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Evaluation of the Water Content and Skin Permeability of Active Pharmaceutical Ingredients in Ketoprofen Poultice Formulations Removed from Their Airtight Containers and Left at Room Temperature

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The poultice formulation is a patch containing a large amount of water. It is known that the water contained in the adhesive polymer layer (ADPL) of poultice affects the cooling sensation and skin permeability of the active pharmaceutical ingredient (API). In this study, we evaluated the relationship between the water content in a ketoprofen poultice formulation and the amount of time the poultice was left out at room temperature after removal from the airtight container, as well as the influence of the decreasing water content on the skin permeability of the API. After removing the poultice from the container for 1 h, the mass of the ADPL decreased by approximately 40%. When the near-infrared (NIR) spectrum of the ADPL of poultice was measured, the peaks reflecting the hydroxyl group were attenuated depending on the time left out at room temperature. It is suggested that the changes in the mass and NIR spectrum of the ADPL are caused by the change in the water content. Moreover, when the permeability of API was evaluated on hairless mouse skin, the cumulative skin permeation amount and flux decreased, while the lag time was prolonged as the time left out increased. These results suggest that the skin permeability of the API is impaired by water evaporation and that maintaining the water in the ADPL in poultice is very important from not only the viewpoint of cooling sensation, tackiness and moisturizing but also the skin permeability of the API.

Key words ketoprofen; poultice; adhesive polymer layer; skin permeability; water

INTRODUCTION

The percutaneous absorption of drugs has attracted considerable attention, particularly in terms of specific advantages, such as avoiding the first pass effect in the liver and gastrointestinal disturbance as well as improving patient compliance.^{1,2)} In the Japanese Pharmacopoeia Seventeenth Edition, a poultice, which is one of the external formulations to be applied to the skin, is described as a patch using a base containing water. The adhesion polymer layer (ADPL) of poultice can be prepared by mixing a high molecular weight compound such as water-soluble polymer and purified water. Therefore, it must be kept in an airtight container to prevent the evaporation of water. A poultice formulation is usually comprised of three layers: an ADPL of crosslinked hydrogel matrix containing the active pharmaceutical ingredient (API), a backing layer consisting of unwoven cloth and a liner. It is fully conceivable that the patient may use a poultice having a reduced water content in the ADPL, for example, when the poultice is stored in a container with insufficient airtightness or left out for a long period of time after opening.

Kawamura *et al.* have reported that the peeling strength of a ketoprofen-containing tape formulation was significantly larger than that of a poultice formulation, that the stratum corneum peeled off by peeling off the tape formulation was significantly more than in case of poultice and that the water content of the skin after peeling it off is significantly lower

than that of poultice.³⁾ Therefore, the water contained in the ADPL of poultice may be involved in the cooling sensation and suppression of swelling symptoms by vaporization heat, tackiness to the skin and relaxation of skin irritation at the time of application. In addition, the water contained in poultice plays an important role in not only moisturizing the skin but also increasing the percutaneous absorption of the drug.⁴⁾ Thus, maintaining the water content in the ADPL during the storage of poultice is considered to be very important from the viewpoint of the proper use of medicine. However, there is no report quantitatively evaluating the relationship between the time left out at room temperature and the water content in the ADPL of poultice in a state taken out from the container. In addition, no case is reported on the direct evaluation of the effect of decreasing the amount of water in the ADPL on the skin migration properties of the API.

Hence, in this study, we aimed to establish a method for measuring the water content of the poultice formulation by near-infrared (NIR) spectroscopy and thermal gravimetry/differential thermal analysis (TG/DTA) targeting a poultice formulation including ketoprofen, a conventional nonsteroidal anti-inflammatory drug by nonselective cyclooxygenase inhibition.^{5,6)} Next, in order to clarify the change in the formulation properties of the poultice that was stored under insufficient airtightness, the water content of the poultice formulation, which had been left out at room temperature when removed from the container, was evaluated. Moreover, the

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influence of the evaporation of water of the poultice on the skin permeability of the API was evaluated by using a skin permeability test with hairless mouse skin.

MATERIALS AND METHODS

Reagents Ketoprofen poultice formulation (Mohrus® pap 30mg, lot. S414T; KP) and ketoprofen poultice formulation XR (Mohrus® pap XR 120mg, lot. U414T; KP_{XR}) were purchased from the Hisamitsu Pharmaceutical Co., Inc. (Tokyo, Japan). These formulations were cut into appropriate sizes, removed from the container and left at 25°C in an air-conditioned laboratory with the liner side up (hereinafter referred to as “left at room temperature”) and used for subsequent experiments.

For the skin permeability test, frozen 7-week-old hairless mouse abdominal skin (Lab-skin) was purchased from Japan SLC, Inc. (Hamamatsu, Japan).

Calculation of the Mass Ratio of ADPL in the Cut Sample Since the removal of the back layer from the cut poultice is difficult, the following calculated value was regarded as an approximate value of the mass ratio of the ADPL. In the mass measurement, the Karl–Fischer (KF) method and TG/DTA, the mass of the ADPL was calculated from this approximate value.

The mass of the back layer and ADPL of one poultice formulation was measured by removing the liner of one container of each formulation (KP: six sheets in one container, KP_{XR}: seven sheets in one container). The ADPL mass ratio in the back layer and ADPL in each formulation “X” was calculated from formula (1) using the fact that the ADPL masses of KP and KP_{XR} are 10 and 6 g, respectively (Table 1).

$$X = \frac{\text{Mass of ADPL (10.0 g or 6.0 g)}}{\text{Mass of back layer and ADPL}} \quad (1)$$

Next, average values of “X” for one container of each formulation were obtained, and they were taken as “X.” Since the sizes of KP and KP_{XR} are both 10 × 14 cm in one sheet (Table 1), we applied this “X” to the ADPL mass ratio in samples cut at similar ratio (*i.e.*, 1 : 1.4).

Mass Measurement of the Poultice Formulation The poultice removed from the airtight container was cut to approximately 1 × 1.4 cm, and then it was used as a sample for mass measurement. The mass of the cut sample was regarded as the total mass of the back layer, ADPL and liner (subscripts

“_b”, “_a” and “_l”, respectively) immediately after removal from the airtight container (M_{bal_0h}). Then, the sample was left at room temperature. After a certain time (T_h), the total mass of the sample (M_{bal_Th}) was measured, and the mass of the liner peeled off from the sample was measured (M_l). The total masses of the back layer and ADPL at 0 h and T_h (M_{ba_0h} and M_{ba_Th}) were calculated by formulas (2) and (3).

$$M_{bal_0h} - M_l = M_{ba_0h} \quad (2)$$

$$M_{bal_Th} - M_l = M_{ba_Th} \quad (3)$$

Subsequently, the amount of the ADPL after immediate removal from the container (M_{a_0h}) and the mass of the back layer (M_b) were calculated from the formulas (4) and (5).

$$M_{a_0h} = M_{ba_0h} \times X \quad (4)$$

$$M_b = M_{ba_0h} - M_{a_0h} \quad (5)$$

The mass of ADPL T_h after removal from the airtight container (M_{a_Th}) was calculated by formula (6). Using this M_{a_Th} , the rate of mass reduction (MR) was calculated by formula (7).

$$M_{a_Th} = M_{ba_Th} - M_b \quad (6)$$

$$MR (\%) = \frac{M_{a_0h} - M_{a_Th}}{M_{a_0h}} \times 100 \quad (7)$$

Measurement of NIR Spectroscopy The poultice formulation was cut into a square with a side length of approximately 1 cm, and the NIR spectrum of the ADPL was measured by a diffuse reflection method using a NIR spectrometer (Spectrum One NTS, PerkinElmer, Inc., Waltham, MA, U.S.A.) with a diffuse reflection unit attached (NIRA, PerkinElmer, Inc.) at a wavenumber resolution of 8 cm⁻¹ employing 32 scans across the wavelength range of 4000–12000 cm⁻¹. The NIR spectra of fluorocarbon polymers were also acquired as background. The obtained original spectrum was subjected to Kubelka–Munk transformation and further normalized treatment was used for analysis.

Determination of the Water Content by the KF Method The KF method was performed by coulometric titration at room temperature using “aqua light GRO-A” (Hiranuma Sangyo Co., Ltd., Ibaraki, Japan) as the KF reagent. Approximately 5 mg (aspect ratio = 1 : 1.4) of sample was precisely

Table 1. Information on Ketoprofen Poultice Formulations Used in This Study

Formulation	Composition	Size	Rules of use
KP	<i>l</i> -Menthol, oxybenzone, crotamiton, synthetic aluminum silicate, perfume, titanium oxide, gelatin, concentrated glycerin, partially neutralized polyacrylic acid, polyvinyl alcohol (partially saponified), three other components 30 mg of JP ketoprofen is contained in 10 g of adhesive polymer layer (one sheet).	10 × 14 cm	Twice a day
KP _{XR}	Edetate sodium hydrate, <i>l</i> -menthol, crotamiton, synthetic aluminum silicate, flavor, gelatin, concentrated glycerin, 4- <i>tert</i> -butyl-4'-methoxydibenzoylmethane, propylene glycol, partially neutralized polyacrylic acid, polyvinyl alcohol (partial saponification), four other components 120 mg of ketoprofen is contained in 6 g of adhesive polymer layer (one sheet).	10 × 14 cm	Once a day

This information was taken from the Interview Forms published by Hisamitsu Pharmaceutical Co., Inc. KP: Morus® pap 30mg, Interview Form, Revised June 2014 (14th version), <http://www.hisamitsu.co.jp/medical/data/mohrusp_i.pdf>, accessed 15 February, 2019. KP_{XR}: Morus® pap XR 120mg, Interview Form, Revised April 2018 (5th version), <http://www.hisamitsu.co.jp/medical/data/mohruspxr_i.pdf>, accessed 15 February, 2019.

weighed, and samples were quickly injected from the sample input port of the KF aquameter (Aquacounter AQ-2200, Hiranuma Sangyo Co., Ltd.) filled with KF reagent. After sample injection, the quantitative value at the time when the potential of the KF reagent became stable was defined as the water content. The amount of the ADPL of the sample was calculated by multiplying the total mass by the ADPL mass ratio "X," and the water content (%) was calculated based on this numerical value.

TG/DTA Measurement Thermal gravimetry/differential thermal analysis (TG/DTA) was carried out using a differential thermal balance (Thermo plus EV 02 TG-DTA 8122, Rigaku, Tokyo, Japan). The poultice was cut out so that the aspect ratio was 1:1.4, and the mass of sample was approximately 5 mg. After precisely measuring the mass, the surface of the ADPL was adhered to the bottom surface of the aluminum pan. Next, a TG/DTA curve was obtained by raising the temperature from 25 to 150°C at 5.0°C/min with reference to air. The amount of the ADPL of sample was calculated by multiplying the total mass by the ADPL mass ratio "X," and the rate of reduction (%) was calculated based on this numerical value.

Skin Permeation Study KP was taken out from the package and molded into round shapes (diameter: 16 mm, 2 cm²). Following the permeation study, molded KPs were left in the incubator maintained 25°C for 0, 1, or 5 h. The *in vitro* skin permeation study was carried out using Franz-type diffusion cells (orifice diameter: 20 mm) with hairless mouse skin as the membrane. Molded KP, calculated as containing 0.43 mg of ketoprofen, was attached with backing tape (Kinesiology Tape, 3M Japan Inc., Tokyo, Japan) as molded round shapes (diameter: 25 mm) on the stratum corneum side of the skin. Initially, the receiver chamber was filled with 23 mL of Dulbecco's phosphate buffered saline (D-PBS (-), pH = 7.4) and was maintained at 32°C. Samples (200 µL) from the receiver chamber were collected at 1, 2, 3, 4, 6, 8, 10, 12 and 24 h. Following sampling, the solution in the receiver chamber was refilled with an equal volume of D-PBS (-). Each sample was centrifuged at 21100 × *g* for 5 min, and the supernatant was stored at -20°C until HPLC analysis.

HPLC for *in Vitro* Samples The samples were assayed using reversed-phase HPLC. The HPLC system was comprised of an organizer, a 5280 autosampler, a 5160 pump, and a 5410 UV detector (Hitachi High-Technologies Co., Tokyo, Japan), and it was equipped with a COSMOSIL 5C18-MS-II column (4.6 × 150 mm; particle size, 5 µm) purchased from Nacalai Tesque, Inc. (Kyoto, Japan). The mobile phase comprised of 70% (v/v) methanol with 2 mM potassium phosphate buffer (pH = 3.0) was delivered at a flow rate of 1 mL/min. The column temperature was maintained at 25°C (room temperature). The injection volume was 10 µL. Ketoprofen was detected at 254 nm by UV detection. Standard curves were linear over the range 0–30 µg/mL (*R*² > 0.9999).

Data Analysis The cumulative amount of ketoprofen that permeated was plotted against time, and then the steady-state skin permeation flux (µg/h/cm²) was estimated from the straight line portion of the curve. The intercept points of the straight line on the x-axis gave the lag time (h).

Fig. 1. Influence of the Time Left out at Room Temperature on the Mass of the Ketoprofen Poultice Formulation

Each point indicates the mean ± standard deviation (S.D.) (*n* = 3).

RESULTS AND DISCUSSION

Mass Reduction of ADPL in the Poultice Formulation

We evaluated the mass change of poultice when left for a certain period of time at room temperature. Both KP and KP_{XR} showed a time-dependent decrease in the mass of the ADPL, which showed the largest reduction at 5 h after removal from the airtight container (Fig. 1). The mass reduction rates at 24 h were approximately 50 and 35% for KP and KP_{XR}, respectively (Fig. 1). As the mass reduction presumed to reflect the disappearance of water contained in the polymer, KP was considered to contain more water than KP_{XR}. Since the liner of the poultice is made of material with low moisture permeability such as polypropylene and polyethylene, it is assumed that moisture will escape to the nonwoven cloth side (backing layer) or laterally in the experimental conditions of this study.

NIR Spectra of Ketoprofen Poultice Formulations The NIR spectra of the ADPL of KP and KP_{XR} were measured by the diffuse reflection method. In the original spectra, peaks were observed at approximately 5200 and 6900 cm⁻¹ in both poultice formulations (Fig. 2a). When poultice was allowed to sit at room temperature for a certain period of time, peaks at approximately 5200 and 6900 cm⁻¹ were attenuated in a time-dependent manner. Since those wavenumber regions reflect the combination and first overtone of the hydroxyl group,^{7,8)} the attenuation of the peaks indicates the disappearance of water due to leaving it sit out at room temperature. A shoulder was observed at approximately 4800 cm⁻¹ in both formulations, and it changed to a peak with an increase in the time after removal (Fig. 2a). As this wavenumber region is derived from a combination including alcoholic hydroxyl groups,⁸⁾ it is considered that these peaks reflect the hydroxyl groups in the polymer molecule in the ADPL. In poultice containing much water, the peak at approximately 4800 cm⁻¹ overlapped with the peak derived from water (at approximately 5200 cm⁻¹). As the water decreased, the peak at 5200 cm⁻¹ disappeared, and it seemed that the peak derived from the hydroxyl group of the polymer appeared. On the other hand, in a study targeting creams, we have reported that when a polyhydric alcohol such as propylene glycol (PG) exists in the sample, a shoulder at approximately 4800 cm⁻¹ continuing from the absorption at approximately 5200 cm⁻¹ was clearly observed.⁹⁾ Therefore, the peak at approximately 4800 cm⁻¹ of KP_{XR} is thought to reflect the presence of PG with the polymer because PG was

Fig. 2. Influence of the Time Left Out at Room Temperature on the NIR Spectrum of the Ketoprofen Poultice Formulation: (a) Original Spectra; (b) Second Derivative Spectra (4500–5500 cm^{-1}); and (c) Second Derivative Spectra (6500–7500 cm^{-1}) of KP (Upper) and KP_{XR} (Lower)

added only in KP_{XR} (Table 1). In the second derivative spectra, downward peaks at 5200 and 6900 cm^{-1} were shown in both formulations, and the downward peak intensities attenuated in a time-dependent manner (Figs. 2b, c). Furthermore, the peak at approximately 5200 cm^{-1} shifted to the lower wavenumber side in a time-dependent manner (Figs. 2b, c). Generally, it is known that as the molecular mobility weakens due to interaction between water molecules and other molecules, the peak in this region shifts to the lower wavenumber side.¹⁰⁾ Thus, it is considered that water molecules with high molecular mobility are evaporated and that water molecules with low molecular mobility partially restrained by polymers remain when left at room temperature, leading to a shift to lower wavenumbers of the peak at approximately 5200 cm^{-1} in time-dependent manner.

In this way, we carried out an experiment assuming a state of being affixed to the skin of poultice. That is, KP was adhered to a plate maintained at 37°C using a temperature controlled device, and the NIR spectrum after leaving out at room temperature for a certain period of time was measured. The peak at approximately 5200 cm^{-1} disappeared at 1 h (Fig. S1), suggesting that water in the ADPL of poultice is consumed in hydration of the stratum corneum in a short time after application to the skin.

As KP is used twice a day by patients (Table 1), the container is opened at least twice a day, even if the formulation is stored in an airtight container. No change was observed in the NIR spectra up to 3 d for KP kept under regular conditions when airtight containers were opened regularly twice a day. However, after 1 week, the peak at approximately 5200 cm^{-1} clearly decreased (Fig. S2). These results suggest that water evaporates several days after repeated opening twice a day even though the formulation is kept in an airtight condition. Hence, this suggests that the long-term storage of poultice after opening is not appropriate.

Table 2. Influence of the Time Left out at Room Temperature on the Water Content of Ketoprofen Poultice Formulations

Formulation	Water content (%)			
	0 h	1 h	3 h	5 h
KP	49.65 $\pm$ 0.54	7.62 $\pm$ 1.29	3.16 $\pm$ 0.14	2.91 $\pm$ 0.18
KP_{XR}	27.12 $\pm$ 1.87	3.89 $\pm$ 0.12	2.43 $\pm$ 0.14	2.00 $\pm$ 0.08

Water content was measured by the Karl–Fischer method. The values indicate the mean $\pm$ S.D. ($n = 3$).

Water Content of the Ketoprofen Poultice Formulations

Determination of the water content in the ADPL of ketoprofen poultice formulations was carried out using the KF method. Immediately after removal from the container, the water contents were 49.7 and 27.1% for KP and KP_{XR} , respectively (Table 2). Thereafter, the water content decreased depending on the time left out at room temperature, and the water contents at 5 h were 2.9 and 2.0% for KP and KP_{XR} , respectively (Table 2). Although the water content in the ADPL in KP was significantly higher than that of KP_{XR} , a reduction of the water content in a time-dependent manner by leaving out at room temperature is revealed in both formulations. These results suggest that the time-dependent change in the mass and NIR spectrum of ADPL by leaving it out at room temperature are due to the reduction of water in the ADPL.

TG/DTA Measurement of Poultice Formulations In KP, an endothermic peak accompanied by mass reduction was observed from 25 to 90°C (Fig. 3a), and both the TG curve and DTA curve had monophasic character. In addition, the rate of mass reduction of ADPL (48.6%, Table 3) was almost consistent with the water content by the KF method (Table 1). These results suggest that the endothermic peak was due to the evaporation of water. On the other hand, in KP_{XR} , the TG curve showed biphasic character with a boundary at approxi-

Fig. 3. TG/DTA Curves of Ketoprofen Poultice Formulations: (a) KP and (b) KP_{XR}

Fig. 4. Relationship between the Water Content and Mass of the Ketoprofen Poultice Formulation Left Out at Room Temperature as Well as the Peak Intensity in the NIR Second Derivative Spectrum: (a) Water Content vs. Rate of Mass Reduction; (b) Water Content vs. Peak Intensity (at Approximately 5200 cm^{-1}); and (c) Water Content vs. Peak Intensity (at Approximately 6900 cm^{-1}) from NIR Spectra of KP (Upper) and KP_{XR} (Lower)

Table 3. Rates of Mass Reduction of Ketoprofen Poultice Formulations Obtained from TG Curves

Formulation	Rate of mass reduction (%)	
KP	48.58 ± 1.57	
KP_{XR}	(1st phase)	(2nd phase)
	33.93 ± 1.17	14.75 ± 0.56

The values indicate the mean $\pm$ S.D. ($n=3$). 1st phase: $25\text{--}90^\circ\text{C}$; 2nd phase: $90\text{--}145^\circ\text{C}$ (see Fig. 3).

mately 90°C . The DTA curve showed a two-step recovery boundary of 90°C after passing through the endothermic peak at 55°C (Fig. 3b). As the mass reduction rate of ADPL (33.9% , Table 3) of the 1st phase (under 90°C) approximated the water content by KF (Table 2), is suggested that this phase reflects the evaporation of water. On the other hand, a TG/DTA measurement was performed on a mixture of PG and water (mass ratio 1:1) in our preliminary study, and a biphasic TG/DTA curve was observed. Furthermore, the temperature range in which the first and second phases were observed was similar to that of KP_{XR} (Fig. S3). Therefore, the 2nd phase (over 90°C)

of the TG/DTA curve of KP_{XR} may be due to the evaporation of PG contained only in KP_{XR} (Table 1).

Relationship between the Water Content, Mass and NIR-spectrum of Ketoprofen Poultice Formulations The correlation between the water content and rate of mass reduction of the ADPL as well as peak intensity of the NIR spectrum at each time point (0, 1, 3 and 5 h) when poultice was left at room temperature was examined. In both KP and KP_{XR} , the rate of mass reduction tended to increase as the water content decreased, and a good negative correlation was found between them (R^2 values: 0.97 and 0.98 for KP and KP_{XR} , respectively, Fig. 4a). These results reveal that the mass reduction by being left at room temperature is caused by a decrease in water in the ADPL of the poultice formulation.

In addition, as the water content increased, there was a tendency that the peak intensity of the combination region of the hydroxyl group in the NIR second derivative spectrum (at approximately 5200 cm^{-1}) tended to increase, and good correlation was also observed between them (R^2 values: 0.84 and 1.00 for KP and KP_{XR} , respectively, Fig. 4b). A similar correlation was also observed between the water content and

Fig. 5. Effect of the Drying Time on the *in Vitro* Skin Permeation Profiles of Ketoprofen across Hairless Mouse Skin

Each point indicates the mean $\pm$ S.D. ($n = 4$).

Table 4. Effect of the Time Left out at Room Temperature on the *in Vitro* Steady-State Skin Permeation Flux of KP and Lag Time across Hairless Mouse Skin

Time	Flux ($\mu\text{g}/\text{h}/\text{cm}^2$)	Lag time (h)
0 h	5.11 ± 0.41	2.51 ± 2.12
1 h	5.00 ± 0.69	4.30 ± 1.00
5 h	3.80 ± 1.10	5.41 ± 1.24

The values indicate the mean $\pm$ S.D. ($n = 4$).

the peak intensity near the first overtone region (6900cm^{-1}) of the hydroxyl group in the NIR second derivative spectrum (R^2 values: 0.95 and 1.00 for KP and KP_{XR} , respectively, Fig. 4c).

These results clearly show that the mass reduction and change in the NIR spectrum of poultice caused by being left out at room temperature are due to the decrease in water. The KF method is destructive, and it is necessary to pay attention to the treatment of waste liquid, although the measurement of this method is very simple. Therefore, the NIR and TG/DTA method have been shown to be useful tools for evaluating the water content of poultice as a substitute for the KF method. In particular, NIR is superior in terms of its nondestructibility and rapidity.

Permeation Profiles of Ketoprofen Poultice Formulations Finally, the skin permeability of the API in KP that was left at room temperature using hairless mouse skin was examined. As the leaving time increased, the cumulative skin permeation amount and flux decreased, while the lag time was prolonged (Fig. 5, Table 4). According to the above results of this study, at least 85% of water disappears by being left at room temperature for 1 h (49.65 to 7.62%, Table 2). Therefore, the results of the permeability test suggest that the disappearance of water not only affects the cooling sensation and tackiness but also the skin permeability of the API. As a result, due to the reduction of water, it is considered that the reduction of adhesion to the skin of the ADPL, the decrease of the drug release rate from the formulation and the decrease of the skin permeability are due to the delay of the stratum corneum hydration. Moreover, it is well known that *l*-menthol added to KP is commonly used as a skin permeation enhancer of the API.^{11–14} The results of this skin permeability test may be partly involved with the evaporation of *l*-menthol with water.

As mentioned above, it is directly revealed that the decrease

in the water content in the ADPL of poultice influences the skin permeability of the API, and it is suggested that maintaining the water content in the ADPL is also very important from the viewpoint of the effectiveness of poultice.

CONCLUSION

When KP was left out at room temperature, within 2–3 h a reduction of the mass of ADPL and attenuation of the peak reflecting the hydroxyl group in the NIR spectrum occurred. It is indicated that those changes were due to a reduction in the water content in the ADPL. In addition, a skin permeability study using hairless mouse skin revealed that the transfer of the API to the skin was impaired by the water content reduction in the ADPL of poultice. Hence, it is suggested that maintaining the water content in the ADPL in poultice is very important from not only the viewpoint of cooling sensation, tackiness and moisturizing but also the skin permeability of the API.

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Conflict of Interest The authors declare no conflict of interest.

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REFERENCES

- Chien YW. Transdermal drug delivery and delivery system. *Novel Drug Delivery Systems*. Vol. 50, Marcel Dekker, New York, pp. 301–380 (1992).
- Subedi RK, Oh SY, Chun MK, Choi HK. Recent advances in transdermal drug delivery. *Arch. Pharm. Res.*, **33**, 339–351 (2010).
- Kawamura N, Shinkai N, Yamauchi H, Takayama S. Comparison of poultice-type and tape-type patches containing ketoprofen on human skin irritation. *J. Toxicol. Sci.*, **28**, 415–425 (2003).
- Wester RC, Mainbach HI. Regional variation in percutaneous absorption. *Percutaneous absorption*. (Bronaugh RL, Maibach HI, eds.) Vol. 97, Marcel Dekker, New York, pp. 107–132 (1999).
- Warner TD, Giuliano F, Vojnovic I, Bukasa A, Mitchell JA, Vane JR. Nonsteroid drug selectivities for cyclo-oxygenase-1 rather than cyclo-oxygenase-2 are associated with human gastrointestinal toxicity: a full *in vitro* analysis. *Proc. Natl. Acad. Sci. U.S.A.*, **96**, 7563–7568 (1999).
- Kay-Mugford P, Benn SJ, LaMarre J, Conlon P. *In vitro* effects of nonsteroidal anti-inflammatory drugs on cyclooxygenase activity in dogs. *Am. J. Vet. Res.*, **61**, 802–810 (2000).
- Diaz-Arnold AM, Arnold MA, Williams VD. Measurement of water sorption by resin composite adhesives with near-infrared spectroscopy. *J. Dent. Res.*, **71**, 438–442 (1992).
- Near-Infrared Spectroscopy*. (Ozaki Y ed.) Kodansha Ltd., Tokyo, pp. 269–270 (2016).
- Yamamoto Y, Fukami T, Onuki Y, Metori K, Suzuki T, Koide T. Comparison of pharmaceutical properties among clobetasol propionate cream formulations: considerations from near-infrared spectra. *Vib. Spectrosc.*, **93**, 17–22 (2017).
- Segtnan VH, Sasić S, Isaksson T, Ozaki Y. Studies on the structure of water using two-dimensional near-infrared correlation spectroscopy and principal component analysis. *Anal. Chem.*, **73**, 3153–3161

- (2001).
- 11) Cornwell PA, Barry BW. Sesquiterpene components of volatile oils as skin penetration enhancers for the hydrophilic permeant 5-fluorouracil. *J. Pharm. Pharmacol.*, **46**, 261–269 (1994).
- 12) Moghimi HR, Williams AC, Barry BW. Enhancement by terpenes of 5-fluorouracil permeation through the stratum corneum: model solvent approach. *J. Pharm. Pharmacol.*, **50**, 955–964 (1998).
- 13) Lane ME. Skin penetration enhancers. *Int. J. Pharm.*, **447**, 12–21 (2013).
- 14) Patil UK, Saraogi R. Natural products as potential drug permeation enhancer in transdermal drug delivery system. *Arch. Dermatol. Res.*, **306**, 419–426 (2014).