TEST Engineering & Management

March - April 2020
ISSN: 0193-4120 Page No. 4539 - 4546

Improvement in Percutaneous Absorption by Modulating the Temperature of oil/water Emulsion

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Article Info
Volume 83
Page Number: 4539 - 4546
Publication Issue:
March - April 2020

Abstract
Background/Objectives: Skin has a low absorption rate for active substances due to the permeation barrier. The present study used oil/water (o/w) emulsions to evaluate trans dermal absorption in vitro and in vivo.
Methods: Comparative evaluation was performed by varying the emulsion's temperature conditions, which could affect transmittance. The absorbed active substances were quantitatively evaluated by high-performance liquid chromatography, and the average transmittance was calculated by examining the flux value of each group.
Findings: As a result, trans dermal absorption at 42°C doubled at 10 minutes after application and tripled at 15 minutes after application compared with the normal skin temperature. The higher the temperature of the emulsion, the higher the trans mittance. Clinical evaluation of the skin revealed that no clinical subject showed specific adverse reactions but all exhibited a significant effect on moisture and oil content.
Improvements/Applications: The o/w emulsion prepared here can be applied to various suitable cosmetic products and beauty devices because of its increased trans dermal permeability according to the temperature proposed in this study.

Article History
Article Received: *24 July 2019*
Revised: *12 September 2019*
Accepted: *15 February 2020*
Publication: *26 March 2020*

Keywords: *Transmittance, Trans dermal, Emulsion's temperature, Beauty devices, Skin barrier.*

1. Introduction

Skin is the largest organ of the human body; it covers the whole body and comprises three layers of epidermis, dermis, and subcutaneous fat. The skin is the outermost tissue of the human body and serves to protect and defend against external stimuli [1]. To effectively protect the human body from external stress or irritation, the skin has a permeation barrier, which prevents skin moisture evaporation and ingredient penetration from the outside. This is a stepped structural property of the lipid matrix, which makes cosmetic active substances not easily absorbable and imparts the barriers with very low permeability to foreign substances [2,3]. The skin penetration route of an active substance involves reaching the dermis through the epidermis; there are generally two routes. The first route is the intracellular pathway, which passes through protein-filled keratinocytes and penetrates through the hydrophilic region of the epidermis, which is relatively high in water content. Only 0.3% of external active substances can pass through the stratum corneum through the passage among the skin cells; moreover, the deliverability of these active substances via this route to the right region can hardly be expected. The second route is the pathway through the skin's appendages such as sweat glands, hair follicles, and sebaceous glands. Although this route delivers

March - April 2020
ISSN: 0193-4120 Page No. 4539 - 4546

rapidly, the amount of external active substances it delivers is very small, given that the effective region of the appendages in the skin is approximately 0.1% [4].

Currently, active research is under way in the cosmetics industry for novel materials with various functions such as antioxidant, anti aging, and whitening effects. Moreover, strategic technologies for improving the skin penetration rate are also being developed. The absorption of cosmetics products and its ingredients in the skin is affected by various conditions such as skin properties and environmental factors depending on the physical and chemical properties of the active ingredients [5]. Various physical and chemical properties include factors such as molecular weight, viscosity, polarity, adsorption, metabolism, and solubility, which can be modified to improve the percutaneous absorption of these active ingredients. Physical approaches include formulation development of polymer hydrogel, polymer micelle, nano emulsion, liposome, elastic liposome, etosome, etc.; iontophoresis that increases the penetration of ionic drugs by changing the skin's electrical environment; sonophoresis that infiltrates drugs using ultrasound; infiltration through stratum corneum removal; and micro needle therapy system and auto microneedle therapy system (AMTS) [6-8]. Although various technologies have been developed, the verification and safety studies on the expression of the original efficacy of the active ingredients are still inadequate, and to date, research has been focused on increasing the transmittance by reducing the size of particles. In the present study, the thermotherapy adopted is a method of complementary replacement therapy that increases body temperature to make the body healthy and has mainly been used as an adjuvant therapy for disease treatment. In the beauty field, thermo therapy also has been mainly used as a heat using tool such as a paraffin device, high frequency machine, and stone massage therapy [9]. Although several studies have been conducted on thermal properties, to the best of our knowledge, no studies have examined the penetration rate in relation to the optimum and elevated temperatures.

Therefore, the present study investigated percutaneous permeability and changes in skin conditions in relation to temperature and examined the optimal temperature and time that can be applied to the future thermal mask pack or personal beauty devices and products.

2. Method

- **Preparation of test solution and safety evaluation**.

The active ingredient used herein was an o/w emulsion that contains 10% niacinamide. Niacinamide is the whitening material recommended by Korean Food and Drug Administration and is stable to heat and light. The oil/water (o/w) emulsion was used to ensure that the sample has no shape properties or ingredients other than butylene glycol and glycerin that may affect absorption.

Five participants were randomly selected before the experiment and were tested for skin safety using the IQ Chamber. Subsequently, 25μL of each test sample was added dropwise to the IQ chamber, fixed to the upper arm, and removed after 24 hours. The test site was monitored after 30 minutes, 24 hours, and 48 hours after removal, and the degree of stimulation was classified according to the criteria of the International Contact Dermatitis Research Group.

- **Skin absorption test using a trans dermal absorption device**.

The temperature of the trans dermal permeation device (Franz Diffusion cell Semi-Auto system: FDC-6T, Logan, USA) used in the experiment was set as the exact temperature (32°C, 37°C, and 42°C) for each experiment, which allows a preheating time of 10 minutes. The artificial skin

March - April 2020
ISSN: 0193-4120 Page No. 4539 - 4546

(Merck MILLIPORE Strat-M Membrane 25mm) was applied with pH 7.4 phosphate-buffered saline buffer at a given temperature to ensure that a constant temperature is maintained, and it was removed after recording the temperature of the skin surface using a thermometer. The permeating samples were prepared with 10% niacinamide emulsion and analyzed using high-performance liquid chromatography.

The O/W emulsions used in the experiments did not have formulation properties or component properties that could affect absorption, other than butylene glycol and glycerin. After evenly applying O/W emulsion on an artificial skin, a 1-mL syringe was used to take 0.5 mL of the emulsion for each given duration (5, 10, 15, 20, and 30 minutes), and the achieved volumes were kept in a 1.5-mL tube. The samples acquired by the Franz Diffusion cell Semi-Auto System were diluted 5-fold, 2-fold with pH 7.4 PBS buffer, and analyzed with HPLC. When the HPLC analysis was performed, the used column was 300 mm × 3.9 mm × 5 µm C18 L1, flow rate was 2 mL/min, wavelength was 254 nm, and retention time was set to 2.1 min. The samples remaining in the donor were used after 25-fold dilution and those remaining in the artificial skin were diluted 10-fold in total with ethanol: pH 7.4, PBS = 1:4, and they were analyzed based on the same analysis condition. The temperature and humidity of the lab were maintained at 23±1℃ and 25±5% RH, respectively.

• **Skin absorption test using Raman spectroscopy**.

Regions termed high wave number regions (2,400–4,000cm^{-1}) and fingerprint regions (400–2,400cm^{-1}) of the Raman spectrum were measured using 671nm and 785nm lasers, respectively. The laser power value for the skin should be maintained at 10–20mW for 671nm and 20–30mW for 785 nm. Corrections were made before monitoring the participants' skin to determine whether the correlation coefficient value is 0.99 and the S/N fingerprint value is ≥ 25. The test was conducted at constant temperature and humidity conditions (temperature: 22°C ± 2°C, humidity: 40%–60%), and the participants were stabilized for at least 15 minutes before initiating the test. The participants had no history of skin disease such as allergy or atopic dermatitis and did not use any skin care product on the day of measurement to investigate the exact amount of penetration of the test ingredient. Two parts to be measured in an area of 4 × 5 cm^{2}were marked on the inside of the lower part of the participants, and the initial skin condition was measured using a Raman spectrophotometer, and then absorbent papers covering an area of 4 × 5 cm^2 that have absorbed 100 µL of the test ingredient solution were attached to the measurement sites. The area was divided into two parts using hot packs: one that exhibits a thermal effect and the other one that does not. The absorbent papers were applied for 5 minutes, and the results were compared.

• **Efficacy test of human skin absorption**.

A total of 10 participants who had no history of skin disease or related drugs in the last 3 months were selected. They were enrolled in the study after providing written informed consent forms. All experiments were performed with the approval of the Institutional Ethics Committee. For the experiment, 0.5 g of the samples were applied to the two inner parts of the upper arm regions in a chamber with constant temperature and humidity, and the nonwoven fabric sheets for mask packs were covered. Subsequently, hot packs were used to divide the region into two parts with and without thermal effect, and their results were compared. In each experiment, the skin surface temperature was maintained at 42°C with regular measuring intervals, and the application time was 15 minutes. Changes in skin conditions were measured with a Multi probe Adapter System MPA5 instrument from the German company

TEST Engineering & Management

March - April 2020
ISSN: 0193-4120 Page No. 4539 - 4546

Courage & Khazaka (C + K electronic GmbH, Cologne, GERMANY).

• Data processing.

All experiments were performed in triplicate. The mean and standard error of the mean were calculated using the SAS (Statistical analysis system, USA) program. P values of <0.05 were considered statistically significant.

3. Results and Discussion

• Effects of treatment on skin absorption according to temperature.

The results of transdermal absorption of the emulsion with 10% niacinamide, the active ingredient of this experiment, are presented in Table 1. Absorption rates were found to vary according to the set temperature, and the highest trans dermal absorption rate was after 15 minutes at 42°C. This temperature was approximately three times more effective than other temperatures. After the initial 5 minutes, the absorption was almost similar at both 42°C and 37°C; however, after 10 min, the difference between the two temperatures almost doubled. In the second experiment Figure 1 summarizes the results of increasing the release time to 20 and 30 minutes. The results were similar to those of the above experiments. After 20 minutes and 30 minutes, the effects of these temperatures were twice higher at 42°C than those at 32°C. Five minutes after initiation of the experiment, the results of the two temperatures displayed a marked difference. This indicates that the thermal effect promotes the percutaneous absorption of the active ingredient. According to the results reported, who studied the blood flow in the skin layer in relation to heat, they were found to have increased blood flow after thermotherapy, and increased blood flow showed physiological responses such as increased cell membrane permeability [10]. Methods for absorbing active ingredients into the skin can be divided into absorption through the epidermis and absorption through the skin appendages. All these methods of absorption are termed trans dermal absorption [11]. In general, the most common pathways/routes through the appendage is absorption through pores; however, when the skin is heated or provided thermal therapy, the pores become wider and more enhanced. These factors must be considered to indicate the same results in this current experiment.

Table 1. Result of permeation test of o/w Niacinamide 10% emulsion (15min)

	5min	10min	15min
32℃	0.078±0.321	0.409±0.177	0.745±0.276
37℃	0.471±0.371	0.469±0.454	0.783±0.783
42℃	0.488±0.137	0.982±0.437	2.015±0.961

Raman spectrophotometer was used to measure the degree of absorption according to the temperature of the sample as shown in Figure 2. Fingerprint regions (400–2,400 cm^{-1}) were measured at 785 nm. The peaks obtained by measuring the sample material were able to identify at a specific part (raman shift cm^{-1}), and the higher the temperature, the higher the absorption rate.

March - April 2020
ISSN: 0193-4120 Page No. 4539 - 4546

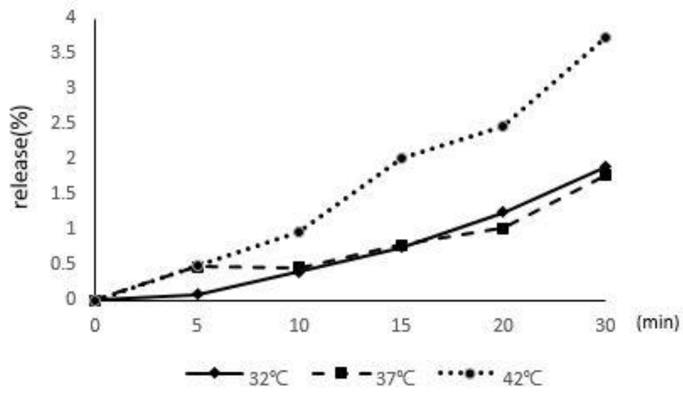


Figure 1. Result of permeation test of o/w Niacinamide 10% emulsion (30min).

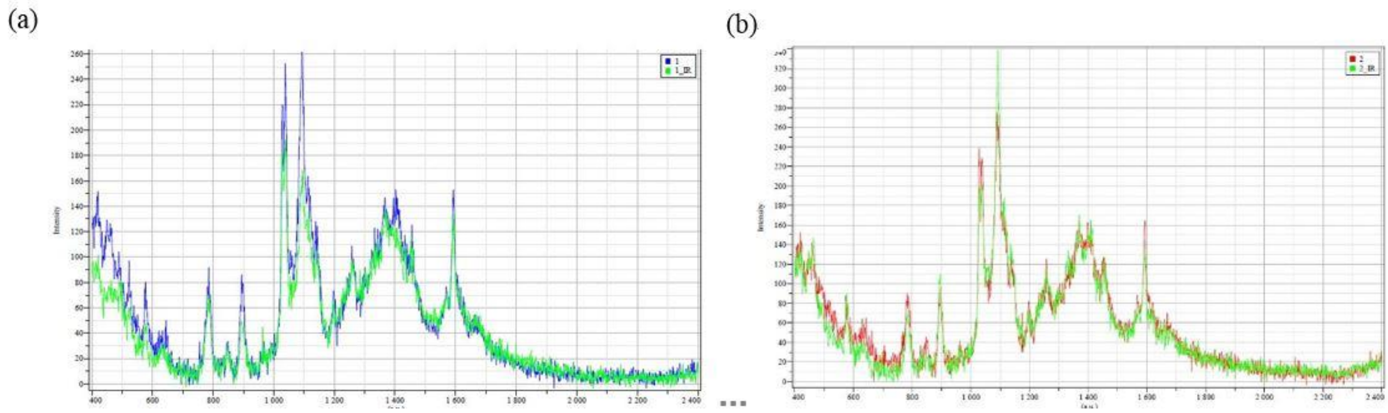
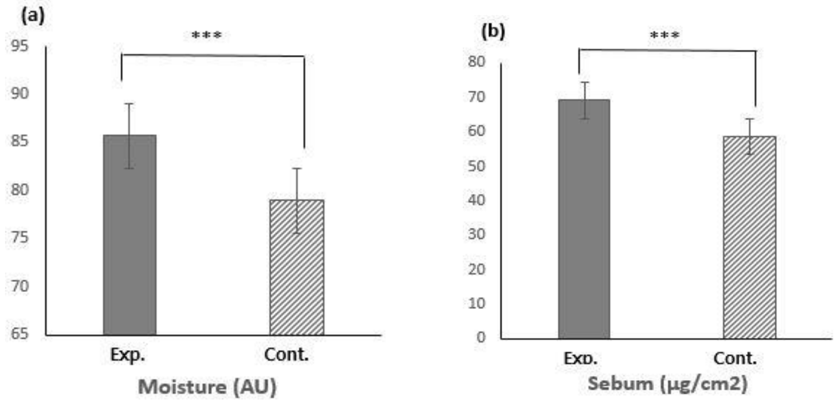


Figure 2. Raman spectrum of o/w Niacinamide 10% emulsion
(laser : 785 nm, power : 25 Mw, spectral range : 400 – 2400 cm^{-1}, a: 15min, b:30min)

• Evaluation of skin absorption for human application.

Changes in skin conditions due to heat are presented in Figure 3. And Table 2 Significant changes were observed in moisture and oil content, but not in skin tone and skin flexibility. Although the number of samples used here is small, and the time used for measurements if short to measure the change, the oil and moisture values of the parts that exhibit the warming effect are remarkably different. It is possibly because of increased blood circulation and capillary expansion due to heat. This is also consistent with the findings reported by a stone therapy using high temperature and high frequency [12,13]. In general, the thermal effect can expand the peripheral blood vessels to increase blood circulation, thereby increasing the amount of water secreted. In addition, it opens pores, smoothens sebum secretion, and increases oil content. Furthermore, heat stimulates the molecular movement to facilitate the penetration of the active ingredient. Hence, providing the skin with some warmth has the advantage of maximizing the characteristics of the active ingredient [14].



*** p < .001

Figure 3. Changes in skin condition(moisture, sebum) after o/w Niacinamide 10% emulsion application (a: moisture(AU), b : sebum(μg/ cm^2))

Table 2: Changes in skin condition (moisture, sebum, skin tone, softness) after o/w Niacinamide 10% emulsion application

variable	Exp.(N=10)	Cont.(N=10)	t	p
Moisture(AU)	85.70±4.13	78.97±8.71	.2034	.000***
Sebum(μg/ cm^2)	69.29±1.78	58.71±1.53	.2457	.000***
Skin tone	147.10±6.81	97.84±3.10	.358	.802
softness	105.37±9.88	101.59±6.27	-.741	.633

*** p < .001

Human body temperature is generally maintained at 36.5°C, but the temperature of the skin surface is different from this overall body temperature. The temperature of normal skin of humans is approximately 31°C. human skin comprises proteins [such as transient receptor potential vanilloid (TRPV)], which detect temperature changes. Among them, the expression of TRPV-1 protein increases skin matrix metalloproteinases (MMP) activity. In other words, the expression of TRPV-1 due to heat increases MMP expression, which may cause skin aging. TRPV-1 reacts at temperatures above 43°C, and strong direct sunlight in August or frequent exposure to sauna baths can cause skin aging [15]. Therefore, the appropriate temperature must be maintained to obtain various skin improvements by heat.

Recently, the cosmetics industry has been developing novel functional materials such as antioxidant, antiaging, and whitening agents, and various studies are being conducted to increase the absorption of these ingredients in the skin. The most common factor for increasing transdermal absorption is skin penetration using small molecular weight formulations. However, it is believed that such a heat therapy can improve absorption only temporarily. If thermal effect is administered to various beauty products, the efficacy of simple ingredients can be further thus aiding in skin enhancement. Therefore, adequate heating is extremely suitable for active short period of time without using any special formulation technology.

increased. Heating allows a smooth blood circulation in the skin, helps with metabolism,

helpful for the development of personalized beauty devices using heat or thermal mask packs in the future.

4. Conclusion

The purpose of this study was to investigate the effect of skin surface temperature on the percutaneous absorption of active ingredients and changes in skin conditions. Percutaneous absorption was set at three temperatures: 32°C, the surface temperature of the skin; 37°C, similar to human body temperature; and 42°C, the highest temperature before aging is expressed. The active ingredient used herein was an o/w emulsion that contains 10% niacinamide, which is stable to light and heat. After 15 minutes of treatment, the highest transdermal absorption was noted at 42°C, which indicates that this temperature is approximately three-fold more effective than other temperatures. After the initial 5 minutes, the absorption was almost similar at both 42°C and 37°C. However, the difference between the two temperatures was almost doubled after 10 minutes of treatment. In the clinical evaluation of the skin using the same emulsion, all the clinical participants displayed no specific adverse reactions and exhibited significant effects on moisture and oil content. Thus, it was found that heat affects the skin absorption of active ingredients and is the most effective at approximately 40°C–41°C, which is the temperature before aging is expressed. However, because body temperatures vary from person to person and they are very sensitive to environmental conditions, it is difficult to determine the optimal temperature. Heat facilitates blood circulation and helps in improving metabolism in the skin. Therefore, appropriate heat and temperature is well suited for the active ingredients to temporarily penetrate in a

short time without any special formulation technique. We believe that our findings will be

Acknowledgment

This work was supported by the Technology development Program (S2634043) funded by the Ministry of SMEs and Startups (MSS, Korea).

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