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Study on Influencing Factors of Nicotinamide Transdermal Absorption In Vitro and the Establishment of an Evaluation Method

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Abstract: The goal of this research was to study the factors influencing the in vitro transdermal penetration of nicotinamide and to establish an evaluation method for the in vitro transdermal absorption of nicotinamide. The permeability of nicotinamide was investigated with Franz diffusion cell in vitro transcutaneous assays, and the effect of the receiving solution composition, receiving solution pH, skin type, diffusion cell temperature, active ingredient concentration, supply quantity, and product dosage form on its permeation was investigated separately by high-performance liquid chromatography. The best assay for the transdermal absorption of nicotinamide was established—there was a better transdermal absorption performance, more stable system, better applicability, and better reproducibility when the receiving solution was PBS (phosphate-buffered saline) solution, the pH was 7.4, the membrane was pig ear skin, the temperature was 37 °C, the concentration of nicotinamide was 3%, and the dose of the test substance was 2 g. In the three cosmetic dosage forms of toning lotion, milk lotion, and gel, the permeability of milk lotion was the highest, followed by toning lotion and gel.

Keywords: nicotinamide; transdermal absorption; Franz diffusion cell; cumulative permeability

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1. Introduction

In recent years, with the continuous development of the social economy, consumers have an increasing demand for cosmetics with whitening properties [1]. Nicotinamide is an amide compound of nicotinic acid and is currently used as an active ingredient in whitening and freckle-removing cosmetics [2]. As one of the B vitamins, its whitening and freckle-removing mechanism is different from other traditional components. Specifically, it can reduce the synthesis of melanin in melanocytes by affecting the transport of substances in melanocytes, thereby effectively inhibiting the transmission of melanin to keratinocytes [3–6]. In addition, when melanin reaches the skin surface, nicotinamide can also accelerate the renewal rate of skin cells, thereby promoting the shedding of cells containing melanin and achieving the effect of improving skin quality and whitening skin from inside to outside [7,8]. However, regardless of its mechanism of action, the whitening agent must first penetrate the skin to exert its whitening effect. Therefore, it is of great significance to study the transdermal absorption of active whitening components to evaluate their whitening effect.

The transdermal absorption of cosmetics can be divided in two ways: 1. The intercellular pathway: chemical substances bypass keratinocytes and penetrate into the subcutaneous tissue through the continuous distribution of intercellular substances between keratinocytes; 2. the transcellular pathway: chemical substances alternately spread directly through the keratinocytes and intercellular substance in the water phase and lipid phase. The direct penetration of chemicals into the dermis through skin appendages such

as hair follicles, sebaceous glands, and sweat duct orifices is also known as the bypass pathway. Macromolecular and ionic substances are difficult to pass through the lipid-rich stratum corneum and may enter the skin through this pathway [9–11].

In this paper, the Franz diffusion cell method and high-performance liquid chromatography were used to study the transdermal permeation of nicotinamide in vitro by measuring the cumulative transmittance. The cumulative permeability of transdermal absorption under different influencing factors was studied to provide the basic value of transdermal absorption of water-soluble whitening and freckle-removing agents for cosmetics R & D personnel.

2. Materials and Methods

2.1. Experimental Materials

Nicotinamide, purity ≥ 98% (Baihaobo, Shanghai, China); octanoic acid/capric acid triacylglycerol ester, dioctyl carbonate, coconut glycerides, and polyethylene glycol E 400, all of which were cosmetic grade (BASF, Ludwigshafen, Germany); PBS buffer solution (tablet, pH 7.2~7.4) (Soleibao, Beijing, China); Tween 20, Tween 60, Span 60, Tween 80, and Span 80 (Aladdin, Shanghai, China); polyglycerol-4-laurate (Yingchuang, Shanghai, China); methanol and formic acid, chromatographic grade, and anhydrous ethanol, analytically pure (Sane Chemical Technology, Shanghai, China).

2.2. Experimental Methods

2.2.1. Preparing Solutions

Nicotinamide (NA) was accurately weighed and configured into a solution with a mass concentration of 10 mg/mL. It was diluted into 2, 10, 50, 100, 250, 500, 750, 1000, 1250, and 1500 μg/mL NA series standard solutions. The standard curve of nicotinamide was obtained under 2.2 chromatographic conditions.

Nicotinamide was accurately weighed and aqueous solutions with mass fractions of 2%, 3%, 4%, and 5% were prepared as supply pool samples.

2.2.2. Instrument Box Parameter

The transdermal diffusion device used in this experiment was improved on the basis of the static vertical diffusion tester designed by Franz. The transdermal absorption process of cosmetics was reproduced by slow penetration of the skin model. The device includes a supply cell (diameter of 2 cm) and a receiving cell (volume of 8 mL). The skin model was placed horizontally between the donor media and the acceptor media, and the stratum corneum was oriented towards the supply pool and fixed with a screw clip to empty the bubbles.

We used an Agilent Plus C18 chromatographic column (4.6 mm × 250 mm, 5 μm); the injection volume was 10 μL, column temperature was 30 °C, and detection wavelength was 261 nm. The mobile phase was a mixed solution of V (methanol)/V (0.1% formic acid water) = 35:65.

2.2.3. In Vitro Skin Treatment

In this study, pig ear skin, pig skin, and Strat-MTM membrane were used as skin models [12]. Pig ears and pig skin (pig back skin) used to make in vitro skin samples for transdermal experiments were purchased from a local market. When making a pig ear skin sample, the pig ear was cleaned with clean water and dried with a soft cloth, and the outer skin of the pig ear was taken off with a scalpel. At the same time, no physical damage and no abnormal skin were selected for the experiment. The skin taken off was full-thickness pig ear skin, including stratum corneum, epidermis, and dermis. The hair was removed with an electric razor to a length of about 3 mm close to human hair. The pig's outer ear skin and excess fat on the pig's skin were scraped off with a scraper and cut into a square with a side length of 3 cm with a scalpel. After treatment, it was wrapped in tin foil paper

and stored at −20 °C for later use. The storage time did not exceed 30 days. The skin was thawed at room temperature before use.

2.2.4. Transdermal Absorption

In vitro transdermal experiments were carried out according to SCCS (2012), OECD (2004a, b) [13] guidelines. After the installation of the diffusion tank, the bubbles were discharged and placed in a water bath with magnetic stirring and temperature control. The experiment was carried out at a constant temperature, and the receptor chamber was continuously stirred at a speed of 300 rpm to ensure that the skin surface temperature was (32 ± 1) °C. After coating the sample, 200 μL of the sample was taken from the receiving pool 1, 2, 4, 6, and 8 h after coating, and the same dose of PBS buffer was added to the receiving pool at the same time. The content of nicotinamide was determined by high-performance liquid chromatography (HPLC). The cumulative permeation amount (Q_n, μg) and cumulative permeation rate (A, %) were calculated according to the following formula:

$$Q_n = \frac{C_n V + \sum_{i=1}^{n-1} C_i \cdot V_i}{s} \quad (1)$$

$$A = \frac{Q_n}{Q_a} \times 100\% \quad (2)$$

- We drew a graph with A to t.
- V is the volume of the receiving pool;
- V_i is the sampling volume;
- Q_a is the total mass of nicotinamide.

2.2.5. Selection of Transdermal Conditions

In order to explore the effects of receiving solution type [13,14], receiving solution pH [15,16], skin type [17–25], receiving cell temperature [26], supply solution concentration, and supply solution application dose [14,27] on the transdermal absorption of nicotinamide, each factor was explored. For the effect of the solution in the receiving cell, the main body of the solution in the receiving cell was PBS buffer solution (pH 7.4), and the effects of 20% anhydrous ethanol, 2% PEG400, and 2% Tween 20 on the transdermal absorption results were investigated. For the effect of the pH of the receiving liquid solution, The receiving pool solution was PBS buffer solution, and the effects of pH 7.4 and pH 5.4 of PBS buffer solution on the transdermal absorption results were investigated. For the effect of skin type, pig ear skin, pig skin, and Start-M^{TM} membrane were used to explore the effect of different skins on transdermal absorption. For the effect of the temperature of the receiving liquid, we kept the room temperature at 25 °C and explored the effect of the water bath temperature of the diffusion liquid at 37 °C, 32 °C, and 25 °C on its transdermal absorption results. For the influence of the concentration of the supply solution, the supply solution was niacinamide aqueous solution, and the influence of concentrations of 2 wt%, 3 wt%, 4 wt%, and 5 wt% on the transdermal absorption results was investigated. For the influence of the dosage, the dosage of the supply solution was applied on the epidermis of the pig ear at a dose of 31.4 mg (limited dose) and 2 g (unlimited dose), respectively, to explore the influence of a limited dose and unlimited dose on the results of transdermal absorption.

2.2.6. Preparation of Nicotinamide Makeup: Water, Gel, and Oil-in-Water Emulsion

To prepare nicotinamide-containing toning lotion, at room temperature, the aqueous phase was accurately weighed: nicotinamide, polyacrylate cross-linked polymer-6, and deionized water were weighed in turn, and three portions were weighed in three beakers. Equal masses of glycerol, 1,3-propanediol, and 1,3-butanediol were added to three beakers, respectively, and stirred until the system was uniform. At the same time, phenoxyethanol was added to the aqueous phase and stirred until the system was uniform, and then the material could be discharged.

Nicotinamide gel was prepared. The water phase was accurately weighed at room temperature. Hansheng gum and polyacrylate cross-linked polymer-6 in the water phase were dispersed in water, fully stirred, and uniformly dispersed. The remaining components of the aqueous phase were added in turn: nicotinamide, glycerol, deionized water. These were stirred and dispersed evenly; then, we added phenoxyethanol to the system, stirred it, and dispersed it until the system was uniform and the material could be discharged.

The nicotinamide oil-in-water emulsion was prepared. At room temperature, the water phase (phase A) was accurately weighed, and nicotinamide, polyacrylate crosslinked polymer-6, deionized water, and glycerol were added in turn. We accurately weighed the oil phase (B phase), added caprylic acid/capric acid triglyceride in three beakers, and added monostearate dehydrated sorbitol ester and polyoxyethylene (20) monostearate dehydrated sorbitol ester, monooleate dehydrated sorbitol ester and polyoxyethylene (20) monooleate dehydrated sorbitol ester, and polyglycerol-4-laurate; the monooleic acid dehydrated sorbitol ester and polyoxyethylene (20) monooleic acid dehydrated sorbitol ester were added to two beakers, and dioctyl carbonate and cocoa butyrate were added, respectively. The A phase and B phase were heated to 80 °C, respectively. The B phase was added to the A phase and stirred at 700 rpm for 5 min. Homogeneous emulsification was performed at 15,000 rpm for 3 min; when the temperature was slowly stirred to 36~38 °C, phenoxyethanol (C phase) was added and stirred until the system was uniform and the material could be discharged.

2.2.7. Data Analysis

Microsoft Excel 2023 (Microsoft) and GraphPad Prism 9.02 (GraphPad) were used to process the data. The experimental data were expressed as mean ± SD (n = 3). One-way analysis of variance (ANOVA) was used to compare the data. $p < 0.05$ indicated significant differences, and $p < 0.01$ indicated extremely significant differences. Origin 2018 (Origin) was used to draw the chart.

3. Results

3.1. Drawing the Standard Curve

The samples were detected independently, and the calibration curve fitting was good ($R^2 > 0.99$). The samples of 0.5 and 100 μg/mL were repeated, and the relative standard deviation was less than 1.0%, indicating that the method had good repeatability and the minimum detection limit was 0.5 μg/mL. The series of standard solutions of nicotinamide were injected in turn, and the linear regression was performed with the standard sample concentration as the abscissa and the peak area as the ordinate to obtain the linear regression equation and correlation coefficient of nicotinamide. The results are shown in Table 1, and the data determined by HPLC are mass concentration (μg/mL).

Table 1. Linear equation, linear range, and correlation coefficient of nicotinamide.

Sample	Linear Equation	Linearity Range/(μg/mL)	Correlation Coefficient
Nicotinamide solution	y = 225,059.50317x + 6,948,230	2~1500	0.99602

3.2. Transdermal Absorption In Vitro

3.2.1. The Influence of the Receiving Liquid

At 37 °C, 300 rpm, with 3% NA aqueous solution, and when the supply amount was 2 g, we used pig ear to subcutaneously explore the effect of different receiving liquids on the penetration of nicotinamide. The receiving liquids were 20% ethanol-PBS solution, 2% Tween 20-PBS solution, 2% PEG 400-PBS solution, PBS solution, and NA. We obtained in vitro percutaneous penetration results within 8 h as shown in Figure 1. The results showed that there was no significant difference in the effect of changing the composition

of the receiving liquid on its penetration within 8 h ($p > 0.05$). At 8 h, the cumulative permeability was 82.14%, 78.64%, 77.69%, and 73.52%, respectively. Considering the actual situation of the human body and that the transdermal raw materials are water-soluble substances, PBS solution was used as the receiving solution in the subsequent experiments.

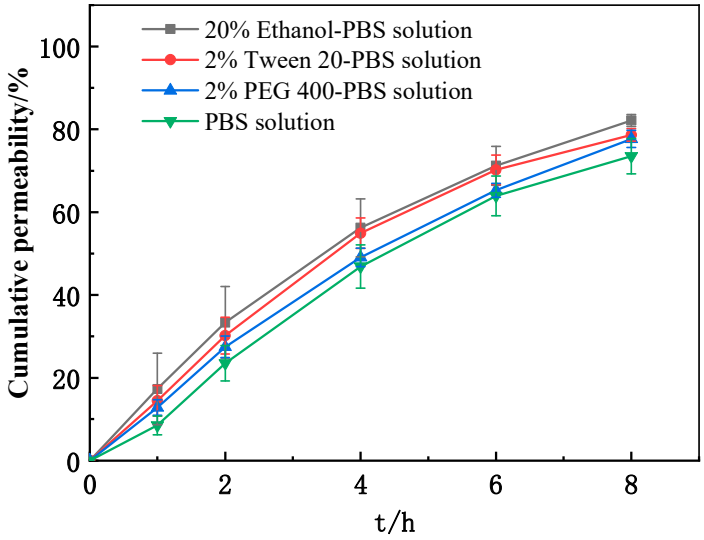

Figure 1. Cumulative permeability of nicotinamide under different receiving fluid conditions (n = 3).

3.2.2. Effect of pH of the Receiving Solution

At 37 °C, 300 rpm, with 3% NA aqueous solution, and when the supply amount was 2 g, the pig ear was subcutaneously used to investigate the effect of the pH of different PBS solutions on the penetration of nicotinamide. The results are shown in Figure 2. When the pH value of the receiving PBS solution was 7.4 and 5.4, respectively, the results showed that within 8 h, changing the pH of the receiving solution had no significant effect on its penetration ($p > 0.05$). At 8 h, the cumulative permeability was 73.52% and 81.48%, respectively. For nicotinamide, weak acidity was beneficial to in vitro percutaneous penetration.

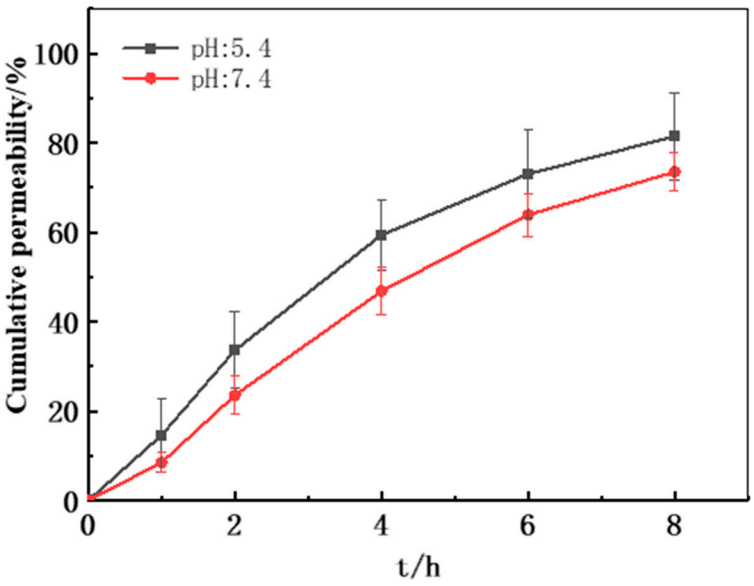

Figure 2. Cumulative permeability of nicotinamide at different pH values (n = 3).

3.2.3. The Influence of Skin Type

At 37 °C, 300 rpm, with 3% NA aqueous solution, a 2 g supply, and PBS solution (pH: 7.4) as the receiving solution, the effects of different skin models on the penetration of nicotinamide were investigated. The skin models were pig ear skin, pig skin, and Start-M^{TM} membrane. The results are shown in Figure 3. The results showed that within 8 h, there was a significant difference in the effect of changing the skin membrane model on its penetration ($p < 0.05$); at 8 h, the cumulative permeability was 73.52%, 18.66%, and 2.28%, respectively, and the cumulative permeability of pig ear skin was four times that of pig skin at 8 h. It was almost impermeable under Start-M^{TM}.

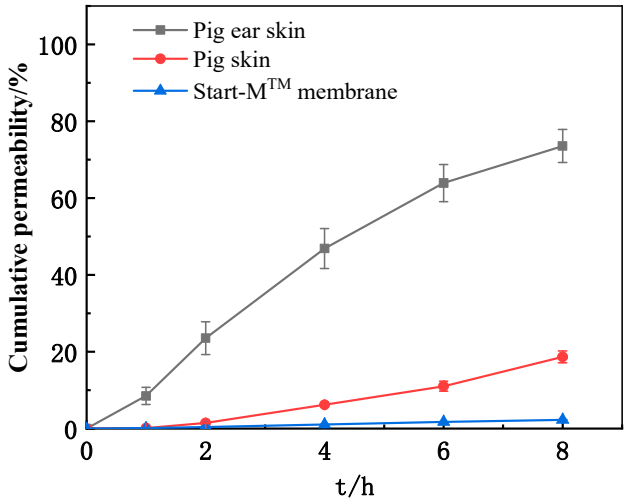

Figure 3. Cumulative permeability of nicotinamide under different skin conditions (n = 3).

3.2.4. The Influence of Diffusion Cell Temperature

The effects of different water bath temperatures on the penetration of nicotinamide were investigated at 300 rpm, with 3% NA aqueous solution, a 2 g supply, and using pig ear skin and PBS solution (pH: 7.4). The water bath temperatures were room temperature, 25 °C; human body surface temperature, 32 °C; and human body temperature, 37 °C. The results are shown in Figure 4. The results showed that within 8 h, there was a significant difference in the effect of changing the water bath temperature on its penetration ($p < 0.05$); the cumulative permeability at 8 h was 73.52%, 72.25%, and 52.25%, respectively.

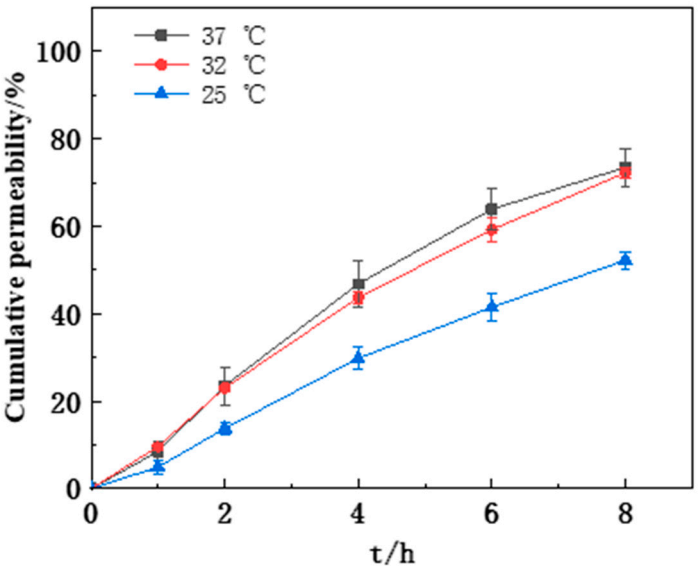

Figure 4. Cumulative permeability of nicotinamide at different temperatures (n = 3).

3.2.5. Effect of Active Ingredient Concentration

The effects of different concentrations on the penetration of nicotinamide were investigated at 37 °C, 300 rpm, 2 g, and using pig ear skin and PBS solution (pH: 7.4). The concentrations of nicotinamide were 2%, 3%, 4%, and 5%, respectively. The results are shown in Figure 5. The results showed that there was no significant difference in the effect of changing concentrations on its permeability within 8 h ($p > 0.05$). At 8 h, the cumulative permeability was 68.44%, 73.52%, 73.30%, and 70.73%, respectively, indicating that the permeability of nicotinamide was not related to the concentration. We simulated the human body to avoid sensitization. This paper takes 3% concentration to perform the follow-up test.

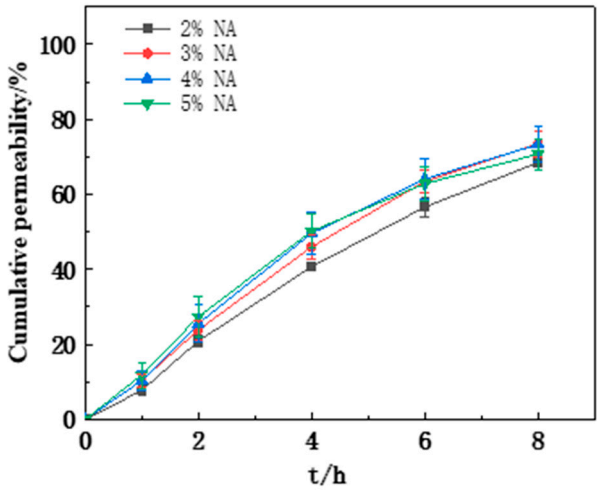

Figure 5. Cumulative permeability of nicotinamide at different concentrations (n = 3).

3.2.6. The Effect of Administration Dose

At 37 °C, 300 rpm, 3% NA aqueous solution, and using pig ear, the effects of different supply amounts on the penetration of nicotinamide were investigated subcutaneously. The supply amounts were 31.4 mg (limited dose) and 2 g (unlimited dose), respectively. The results are shown in Figure 6. The results showed that within 4 h, there was a significant difference in the effect of changing the supply on its penetration ($p < 0.05$); when the supply amount was limited, the transdermal penetration of nicotinamide reached saturation at 2 h. Considering the exploration of other influencing factors of nicotinamide, 2 g was used as the supply in subsequent experiments.

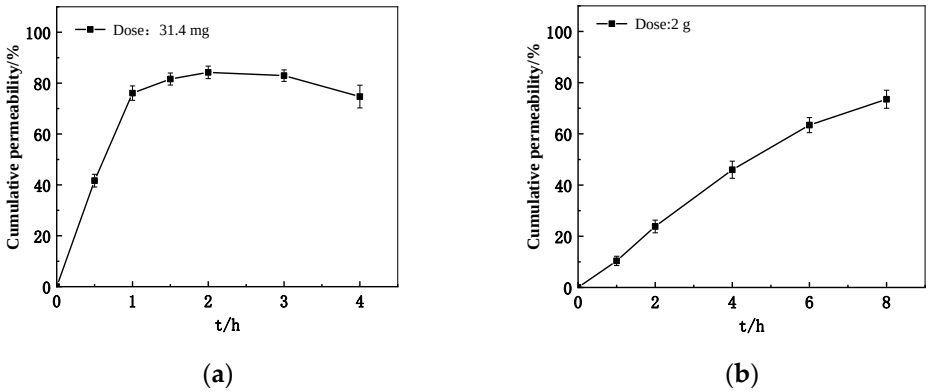

Figure 6. Cumulative permeability of nicotinamide under different doses conditions (n = 3). (**a**) Doses: 31.4 mg; (**b**) Doses: 2 g.

3.3. The Influence of Product Dosage Form

Based on the above results of the factors affecting the transdermal absorption of nicotinamide, a method model for the in vitro transdermal absorption of nicotinamide was established based on the Franz diffusion cell method: the rotation speed was 300 rpm, the water bath temperature was 37 °C, the skin was pig ear skin, the receiving solution was PBS buffer solution (pH: 7.4), the concentration of nicotinamide was 3%, and the supply amount was 2 g. In this method, we explored the effect of the matrix carrier on the penetration of nicotinamide.

3.3.1. The Effect of Administration Dose

In the makeup water, we explored the influence of different polyols on its penetration, and selected glycerol (S-NA-P1), 1,3-propanediol (S-NA-P2), and 1,3-butanediol (S-NA-P3) as the objects of investigation. The results are shown in Figure 7. The results showed that the cumulative permeability was 81.48%, 77.64%, and 76.59% at 8 h, respectively, indicating that the penetration effect of glycerol was better. Using One-way ANOVA comparative analysis, there was no significant difference in the cumulative permeability between the three ($p > 0.05$).

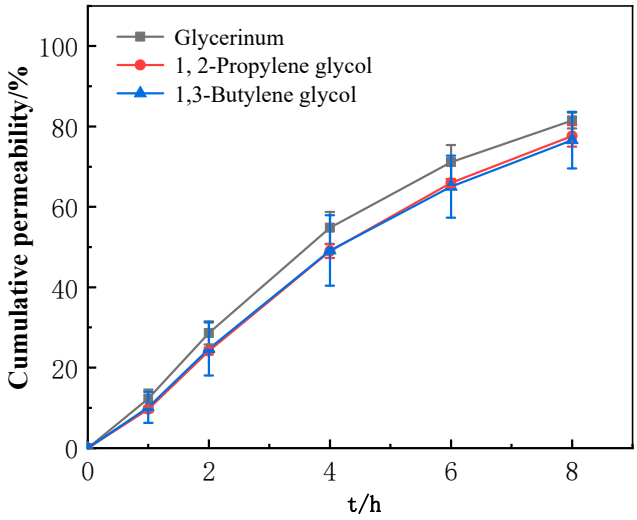

Figure 7. Cumulative permeability of nicotinamide under different polyols (n = 3).

3.3.2. Effects of Rheological Modifiers

In the gel, we explored the effects of different rheological regulators on its penetration, and selected polyacrylate crosslinked polymer-6 (N-NA-1) and Hansheng gum (N-NA-2) as the research objects. The results are shown in Figure 8. The results showed that within 8 h, through One-way ANOVA comparative analysis, at 6 h, there was a significant difference between the two ($p = 0.0417$); at the 8th hour, the cumulative permeability was 61.79% and 50.54%, respectively. At the same mass fraction of the rheological regulator, Hansheng gum had better fluidity, so the cumulative permeability was high.

3.3.3. Effect of Emulsifiers

In the oil-in-water emulsion, we explored the effect of different emulsifiers on its penetration. For emulsifiers, Span 60 Tween 60 (W-NA-O1-RI), Span 80 Tween 80 (W-NA-O1-R2), and polyglycerol-4-laurate (W-NA-O1-R3) were selected as the investigation objects. The results are shown in Figure 9. The results showed that the cumulative permeability was 67.68%, 64.91%, and 64.41% at 8 h, respectively. Using One-way ANOVA comparative analysis, there was no significant difference in the cumulative permeability between the three ($p > 0.05$).

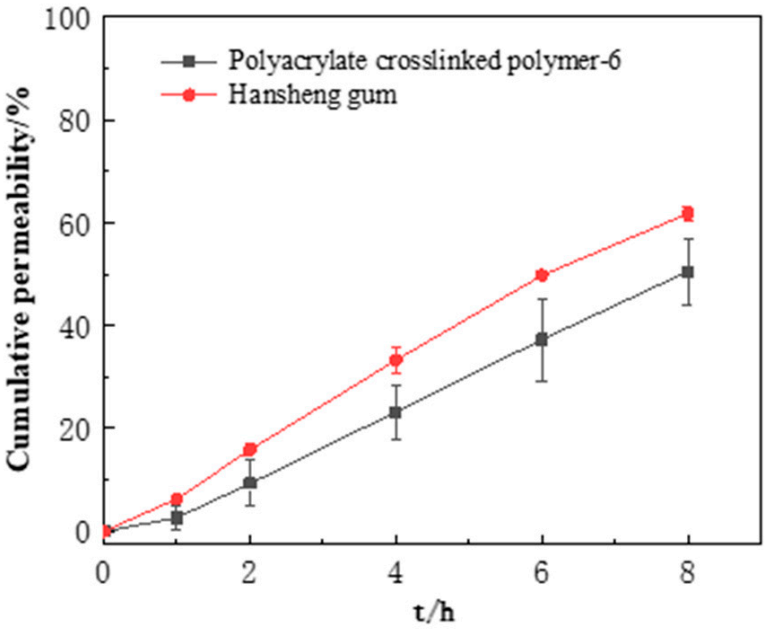

Figure 8. Cumulative permeability of nicotinamide under different rheological regulators (n = 3).

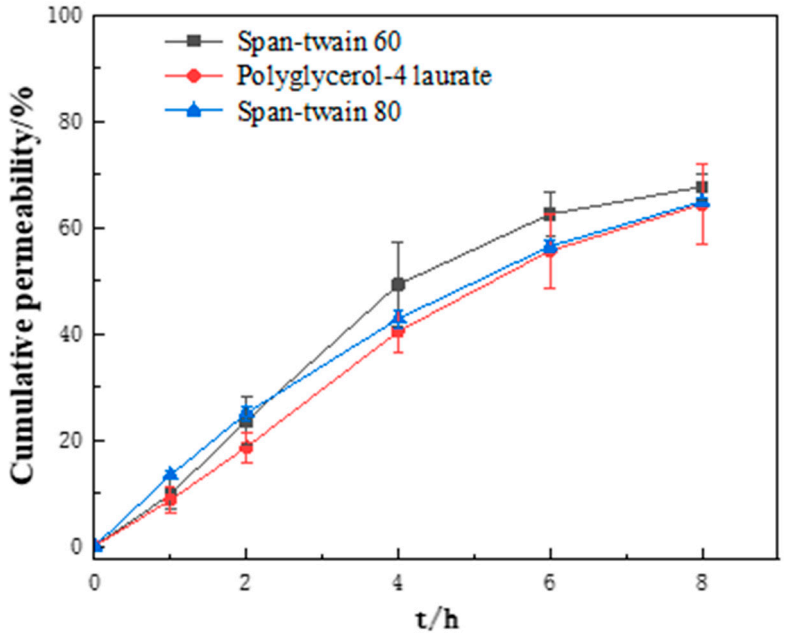

Figure 9. Cumulative permeability of nicotinamide under different emulsifiers (n = 3).

3.3.4. The Effect of Oil

In the oil-in-water emulsion, we explored the effects of different oils on its penetration. For oils, octanoic acid/capric acid triglyceride (W-NA-O1-R2), dioctyl carbonate (W-NA-O2-R2), and coconut acid glycerides (W-NA-O3-R2) were selected as the investigation objects. The results are shown in Figure 10. The results showed that the cumulative permeability was 64.41%, 65.96%, and 71.13% at 8 h, respectively. Using One-way ANOVA comparative analysis, there was no significant difference in the cumulative permeability between the three ($p > 0.05$).

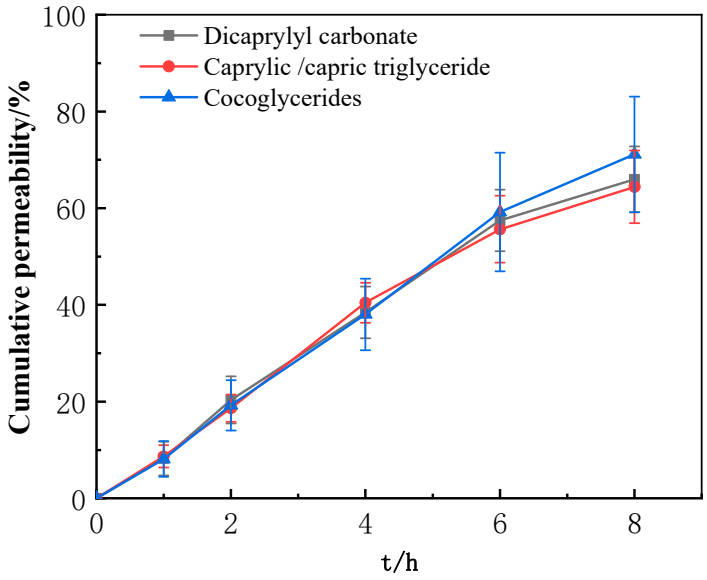

Figure 10. Cumulative permeability of nicotinamide under different oil conditions (n = 3).

The permeability of different formulations of makeup water (S-NA-P1), gel (N-NA-1), and oil-in-water emulsion (W-NA-O1-R2) to nicotinamide was investigated. The results showed that at 8 h, the cumulative permeability was makeup water > oil-in-water emulsion > gel, indicating that makeup water is most conducive to nicotinamide penetration. Using One-way ANOVA analysis, there were significant differences between gel and emulsion (p = 0.0488) and makeup water (p = 0.0037) at 1 h. At 2 h, there was a significant difference between makeup water and emulsion (p = 0.0435) and gel (p = 0.0010). At 4 h, there were significant differences among the three groups ($p < 0.05$). At 6 h, there were significant differences among the three groups ($p < 0.05$). At 8 h, there was a significant difference between makeup water and emulsion (p = 0.0215) and gel (p = 0.0006), and there was a significant difference between aqueous solution and gel (p = 0.0039).

4. Discussion

According to the national standard GB/T 27818-2011 chemical skin absorption in vitro experimental method, the receiving solution should be preferentially selected as the receiving solution [28], such as PBS buffer (pH 7.4), but also to meet the requirements of the leak test. Generally, a water-soluble solution is selected for drugs with good water solubility, while organic solvents or surfactants such as ethanol may be considered for drugs with poor water solubility, but it may change skin characteristics or dissolve lipid components, and should be added carefully. Although other solutions can also be used as the receiving solution, it is necessary to provide accurate components of the receiving solution and confirm that the test substance can be fully dissolved in the receiving solution without affecting skin absorption; in addition, the receiving fluid does not affect the integrity of the skin. The composition of the receiving solution has a significant effect on the amount of transdermal absorption [29]. Liu Yafeng et al. [14] explored the effect of three different cosolvents on the measurement results in the transdermal absorption of sunscreens by adding 2% Tween 20, 20% ethanol, and 4% PEG to the receiving solution in PBS. These three solvents had little effect on the experimental results. Therefore, the co-solvent with a lower dosage and less effect on the physiological state of the skin was preferred. Therefore, 2% Tween 20 was added to PBS as the receiving solution.

Nowak et al. [30] found that the permeation amount of nicotinamide through the nicotinamide cellulose acetate membrane (CA membrane) was 883 ± 62 μg when the pH value of the receiving solution was 5.4 and 679 ± 21 μg when the pH value of the receiving solution was 7.4, indicating that it was easier to promote the penetration of nicotinamide under acidic conditions. Zhang Tingting et al. [16] also pointed out that weak acid is

conducive to percutaneous penetration in vitro. The pH value of the human skin surface is 5.4, and the pH value of deeper skin layers is 7.4. Combined with the actual human condition, the PBS buffer solution with a pH of 7.4 was used as the receiving solution in the subsequent experiments.

Huang Xiaoping [11] found that the permeability of pig ear and pig abdominal skin were similar to that of human thigh skin when comparing the difference of drug permeability between different parts of pig skin and human skin. Zhang et al. [19] found that the permeability of nicotinamide in pig skin was higher than that in human skin. Pulsoni et al. [25] evaluated the permeability of caffeine (reference to OECD 428 substance) and LIP1 (lipophilic substance), two molecules with similar molecular weights but different lipophilicity, by using Strat-M^{TM} membrane and pig ear skin for comparative study. By applying this to the skin under infinite dose conditions, it was found that the Strat-MTM membrane was more permeable than the pig ear skin. According to the results, the pig ear skin will be used as an in vitro penetration skin model.

The passive absorption of chemicals is affected by temperature. Zhang Hongyan et al. [26] studied the transdermal absorption characteristics of fluorescent whitening agents in facial masks by conducting cosmetic transdermal absorption experiments at a constant temperature (37 °C ± 1 °C). According to the national standard GB/T 27818-2011 chemical skin absorption in vitro experimental method, the supply pool and skin should be kept constantly close to the normal skin temperature (32 °C ± 1 °C). Different diffusion cells require different water baths or heating to a certain temperature to ensure that the skin temperature is at its normal physiological standard.

According to a number of test results, the expert panel of the American Cosmetics Raw Materials Review Committee found that the use of 3% NA in cosmetics had no significant irritation, sensitization, and photosensitivity to the skin. When cosmetics containing nicotinamide are used for a long time, the daily dosage is not more than 3 g. The content of nicotinamide in cosmetics is generally 2–5%. A total of 4% of nicotinamide may cause intolerance in 20% of people. Cosmetics containing 5% nicotinamide can block ultraviolet rays in large quantities and reduce skin immunosuppression by smearing. In this experiment, the effect of 2–5 wt% NA on its permeability was explored, and it was found that the concentration had no effect on the cumulative permeability, but had a significant effect on the cumulative permeability ($p < 0.05$), and the cumulative permeability increased with the increase in concentration.

The application amount of the formula dose applied to the donor room was divided into limited doses and unlimited doses, limited dose $\leq$ 10 μL/cm^2 and unlimited dose $\geq$ 10 μL/cm^2. The finite dose represents a closer application and use condition, while the infinite dose helps us to understand and explain the steady-state permeation and the permeation capacity of a constant high formulation concentration. In the study of the effect of dose on the penetration of nicotinamide, Zhang Y et al. [27] found that the penetration percentage of nicotinamide in the finite dose study was relatively higher than that in the infinite dose study ($p < 0.05$).

The formulation also affects the transdermal absorption of chemicals, and the viscosity of the carrier is one of the factors affecting the diffusion of active ingredients and the release rate of the dosage form. Nabiee et al. [31] found the inter-group difference in the permeation of vitamin K1 by matrix carriers (O/W $\geq$ W/O > Aqueous) in exploring the transdermal absorption of vitamin K1. López-Sánchez et al. [30] explored the effects of aqueous solution, oil-in-water emulsion, and gel on the in vitro transdermal absorption of preservatives. It was found that the absorption of Bronidox and Bronopol depended on the formulation. The O/W emulsion was the system that promoted the least absorption of Bronidox, while the absorption of Bronopol by the gel was lower. The aqueous solution provided the maximum transdermal absorption of the two preservatives.

5. Conclusions

In this study, the effects of different types of receiving solution, pH values of receiving solution, skin, temperature, concentration of supply solution, and application amount of supply solution on the transdermal absorption of nicotinamide were studied. The best detection method for the transdermal absorption of nicotinamide was established. When the receiving solution was PBS solution, the pH was 7.4, the skin was pig ear skin, the temperature was 37 °C, the concentration of nicotinamide was 3%, and the application amount was 2 g, the transdermal absorption performance of nicotinamide was better, the system was more stable, the applicability was stronger, and the repeatability was better.

The effects of different formulations on the transdermal absorption of nicotinamide were investigated by using the established nicotinamide transdermal absorption system. The results showed that in the makeup water, the change in polyols had little effect on the transdermal absorption of NA; in the gel, changing the rheological regulator had a significant effect on the transdermal absorption of NA, that is, cumulative permeability: Hansheng gum > polyacrylate cross-linked polymer-6; and in the oil-in-water emulsion, the change in emulsifier and oil had little effect on the transdermal absorption of NA. Different product formulations of nicotinamide have a significant effect on its permeability. Among them, makeup water is most conducive to the penetration of nicotinamide, followed by emulsion, and finally gel.

The construction of this method makes the detection of nicotinamide transdermal absorption more applicable and accurate, provides the basic value of the transdermal absorption of water-soluble whitening and freckle-removing agents for cosmetics R & D personnel, and also provides reference for the future research and development of whitening and freckle-removing agent formulas.

We encourage researchers to build upon our findings and explore testing a range of nicotinamide concentrations to understand their impact on skin response.

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