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Swollen Micelle을 이용한 Tocopheryl Acetate의 피부흡수 연구

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Study on Skin Permeation of Tocopheryl Acetate Using Swollen Micelle

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요 약: 본 연구에서는 탈모방지 토너의 활성 성분인 tocopheryl acetate, salicylic acid 및 niacinamide 성분 중 유용성 성분인 tocopheryl acetate의 피부흡수를 높이기 위한 연구를 진행하였다. Salicylic acid와 niacinamide를 함유함과 동시에 tocopheryl acetate를 투명하게 가용화시키려는 방법으로, 계면활성제가 회합하여 형성되는 micelle의 크기를 증가시킨 swollen micelle 구조를 이용하였다. 제조한 swollen micelle 용액은 3종의 활성 성분을 함유하며 투명한 성상과 안정성을 높이기 위해 계면활성제로 poloxamer 407과 octyldodeceth-16을 사용하였다. 또한 micelle의 크기를 증가시키기 위한 보조계면활성제로서 isostearic acid를 사용하였다. 제조한 swollen micelle의 물리적 특성을 평가하기 위해 실온(25 ℃)에서의 탁도를 측정하였다. Swollen micelle에 함유된 탈모방지 성분 중, tocopheryl acetate의 피부흡수율을 평가하기 위해 Franz diffusion cell method를 이용하였다. 24 h 후 tocopheryl acetate의 경우 대조군보다 6 배 향상된 피부흡수량을 보여주었다. 따라서 본 연구에서 개발한 swollen micelle은 탈모방지 제품 또는 여러 기능성 화장품의 가용화 제형에 응용될 수 있음을 알 수 있다.

Abstract: A study was performed to increase skin permeation of tocopheryl acetate, an oil-soluble component among tocopheryl acetate, salicylic acid, and niacinamide, which are the active ingredients of the anti-hair loss toner. As a method of transparently solubilizing tocopheryl acetate while containing salicyl acid and niacinamide, we used a swollen micelle structure that increased the size of the micelle formed by the aggregation of surfactants. The prepared swollen micelle solution contains three kinds of active ingredients, and poloxamer 407 and octyldodeceth-16 were used as surfactants to increase transparent properties and stability. In addition, isostearic acid was used as a co-surfactant to increase the size of micelles. To evaluate the physical properties of the prepared swollen micelles, turbidity at room temperature (25 ℃) was measured. The Franz diffusion cell method was used to evaluate the skin permeation rate of tocopheryl acetate among the hair-loss prevention components contained in swollen micelles. After 24 h, tocopheryl acetate showed a 6-fold improvement in skin permeation compared to the control group. Therefore, it can be seen that the swollen micelles developed in this study can be applied to hair-loss prevention products or solubilized formulations of various functional cosmetics.

Keywords: *swollen micelle, anti-hair loss, tocopheryl acetate, poloxamer 407, Franz diffusion cell*

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1. Introduction

The stratum corneum, the outermost layer of the skin, forms a lamellar structure with a depth of 10 to 15 μm[1]. This stratum corneum consists of keratinocytes and keratinocyte interstitial lipids and is called “Brick and Mortar” structure[2]. When cosmetics are applied to the skin, the route that can flow into the skin is the intercellular route that mainly passes between the keratinocytes and interlipids[3,4]. Therefore, small particle size and membrane flexibility are important for material permeation. To this end, a drug delivery system (DDS) is a concept introduced to improve skin permeation of active substances having a large molecular weight and properties such as poor solubility[5,6].

Formulations used for DDS include liposome[7], ethosome[8], transfersome[9], niosome[10], and nanoemulsion[11]. For these formulations, the method using a high-pressure emulsifier is mainly introduced[3,12]. However, the commonly used liposome has limitations in the capture efficiency of active ingredients and drug release time[13]. In addition, manufacturing methods such as thin film hydration have disadvantages such as a long manufacturing time and use of an organic solvent. The method using a high pressure emulsifier also has limitations because, when a polymer is contained in the formulation, fracture of the polymer may occur[14]. Therefore, in this study, the swollen micelle structure was used as an alternative method[15].

A micelle is an aggregate in aqueous solution with a surfactant. When a surfactant collects in an aqueous solution, surface adsorption occurs due to interfacial tension on the surface of the aqueous solution. After that, above the critical micelle concentration (CMC) at which micelles begin to form, spherical micelles with hydrophilic groups facing outward are formed in the aqueous solution. This means an increase in solubility of poorly soluble components above CMC[16]. Micelles are about 0 to 20 nm in size, and there is a limit to containing poorly soluble active ingredients. Therefore, the disadvantage can be solved by using the swollen micelles, which are increased in size by adding a co-surfactant[17].

Swollen micelles vary by several hundred nm depending on the surfactant. Recently, studies have been performed on the stability of micelles using polymers[18-20]. Among them, poloxamer 407 is a general class of hydrophilic nonionic surfactants. It is a triblock ABA copolymer with two polyethylene glycol (PEG) hydrophilic blocks on the side and polypropylene glycol (PPG) on the central hydrophobic block. It has a reticulated structure, allowing the capture of amphiphilic substances. It is also hypoallergenic and is often used as a polymer for DDS due to its high stability[21].

Tocopheryl acetate protects the skin from harmful oxygen and is used as an alternative to tocopherol. It is also effective in preventing hair loss[22]. One of the causes of hair loss is oxidative stress. At this time, vitamin E series and derivatives play a role in reducing stress caused by oxidation. Also, salicylic acid and niacinamide are effective ingredients for preventing hair loss and can be used in cosmetics[23,24]. In a previous study[21], poloxamer 407 and octyldodeceth-16 were used as surfactants for transparently solubilizing the oil-soluble component tocopheryl acetate.

In this study, as an extension thereof, it was attempted to develop a transparent and stable swollen micelle formulation according to the content and ratio of poloxamer 407, octyldodeceth-16, and isostearic acid. Since an increase in the surfactant content has adverse effects such as increased feeling of use and irritation[25], it is important to develop an optimized formulation by lowering the surfactant content. As active ingredients, tocopheryl acetate (2.0%), salicylic acid (0.5%) and niacinamide (0.3%) were used. By comparing the skin permeation rate between the test group and the control group, the possibility of use as data applicable to hair loss prevention products or various functional products was confirmed.

2. Materials and Methods

2.1. Reagents and Instruments

The cosmetic raw materials used in the experiment are written in Table 1. Purified water was prepared using a distilled water maker (Pure RO 130, Human Corporation, Korea). Swollen micelles were prepared using an Agitator (PA1180, LK Lab, Korea). Turbidity meter (TU-2016, Lutron, USA) and pH meter (Orion star A111, Thermo Scientific, USA) were used for turbidity and pH of the prepared swollen micelle solution, respectively.

Table 1. Information About the Raw Materials Used in the Experiment

Ingredient	INCI name	Company, Nation
Emalex OD-16	Octyldodeceth-16	Nihon Emulsion, Japan
Ethanol	Ethanol (ethyl alcohol 95%)	Daejung, Korea
Eumulgin CO 60	PEG-60 hydrogenated castor oil	BASF, Germany
Isostearic acid	Isostearic acid	Kokyu Alcohol Kogyo, Japan
Na-citrate	Sodium citrate	Jungbunzlauer, Swiss
Niacinamide USP	Niacinamide	Western Drugs, India
Pluracare F 127	Poloxamer 407	BASF, Germany
Rheodol TW-O120V	Polysorbate 80	Kao, Japan
Salicylic acid	Salicylic acid	Duksan, Korea
TW-HD	1,2-Hexanediol	Twin Coschem, Korea
Vitamin E. acetate	Tocopheryl acetate	DSM, UK

To analyze the contents of tocopheryl acetate, salicylic acid and niacinamide contained in swollen micelles, HPLC (2695 & 2998, Waters, USA) with a photodiode array detector was used. As a column, Kromasil 100-5-C18 (AkzoNobel, Netherlands) was used. At this time, isopropanol, methanol, acetic acid, trifluoroacetic acid, and water used for analysis were purchased and used with commercially available special reagents for analysis from Sigma-Aldrich (USA). The analysis conditions for HPLC analysis are shown in Table 2.

In addition, to evaluate the skin permeation rate of tocopheryl acetate and salicylic acid contained in the swollen micelle, Franz diffusion cells (DKC-6DT, Logan, USA) were used, and an artificial skin model simulating human skin, Strat-M membrane (25 mm discs, Merck Millipore, USA) was used. Strat-M membrane dimmers from human skin, but the Strat-M membrane used in this study showed a strong correlation with human cade var skin[26], and high correlation between Strat-M membrane and porcine skin has been described [27].

2.2. Preparation Method

Swollen micelles containing three active ingredients were prepared by heating the oil phase containing tocopheryl acetate, part A, to 75 ℃, followed by cooling to 35 ℃. After that, salicylic acid was dissolved in ethanol, which is part A', and added to part A. After agitating while adding part A including part A' to part B, which is an aqueous phase, very slowly, it was prepared by further agitating for 3 min (Figure 1).

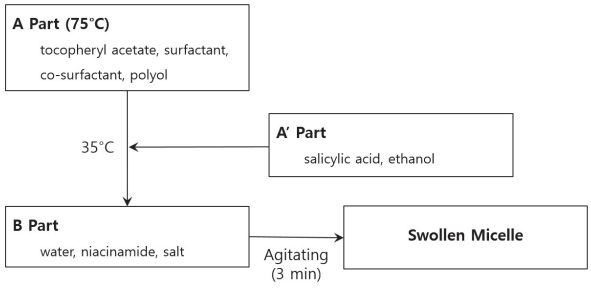

Figure 1. Manufacturing method of swollen micelle contained active materials.

2.3. Stability Evaluation

The prepared test articles were stored at room temperature (25 ℃), low temperature (4 ℃), and high temperature (45 ℃) for 90 d. The turbidity (Nephelometric Turbidity Units; NTU) over time was measured after 1 d, 15 d, 30 d, 60 d, and 90 d with the stock solution. At this time, turbidity measurement was repeated three times (N = 3). In addition, the stability was evaluated by visually confirming the transparency at 4 ℃ and 45 ℃.

2.4. Active Ingredients Analysis

In order to analyze the content of three active ingredients contained in swollen micelles, quantitative analysis was performed using HPLC. To prepare a calibration curve for each active ingredient, tocopheryl acetate, salicylic acid, and niacinamide standards were dissolved in methnol to make 25, 50, 100, and 500 ppm solutions, respectively, and each step

Table 2. HPLC Conditions for Analysis of Active Materials

System	Tocopheryl acetate	Salicylic acid	Niacinamide
Column	Kromasil 100-5-C18 (5 μm, 4.6 × 250 mm)		
Temperature	Column 40 °C, Sample 25 °C		
Mobile phase	Isopropanol : Methanol (25 : 75)	5.45% Acetic acid in Water : Methanol (55 : 45)	0.01% Trifluoroacetic acid in Water : Methanol (95 : 5)
Flow rate	1.0 mL/min		
Injection volumn	20 μL	10 μL	
Detector	Photodiode array (PDA)		
Wavelength	284 nm	254 nm	261 nm

was subjected to sonication for 1 min for homogenization. Then, 20,000 ppm of tocopheryl acetate and 5,000 ppm of salicylic acid contained in the swollen micelle solution were diluted at a ratio of 1/100 and niacinamide 3,000 ppm at a ratio of 1/10, and sonication was performed for 2 h, followed by HPLC analysis according to the conditions in Table 2.

2.5. *In Vitro* Skin Permeation Test

Through the basic experiment, the most transparent and stable test product was selected as the test group, and the skin permeation rate with the control group was compared. For the control group, not the surfactant used in the test group, but a micelle solution containing a surfactant, which is generally used as a solubilizer in preparing toners, was prepared and used. To evaluate the skin permeation rate of tocopheryl acetate, a Franz diffusion cell test using a 25 mm Strat-M membrane simulating human skin was used.

The receptor solution used in the experiment was polysorbate 20 : ethanol : water = 2.0 : 50.0 : 48.0 (wt%) ratio. To proceed with the experiment, first, a membrane was fixed between the donor phase and the receptor phase, and then 12 mL of the receptor solution was injected into the receptor phase. Membrane was stabilized for 20 min by maintaining the magnetic bar in the receptor phase at a speed of 600 rpm, and the temperature at 37.0 ± 1.0℃. After that, 0.4 mL of each of the test group and the control group was added to the donor phase, and 12 mL of the receptor phase was collected each time at intervals of 8, 16, and 24 h (3 times in total) with a pipette through the sampling port and stored in a conical tube. Immediately after collection, the same amount of receptor solution was injected into the receptor phase. Tocopheryl acetate contents were determined according to HPLC conditions in the receptor phase obtained by time period.

To measure the amount of active ingredient remaining in the membrane after 24 h, it was washed with 10 mL of receptor solution immediately after removal from the diffusion cell, and diluted with 20 mL of methanol for HPLC analysis. Again, the membrane was cut into 8 pieces, put in a conical tube, and 10 mL of methanol was added to perform HPLC analysis. The conditions for HPLC analysis are shown in Table 2.

2.6. Measurement of Particle Size

A particle size analyzer (zetasizer nano ZS system, Malvern Instrument Ltd., UK) using a dynamic light scattering method to measure the particle size of swollen micelles was used. At this time, the temperature was kept constant at 25 ℃, and the sample was measured as a stock solution.

2.7. Statistical Analysis

Experiments were repeated 3 times and data were presented as means ± SD. Significance in difference was tested by Student's *t*-test. Differences were considered significant at $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$.

3. Results and Discussion

3.1. Solubilizer Selection for Solubilization of Tocopheryl Acetate

An experiment was performrd to select a surfactant that can transparently solubilize tocopheryl acetate, an oil-soluble substance, while containing salicylic acid and niacinamide. As

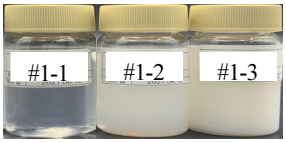

Figure 2. Results of composition experiment for the selection of surfactants in Table 3.

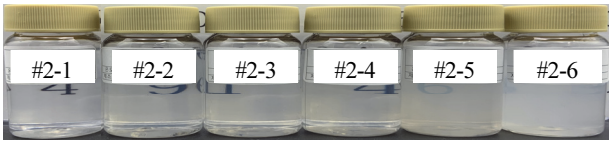

Figure 3. Results of composition experiment for turbidity test according to different polyols in Table 4.

shown in Figure 1, part A consists of tocopheryl acetate (2.0%) and polyol 1,2-hexanediol (4.5%) as surfactants and active ingredients, and part A' consists of salicylic acid (0.5%) and It was composed of ethanol (3.0%), and part B was composed of niacinamide (0.3%), sodium citrate (0.6%) and purified water so that the whole was 100%.

The surfactant is a combination of poloxamer 407, octyldodeceth-16, and isostearic acid (#1-1) at a total level of 5.0%, and PEG-60 hydrogenated castor oil (#1-2) and polysorbate 80 (#1-3) was tested in the same manner as in Figure 1 (Table 3). As a result of the experiment, #1-1 was transparent, but #1-2 and #1-3 were opaque white (Figure 2). From Figure 2 (Table 3), it was determined that poloxamer 407, octyldodeceth-16, and isostearic acid were suitable for preparing a swollen micelle solution in which tocopheryl acetate (2%) was solubilized.

Referring to Figure 2, when poloxamer 407 (2.0%), octyldodeceth-16 (2.0%), and isostearic acid (1.0%) as a co-surfactant were used as surfactants, the degree of solubilization according to the type of polyol was visually observed and turbidity measurement were performed.

As shown in Table 4, when 4.5% of each of 6 polyols (1,2-hexanediol, butylene glycol, dipropylene glycol, pentylene

Table 3. Composition Experiment for the Selection of Surfactants

Part	Ingredient	#1-1	#1-2	#1-3
A	Poloxamer 407	2.0	—	—
	Octyldodeceth-16	2.0	—	—
	Isostearic acid	1.0	—	—
	PEG-60 Hydrogenated castor oil	—	5.0	—
	Polysorbate 80	—	—	5.0

Table 4. Composition Experiment for Turbidity Test According to Different Polyols

Part	Ingredient	#2-1	#2-2	#2-3	#2-4	#2-5	#2-6
A	1,2-Hexanediol	4.5	—	—	—	—	—
	Butylene glycol	—	4.5	—	—	—	—
	Dipropylene glycol	—	—	4.5	—	—	—
	Pentylene glycol	—	—	—	4.5	—	—
	PEG-400	—	—	—	—	4.5	—
	Propanediol	—	—	—	—	—	4.5

Table 5. Results of Turbidity (NTU, mean ± SD) of Composition Experiment (Table 4) in 1 ~ 90 d at 25 °C

Time	#2-1	#2-2	#2-3	#2-4	#2-5	#2-6
1 d	9.11 ± 0.09	28.53 ± 0.09	28.90 ± 0.08	29.52 ± 0.06	207.67 ± 0.58	188.00 ± 0.00
15 d	16.67 ± 0.09***	31.65 ± 0.17***	33.89 ± 0.03***	52.33 ± 0.58***	213.33 ± 0.58***	178.00 ± 0.00**
30 d	23.86 ± 0.03***	32.34 ± 0.08***	37.41 ± 0.12***	72.00 ± 0.00***	225.00 ± 0.00***	173.00 ± 0.00
60 d	56.00 ± 0.00***	32.14 ± 0.25***	36.85 ± 4.19**	90.00 ± 0.00***	233.00 ± 0.00***	213.67 ± 2.08**
90 d	opaque	36.47 ± 0.16*	46.06 ± 0.09***	154.33 ± 0.58***	257.00 ± 0.00***	opaque

The results were expressed as the mean ± SD (N = 3), $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared with each 1 d data.

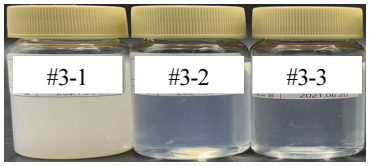

Figure 4. Results of composition experiment for turbidity test according to the ratio of surfactants in Table 6.

glycol, PEG-400, propanediol) was added, as in Figure 3, except for #2-5 (PEG-400) and #2-6 (propanediol), all other cases were relatively transparent.

However, as a result of measuring the turbidity at 25 ℃ (Table 5), it was shown that the turbidity gradually increased over time, although there was a difference in degree.

Through experiment for turbidity test according to different polyols, changes in turbidity were observed for various types of polyols. Based on this, when 1,2-hexanediol (4.5%) was added, an experiment was performed to confirm the ratio of isostearic acid (1.0%) and the surfactant poloxamer 407 and octyldodeceth-16 and the change in turbidity over time (Table 6).

When Poloxamer 407 and octyldodeceth-16 were added alone or mixed to 4.0%, it was opaque in #3-1 (Figure 4) using only poloxamer 407 alone. However when poloxamer 407 and octyldodeceth-16 were mixed, it was relatively transparent (Figure 4).

As a result of measuring the turbidity at 25 ℃ (Table 7), it was shown that the transparency gradually decreased over time, #3-3 showed relatively more solubilization stability.

In experiment of Table 6, poloxamer 407 alone or mixed with octyldodeceth-16 was compared to compare the turbidity according to the ratio. Additional experiments were performed to determine the change in turbidity over time when performed with isostearic acid (1.0%) and octyldodeceth-16 alone or in combination with poloxamer 407 (Table 8).

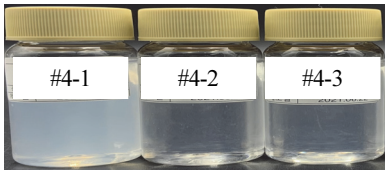

Figure 5-1. Results of composition experiment for turbidity test according to the ratio of surfactants in Table 8.

Figure 5-2. Results of composition experiment #4-2 (poloxamer 407 1.0% and octyldodeceth-16 3.0%) in Table 8 after 90 d.

As in Figure 4, the transparency was relatively higher when mixed with poloxamer 407 than when octyldodeceth-16 was used alone (Figure 5-1).

As a result of measuring the turbidity at 25 ℃ (Table 8), it was found that the transparency gradually decreased over time in the case of # 4-2, but it was confirmed that the transparency was almost maintained in # 4-3 (Figure 5-2, Table 9).

Table 6. Composition Experiment for Turbidity Test According to the Ratio of Surfactants

Part	Ingredient	#3-1	#3-2	#3-3
A	Poloxamer 407	4.0	3.0	2.0
	Octyldodeceth-16	—	1.0	2.0

Table 8. Composition Experiment for Turbidity Test According to the Ratio of Surfactants

Part	Ingredient	#4-1	#4-2	#4-3
A	Poloxamer 407	—	1.0	2.0
	Octyldodeceth-16	4.0	3.0	2.0

Table 7. Results of Turbidity (NTU, mean ± SD) of Composition Experiment (Table 6) in 1 ~ 90 d at 25 ℃

	1 d	15 d	30 d	60 d	90 d
#3-2	21.73 ± 0.00	246.00 ± 0.00	white opaque	white opaque	white opaque
#3-3	9.30 ± 0.00	11.83 ± 0.09***	18.74 ± 0.01***	64.00 ± 0.00	235.33 ± 0.58***

The results were expressed as the mean ± SD (N = 3), $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared with each 1 d data.

Table 9. Results of Turbidity (NTU, mean ± SD) of Composition Experiment (Table 8) in 1 ~ 90 d at 25 ℃

	1 d	15 d	30 d	60 d	90 d
#4-2	7.69 ± 0.36	9.20 ± 0.19**	6.35 ± 0.03*	7.23 ± 0.31**	6.82 ± 0.13**
#4-3	10.37 ± 0.04	11.30 ± 0.08**	13.26 ± 0.06***	31.31 ± 0.03***	97.67 ± 0.58***

The results were expressed as the mean ± SD (N = 3), $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared with each 1 d data.

Through the above experiment, it was confirmed that a specific ratio of poloxamer 407 and octyldodeceth-16 affects solubilization. Therefore, #4-2 in Table 8 was finally selected as the swollen micelle test group.

3.2. Control Group Selection

Table 10 shows the experimental composition for preparing a control group for the skin permeation test for test group #4-2. The control group is the same composition as the test group, and as shown in Figure 1, part A consists of a surfactant, tocopheryl acetate (2.0%) as an active ingredient, and 1,2-hexanediol (4.5%) as polyol, part A' consists of salicylic acid (0.5%) and ethanol (3.0%) for dissolving it, and part B consists of niacinamide (0.3%), sodium citrate (0.6%) and purified water containing 100% of the whole.

As shown in Table 3, PEG-60 hydrogenated castor oil and polysorbate 80, which are generally used as solubilizers in the manufacture of transparent toners, were used alone or mixed with the surfactant according to the process shown in Figure 1.

As a result of the experiment for preparation of control group (Table 10), all exhibited opaque properties (Figure 6).

In the case of #5-2 using only PEG-60 hydrogenated castor oil, transparency was relatively high when observed with the naked eye (Figure 6), so #5-2 was selected as a control group for test group #4-2.

Table 10. Composition Experiment for Preparation of Control Group

Part	Ingredient	#5-1	#5-2	#5-3
A	PEG-60 hydrogenated castor oil	3.0	5.0	—
	Polysorbate 80	2.0	—	5.0

3.3. Analysis of Active Ingredients for Preventing Hair Loss

The contents of tocopheryl acetate, salicylic acid and niacinamide contained in swollen micelle test group #4-2 (Table 8) were quantitatively analyzed by HPLC according to the conditions shown in Table 2. The following is the HPLC

Figure 6. Results of composition experiment for preparation of control group in Table 10.

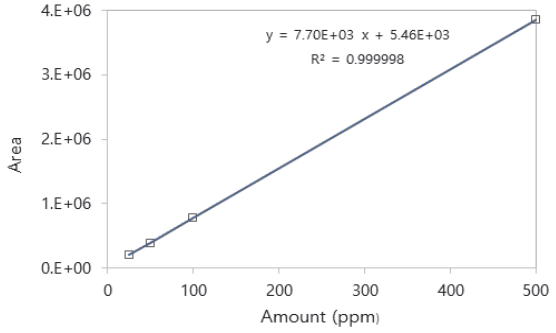

Figure 7-1. HPLC calibration curve of tocopheryl acetate standard solution.

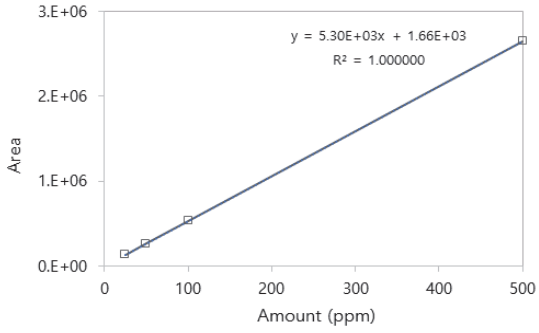

Figure 7-2. HPLC calibration curve of salicylic acid standard solution.

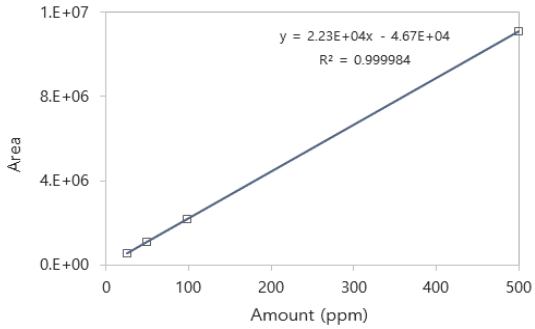

Figure 7-3. HPLC calibration curve of niacinamide standard solution.

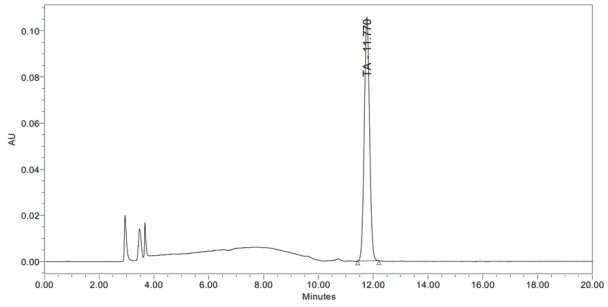

Figure 8-1. HPLC chromatogram for content analysis of tocopheryl acetate in test group.

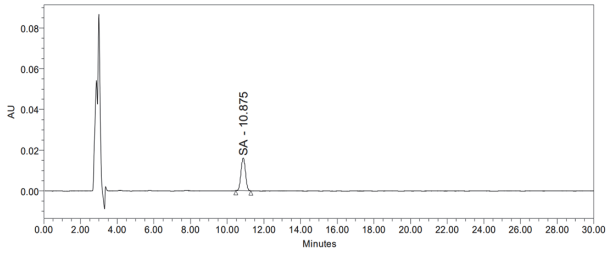

Figure 8-2. HPLC chromatogram for content analysis of salicylic acid in test group.

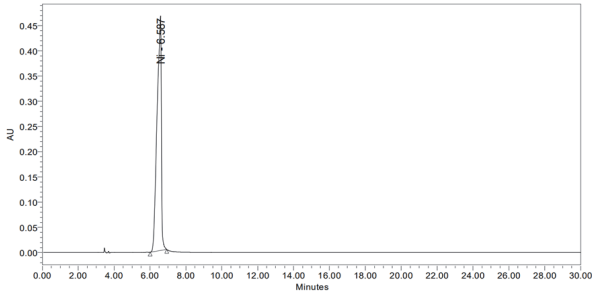

Figure 8-3. HPLC chromatogram for content analysis of niacinamide in test group.

calibration curve analyzed in advance to obtain the reference concentration of the active ingredients (Figure 7).

Using this, HPLC chromatograms were obtained to obtain the concentrations of three active ingredients (Figure 8).

The retention times of tocopheryl acetate, salicylic acid, and niacinamide were about 12, 11, and 7 minutes, respectively. Considering the purity of the reagent, 1.90% for tocopheryl acetate (2.0%), 0.50% for salicylic acid (0.5%), and 0.35% for niacinamide (0.3%). Therefore, it was confirmed that all three types of active ingredients in test group #4-2 were contained at least 95%.

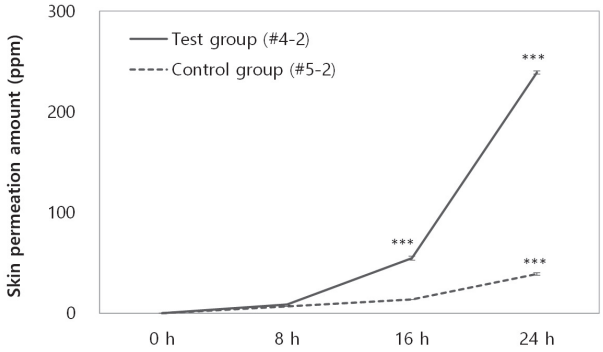

Figure 9-1. Cumulative skin permeation amount of tocopheryl acetate in accordance with time by Franz diffusion cell test in test group #4-2 and control group #5-2; the results were expressed as the mean ± SD, $^{*}p < 0.05$, $^{***}p < 0.001$ compared with each 8 h data.

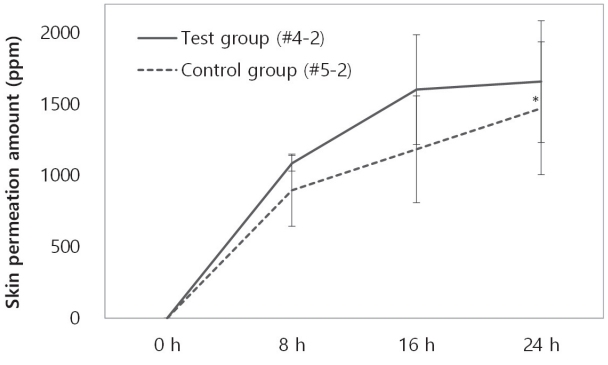

Figure 9-2. Cumulative skin permeation amount of salicylic acid in accordance with time by Franz diffusion cell test in test group #4-2 and control group #5-2; the results were expressed as the mean ± SD, $^{*}p < 0.05$, $^{***}p < 0.001$ compared with each 8 h data.

3.4. *In Vitro* Skin Permeation Test

To investigate the skin permeation rates of tocopheryl acetate and salicylic acid contained in test group #4-2 (Table 8) and control group #5-2 (Table 10), a skin permeation test using the Franz diffusion cell method was performed. Figure 9 and Table 11 show the results of skin permeation analysis of tocopheryl acetate and salicylic acid of #4-2 after 8, 12, and 24 h. At this time, the initial dose of tocopheryl acetate is 7,600 ppm and salicylic acid is 2,000 ppm. In the case of tocopheryl acetate, it was found that there was statistical significance at each time between test group and control group ($^{**}p < 0.01$ at 8 h, $^{***}p < 0.001$ at 16 h and 24 h), but in the case of salicylic acid, it was found that there was no statistical significance.

The transparently solubilized active ingredient of test group #4-2 showed high skin permeation even in a micelle solution (control group, #5-2) prepared with a common solubilizer[28], PEG-60 hydrogenated castor oil. Tocopheryl acetate located inside the micelle of the swollen micelle appears to be a sufficiently predictable result in a formulation in which oil-soluble components are transparently solubilized. Instead, salicylic acid located in the outer phase of the swollen micelle also showed good skin permeation in the transparent solubilized formulation. This is thought to be the result of salicylic acid being mostly present in the outer phase, but some adsorbed to the micelle interface with small particle size.

The content of tocopheryl acetate, an oil-soluble active ingredient, adsorbed to the membrane was higher than the content penetrated into the receptor phase. On the other hand, the amount of salicylic acid adsorbed to the membrane was analyzed to be less than that of penetration.

3.5. Measurement of Particle Size

The particles of the test group and control used in skin permeation test were measured with a zetasizer nano ZS. At this time, samples without tocopheryl acetate were prepared in the test group and the control group, respectively, and used as positive control groups.

The measurement results are shown in Table 12. The particle size of the test group was 21.85 nm, and the positive control group (without TA) was 9.90 nm.

The particle size of the control group was 133.77 nm, and the positive control group (without TA) was 15.55 nm.

Although it is difficult to clearly distinguish the boundary between micelles and swollen micelles, micelles are surfactant aggregates capable of sufficiently solubilizing fragrance ingredients and have a size of about 20 nm or less[17].

On the other hand, swollen micelles can be prepared by a simple manufacturing process like micelles, and can be prepared into swollen micelles to support oil-soluble components such as tocopheryl acetate in micelle cores. Formulations containing a higher amount of oil-soluble active substances than in micelles

Table 11. Cumulative Skin Permeation Amounts of Tocopheryl Acetate and Salicylic Acid at Time

Time	Tocopheryl acetate (ppm)		Salicylic acid (ppm)	
	Test group (#4-2)	Control group (#5-2)	Test group (#4-2)	Control group (#5-2)
8 h	8.61 ± 0.44	6.84 ± 0.00	1,085.80 ± 54.59	896.07 ± 253.07
16 h	54.72 ± 2.01***	13.68 ± 0.00	1,601.80 ± 383.63	1,183.47 ± 373.68
24 h	239.15 ± 1.58***	39.01 ± 1.16***	1,658.00 ± 426.59	1,471.00 ± 464.86*

The results were expressed as the mean ± SD (N = 3), $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared with each 8 h data.

Table 12. Comparison of Particle Size Between Test Group and Control Group According to the Presence or Absence of Tocopheryl Acetate (TA)

Test group (#4-2)		Control group (#5-2)	
With TA	Without TA	With TA	Without TA
22.85 ± 0.27 nm	9.90 ± 0.02 nm	133.77 ± 0.32 nm	15.55 ± 0.12 nm

while maintaining a nano-sized particle size can be viewed as swollen (expanded) micelles [15,21].

As such, the degree of skin absorption of oil-soluble active ingredients is greatly influenced by the size of the particles [3,6].

In addition, the factors affecting the particles are considered to be influenced by the type of surfactant[13], the concentration of surfactant, the ratio of selected surfactants, and the interfacial adsorption of co-SAA[15].

4. Conclusion

Through this study, transparently solubilized swollen micelles containing tocopheryl acetate (2.0%), salicylic acid (0.5%) and niacinamide (0.3%), which are active ingredients for preventing hair loss, were prepared.

The turbidity and pH of the swollen micelle test group prepared by applying poloxamer 407 (1.0%), octyldodeceth-16 (3.0%) as a surfactant (solubilizer), and isostearic acid (1.0%) as a co-surfactant, respectively, were 6.82 ± 0.13 NTU, 4.62 ± 0.06, it was confirmed that stability is maintained for 90 d at room temperature (25℃).

As a result of comparing the skin permeation rate of the test group with the control group prepared with PEG-60 hydrogenated castor oil, which is generally used as a solubilizer in solubilizing formulations. It was confirmed that tocopheryl acetate and salicylic acid cumulatively increased the amount of skin permeation measured after 8 h, 12 h, and 24 h.

The active ingredient, tocopheryl acetate, is located inside the micelle as an oil-soluble component, and the water-soluble niacinamide component is located in the outer phase.

The relatively low solubility of salicylic acid appears to exist in the outer phase mostly by dissolving it in ethanol. However, it is thought that some of it was also adsorbed to the micelle interface in the measurement of skin permeation over time.

In particular, the tocopheryl acetate component has a large molecular weight, and as an oil-soluble component, it is not easy to solubilize into a transparent formulation, and it has a low skin permeation rate. In this study, swollen micelles in a transparent formulation containing tocopheryl acetate (2.0%) were prepared, and the final test group showed stability for 90 d, and it was confirmed that it could relatively improve skin permeation.

According to the results of this study, it is expected that the optimized swollen micelles with a small amount and ratio of surfactants (for example, test group) will be applied to solubilization formulations for hair loss prevention products or various functional cosmetics.

Acknowledgement

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References

1. J. A. Bouwstra and P. L. Honeywell-Nguyen, Skin structure and mode of action of vesicles, *Adv. Drug Deliv. Rev.*, **54**(1), 41 (2002).
2. K. M. Gopinathan, W. C. Gary, and E. L. Majella, The structure and function of the stratum corneum, *Int. J. Pham.*, **435**(1), 3 (2012).
3. X. Wu and R. H. Guy, Applications of nanoparticles in topical drug delivery and in cosmetics, *J. Drug Deliv. Sci. Techno.*, **19**(6), 371 (2009).
4. K. Sakamoto, R. Y. Lochhead, H. I. Maibach, and Y. Yamashita, Cosmetic science and technology: theoretical principles and applications, *Elsevier Inc.*, Chapter 46, 741 (2017).
5. L. M. Ickenstein and P. Garidel, Lipid-based nanoparticle formulations for small molecules and RNA drugs, *Expert Opin. Drug Deliv.*, **16**(11), 1205 (2019).
6. G. E. Flatena, Z. Palacb, A. Engeslanda, J. Filipovic-Grcicb, Z. Vanićb, and N. Skalko-Basneta, *In vitro* skin models as a tool in optimization of drug formulation, *Eur. J. Pharm. Sci.*, **75**, 10 (2015).
7. D. T. Rani, Liposome as a potential drug delivery system: a review, *Int. Res. J. Pharm.*, **4**(1), 6 (2013).
8. N. Akhtar, A. Varma, and K. Pathak, Ethosomes as vesicles for effective transdermal delivery: from bench to clinical implementation, *Curr. Clin. Pharmacol.*, **11**(3), 168 (2016).

9. J. R. Walve, S. R. Bakliwal, B. R. Rane, and S. P. Pawar, Transferosomes: a Surrogated carrier for transdermal drug delivery system, *Int. J. Appl. Biol. Pharm.*, **2**(1), 204 (2011).
10. R. Khan and R. Irchhaiya, Niosomes: a potential tool for novel drug delivery, *J. Pharm. Investig.*, **46**, 195 (2016).
11. M. N. Yukuyama, D. D. M. Ghisleni, T. J. A. Pinto, and N. A. Bou-Chacra, Nanoemulsion: process selection and application in cosmetics - a review, *Int. J. Cosmet. Sci.*, **38**(1), 13 (2016).
12. W. Y. Jeong, M. Kwon, H. E. Choi, and K. S. Kim, Recent advances in transdermal drug delivery systems: a review, *Biomater. Res.*, **25**(24), 1 (2021).
13. Z. Ahmad, A. Shah, M. Siddiq, and H. B. Kraatz, Polymeric micelles as drug delivery vehicles, *R. Soc. Chem. Adv.*, **4**, 17028 (2014).
14. W. G. Cho, Nanoemulsions: a novel vehicle for cosmetics, *J. Soc. Cosmet. Sci. Korea*, **37**(1), 1 (2011).
15. S. K. Swafford, W. R. Bergmann, K. G. Migliorese, J. L. Lichtin, and A. Sark, Characterization of swollen micelies containing linoleic acid in a microemulsion system, *J. Soc. Cosmet. Chem.*, **42**(4), 235 (1991).
16. E. Ruckenstein and R. Krishnan, Swollen micellar models for solubilization, *J. Colloid Interface Sci.,* **71**(2), 321 (1979).
17. S. J. Kim, Y. L. Jeong, J. J. Nam, J. H. Jang, H. L. Yeo, M. S. Yoon, K. J. Yoo, and J. B. Lee, A study of stabilization for insoluble active ingredients using swollen micelles, *J. Soc. Cosmet. Sci. Korea*, **42**(1), 9 (2016).
18. S. C. Owen, D. P. Y. Chan, and M. S. Shoichet, Polymeric micelle stability, *Nano Today*, **7**(1), 53 (2012).
19. N. H. Hoang, C. Lim, T. Sim, and K. T. Oh, Triblock copolymers for nano-sized drug delivery systems, *J. Pharm. Investig.*, **47**, 27 (2017).
20. G. S. Kwon and M. L. Forrest, Amphiphilic block copolymer micelles for nanoscale drug delivery, *Drug Dev. Res.*, **67**(1), 15 (2006).
21. M. S. Kim and K. S. Yoon, Stabilization of tocopheryl acetate of swollen micelle by poloxamer, *J. Appl. Sci. Technol.*, **36**(2), 609 (2019).
22. L. A. Beoy, W. J. Woei, and Y. K. Hay, Effects of tocotrienol supplementation on hair growth in human volunteers, *Trop. Life Sci. Res.*, **21**(2), 91 (2010).
23. H.-T. Kim, H.-S. Park, Y.-M. Kim, I.-C. Lee, S.-J. Lee, and J.-S. Choi, Double-blind randomized placebo-controlled study of the efficacy and safety of hair loss prevention shampoo containing salicylic acid, panthenol, and niacinamide in alopecia patients, *Toxicol. Environ. Health Sci.*, **4**, 1 (2022).
24. Y.-H. Choi, J. Y. Shin, J. Kim, and S. Lee, Niacinamide down-regulates the expression of DKK-1 and protects cells from oxidative stress in cultured human dermal papilla cells, *Clin. Cosmet. Investig. Dermatol.*, **14**, 1519 (2021).
25. P. Dykes, Surfactants and the skin, *Int. J. Cosmet. Sci.*, **20**(1), 53 (1998).
26. Strat-M membrane brochure, Experience the unmatched predictability of Strat-M membrane, EMD Millipore Corporation, USA (2012).
27. F. J. Arce, N. Asano, G. L. See, S. Itakura, H. Todo, and K. Sugibayashi, Usefulness of artificial membrane, Strat-M, in the assessment of drug permeation from complex vehicles in finite dose conditions, *Pharmaceutics*, **12**(173), 1 (2020).
28. A. Hisatomi, M. Kimura, M. Maeda, M. Matsumoto, K. Ohara, and H. Noguchi, Toxity of polyoxyethylene hydrogenated castor oil 60 (HCO-60) in experimental animals, *Toxicol. Sci.*, **18**, 1 (1993).