmons, L. Williams, and R. McNulty. Efficacy and safety of two-year therapy with transdermal clonidine for essential hypertension. *Chest* **93**:941–945 (1988).
15. W. H. Utian. Transdermal estradiol overall safety profile. *Am. J. Obstet. Gynecol.* **156**:1335–1338 (1987).
16. A. Karlsberg, K. Magnusson, and U. Nilsson. Air oxidation of d-limonene (the citrus solvent) creates potent allergens. *Contact Dermatitis* **26**:332–340 (1992).
17. J. Roed-Petersen and N. Hjorth. Contact dermatitis from antioxidants. *British J. Dermatol.* **94**:233–241 (1976).

18. S. M. Peck, T. J. Michelfelder, and L. L. Paritz. Further studies on the mechanism of adhesive tape dermatitis. *AMA. Arch. Derm.* **63**:289–311 (1951).
19. T. Golden. Non-irritating, multipurpose surgical adhesive tape. *Am. J. Surg.* **100**:789–796 (1960).
20. S. Sjöborg and S. Fregert. Allergic contact dermatitis from a colophony derivative in a tape skin closure. *Contact Dermatitis* **10**:114–115 (1984).
21. B. M. Hausen and J. Mohnert. Contact allergy due to colophony. *Contact Dermatitis* **20**:295–301 (1989).
22. H. Takiwaki, L. Overgaard, and J. Serup. Comparison of narrow-band reflectance spectrophotometric and tristimulus colorimetric measurements and skin color. *Skin Pharmacol.* **7**:217–225 (1994).
23. S. Weltfriend, M. Ramom, and H. I. Maibach. "*Dermatotoxicology 6th edition*" CRC (2004).