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Synthesis and characterization of ethosomal carriers containing cosmetic ingredients for enhanced transdermal delivery of cosmetic ingredients

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Abstract−The transdermal delivery of cosmetic ingredients is often resisted by the outer layer of the skin, which can prevent diffusion of cosmetic ingredients through the skin. Ethosomes, lipid carriers composed mainly of phospholipids and ethanol, are one of the methods used to overcome the limitation of the skin barrier for cosmetic ingredients. We prepared ethosomes containing Rhodamine B (Rh-B) and investigated the correlation between the synthesis conditions of ethosomes and ethosome size and entrapment efficiency of Rh-B within the ethosome. Based on the correlation results, we examined the effect of ethosome size and entrapment efficiency of Rh-B within the ethosome on skin permeation of Rh-B. The skin permeability of Rh-B increased as ethosome size decreased and entrapment efficiency of Rh-B increased. Finally, the Rh-B or niacinamide loaded within ethosomes showed significantly higher skin permeation. The results indicated that using ethosomes can improve the skin permeability of niacinamide, and the same effect can be expected for other cosmetic ingredients.

Keywords: Ethosomes, Cosmetic Ingredients, Skin Permeation, Niacinamidem, Transdermal Delivery

INTRODUCTION

There have been many reports mentioning that niacinamide, known as vitamin B_3, may have various beneficial effects on the skin, including anti-aging effects, anti-inflammatory effects in acne, suppression of melanosome transfer leading to the reduction of cutaneous pigmentation, reduction of transepidermal water loss, and prevention of photocarcinogenesis. In addition, it increases the synthesis of stratum corneum lipids and ceramides with enhanced epidermal permeability barrier function [1-4]. Therefore, niacinamide has been introduced into a number of cosmetics to improve not only skin appearance but also skin health. However, the traditional methods for administering cosmetic ingredients to the skin, such as creams and patches, restrict the transfer of ingredients that can passively cross the skin. The skin is the largest organ in the body, approximately 1.5 mm in thickness and has as a primary function to keep water inside the body and prevent foreign substances from entering the body from the environment [5-8].

Thus, various technological advances have been developed to overcome the inefficient skin penetration of cosmetic ingredients. One such technique is the use of liposomes as a carrier to penetrate through the skin barrier layer. Liposomes are small vesicles composed of a lipid bilayer envelope surrounding a central aqueous core and were reported as the first topical lipid vesicular system for enhanced drug delivery to the skin in the early 1980s. Since then, many studies have shown that they are able to increase the accumulation of various molecules in the stratum corneum, the upper skin layer or the outermost layer of the skin [9-13]. Ethosomes, lipid carriers composed mainly of phospholipids and ethanol, are interesting vesicular systems that have used in drug delivery and pharmaceutical technology in recent years [14-18]. They are similar in physical structure and form to liposomes; however, they have some advantages in comparison to classical liposomes. Ethosomes are easy to prepare, have shown high encapsulation efficiency and transdermal flux for a wide range of molecules, can permeate through the skin layers more rapidly and deeply, and are small relative to liposomes, when both are prepared by methods not involving any size reduction steps such as sonication and extrusion [19-22]. Liposomes, including ethosomes, have been used to improve skin permeation, but their activity as penetration enhancer is not completely elucidated. Some authors suggest that liposomes are able to pass though the intercellular route of the stratum corneum, while other papers indicate that phospholipids, a main component of liposomes, would either supplement the lipid content of the skin or influence the structure and organization of the lipid matrix of the skin, facilitating the absorption of active compounds [23,24].

The goal of this study was to investigate the effect of ethosome size and entrapment efficiency of ingredients loaded within ethosomes on skin permeation of ingredients for enhancing the skin permeation of cosmetic ingredients using ethosomes. At first, using Rhodamine B (Rh-B), we determined the effect of the synthesis conditions of the ethosomes on ethosome size and Rh-B entrapment efficiency and then investigated the effect of ethosome size and Rh-B entrapment efficiency on skin permeation of Rh-B to determine the optimal ethosome size and encapsulation efficiency of ingredients within the ethosome for the highest skin permeation. Finally, we looked at in vitro skin permeation of Rh-B or niacina-

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mide with and without ethosomes.

EXPERIMENTAL

1. Materials

Rhodamine-B (Rh-B), ethanol, Triton X-100, phosphate buffer saline (PBS), and niacinamide were obtained from Sigma-Aldrich. Lecithin was purchased from Lucas Meyer.

2. Preparation of Ethosomes Containing Rh-B or Niacinamide

Ethosomes containing Rh-B were prepared based on the method reported by Touitou [17]. Briefly, 1 g of lecithin was dissolved in 1 ml of ethanol at 60 °C, and then 1 ml of Rh-B solution (50 mg/ml) was added to the lecithin-dissolved ethanol. This solution was stirred with a magnetic bar at 600 rpm for 10 min, and then 20 ml of PBS was added slowly to the solution using a syringe pump at a flow rate of 1 ml/min while being agitated with a magnetic stirrer. At this step, ethosomal vesicles formed and were suspended in PBS. While the temperature was down to room temperature, stirring of this suspension was continued for an additional 10 minutes. The prepared Rh-B-loaded ethosomes were washed using PBS and centrifuged at least three times. To prepare niacinamide-loaded ethosomes, the same procedure was used except 1 ml of niacinamide solution (1 mg/ml) was added instead of 1 ml of Rh-B solution.

3. Characterization of Ethosome Size and Entrapment Efficiency

Size and size distribution of the prepared ethosomes were determined by dynamic light scattering (DLS, Nano ZS, Malvern). The entrapment efficiency of Rh-B or niacinamide within the ethosomes was defined as the ratio of the concentration of Rh-B or niacinamide within the ethosomes to the initial concentration of Rh-B or niacinamide solution. To measure the concentration of ingredients within the ethosomes, the ethosomes were destroyed with Triton X-100, which is used to disrupt the lipid bilayer [25, 26]. The concentrations of Rh-B and niacinamide were measured using a UV-Visible spectrophotometer (Cary 100, Varian) at 553 nm and HPLC (1260 Infinity LC, Agilent) at 263 nm, respectively. Agilent Zorbax Eclipse XDB (C18, 5 μm, 4.6×250 mm) column and a mobile phase of 75% of 0.05M KH_2PO_4 solution and 25% methanol were used to operate the HPLC.

4. *In Vitro* Skin Permeation Analysis

An *in vitro* skin permeation experiment with Rh-B or niacinamide was performed using Franz diffusion cells (FCDV-15, Labfine) with porcine skin (Medikinetics) having an area of 2×2 cm^2 and a thickness of 1.7-2.0 mm. A diffusion cell was composed of a donor section and a receptor section with the skin positioned between the sections. The receptor was filled with 5 ml of PBS, and the absorption surface area of the porcine skin was 0.785 cm^2. The ingredient-loaded ethosome suspension was applied to the surface of the porcine skin which was treated with the receptor fluid of PBS. The receptor fluid was kept in contact with the underside of the skin until the end of the experiment. The diffusion cell and skin were maintained at a constant temperature of 36 °C. The receptor fluid was agitated continuously with a magnetic stirrer at 500 rpm. After 24 hours, the sample was withdrawn from the receptor and the Rh-B or niacinamide concentration was measured using a UV-Visible spectrophotometer and HPLC, respectively. For control experiments, the same amount of Rh-B or niacinamide that was encapsulated in the ethosomes was applied directly on the skin in the diffusion cell and the transferred concentration of Rh-B or niacinamide was measured using the same methods.

RESULTS AND DISCUSSION

1. Synthesis and Characterization of Rh-B-loaded Ethosomes

We assumed that as ethosome size became smaller and entrapment efficiency of the ingredients within the ethosomes became higher, then skin permeability of the ingredients would be higher. So we investigated synthesis conditions where the ingredient-loaded ethosomes having the smallest size and the highest entrapment efficiency of ingredients could be produced using Rh-B. Ethosomal vesicles containing Rh-B were produced by dissolution of lecithin in ethanol, followed by the slow addition of a Rh-B solution and PBS under continuous stirring. Initially, before adding PBS, the solution was transparent, since the high ethanol concentration was able to make the lecithin soluble. However, by adding PBS, the solution became turbid because of the formation of ethosomal vesicles by the reorganization of lipid within the system. Fig. 1 shows optical and fluorescent images of ethosomes containing Rh-

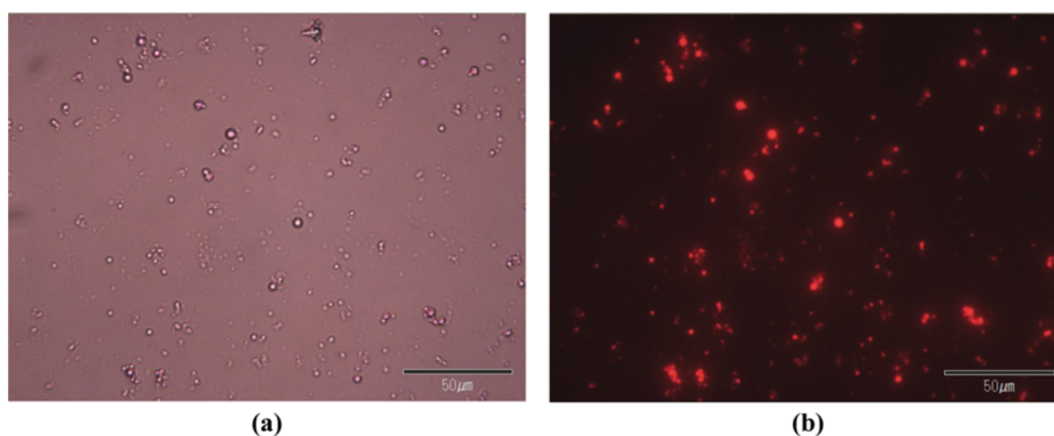

Fig. 1. Optical (a) and fluorescent (b) images of Rh-B-loaded ethosomes.

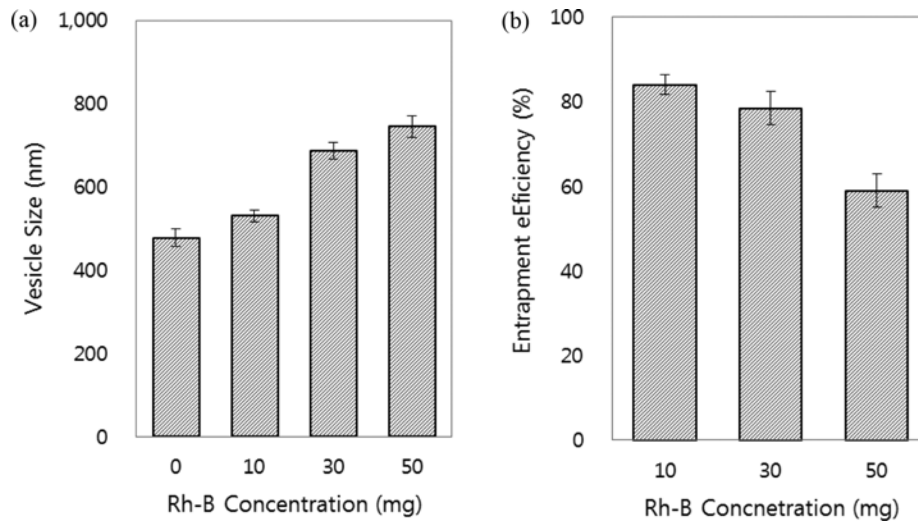

Fig. 2. Effect of initial Rh-B concentration on size of Rh-B-loaded ethosomes (a) and entrapment efficiency of Rh-B within ethosomes (b) (n=3-5).

B. Spherical-shaped particles were observed and the particles on the fluorescent image (Fig. 1(b)) emitted a strong red color, indicating that the Rh-B was successfully incorporated within the ethosomes and the fluorescence property was maintained after encapsulation. Fig. 2 presents the effect of initial Rh-B concentration on ethosome size and entrapment efficiency of Rh-B within the ethosomes. The size of Rh-B-loaded ethosomes increased while the entrapment efficiency decreased, as the initial Rh-B concentration increased. The ethosome size without Rh-B was 477.3 (±21.5) nm, whereas the size of Rh-B-loaded ethosomes was larger than that of ethosomes without Rh-B. When the lecithin, which is a phospholipid, is suspended in an aqueous phase, it spontaneously forms spherical vesicles composed of a lipid bilayer envelope, surrounding a center aqueous core (Fig. 3(a)). Thus, Rh-B mainly existed in the core of ethosomes as it was dissolved in the aqueous phase. However, some Rh-B would be incorporated in the lipid bilayer of the ethosome because of its hydrophobicity coming from the aromatic rings in its structure (Fig. 3(b)). For this reason, as the initial Rh-B concentration increased, more Rh-B could be incorporated in the lipid bilayer, which would make the final formation of the ethosome lipid bilayer interrupted, leading to loose and large ethosome vesicles. Fig. 4 shows the effect of initial ethanol content on the size of Rh-B-loaded ethosomes and the entrapment efficiency of Rh-B within the ethosomes. As the initial ethanol content increased, the ethosome size increased, while the entrapment efficiency decreased. A similar explanation as previously mentioned can be applied to the results shown in Fig. 4. When the initial ethanol content increased, the ethosomes formed looser lipid bilayers due to more ethanol incorporated in the lipid bilayer of the ethosomes, so that the size of the final ethosomes increased and the entrapment efficiency of Rh-B within the ethosomes decreased. The effect of stirring speed on the size of Rh-B-loaded ethosomes and the entrapment efficiency of Rh-B within the ethosomes is shown in Fig. 5. As previously mentioned, when the lecithin was suspended in an aqueous phase, spherical vesicles were obtained and the vesicle size was affected by the applied shear force resulting from the stirring. As shown in Fig. 5, the size of Rh-B-loaded ethosomes decreased but the Rh-B entrapment efficiency increased with the stirring speed. This was because the faster stirring speed led to stronger shear force and produced smaller vesicles.

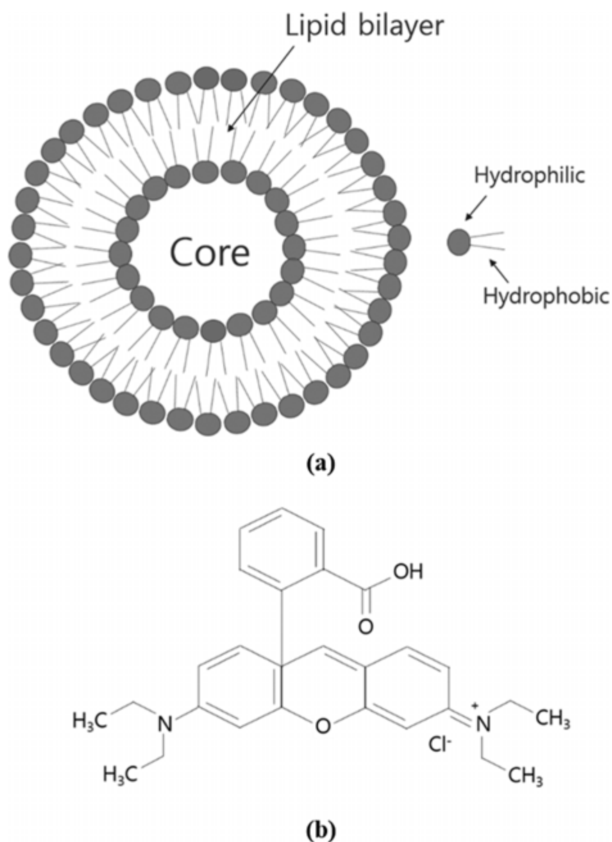

Fig. 3. Schematic diagram of ethosome (a) and structure of Rhodamine B (b).

2. *In Vitro* Skin Permeation of Rh-B-loaded Ethosomes

To evaluate the influence of ethosome size and entrapment effi-

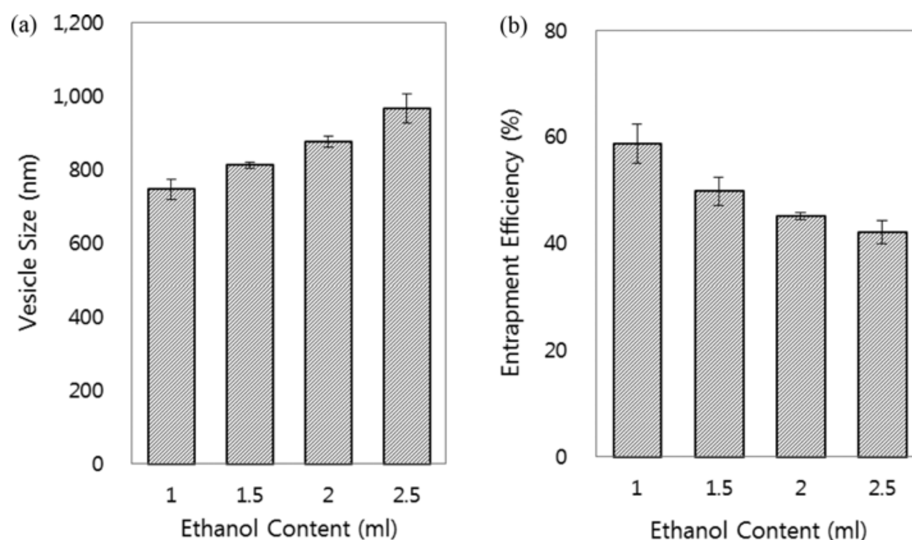

Fig. 4. Effect of initial ethanol content on size of Rh-B-loaded ethosomes (a) and entrapment efficiency of Rh-B within ethosomes (b) (n=3-5).

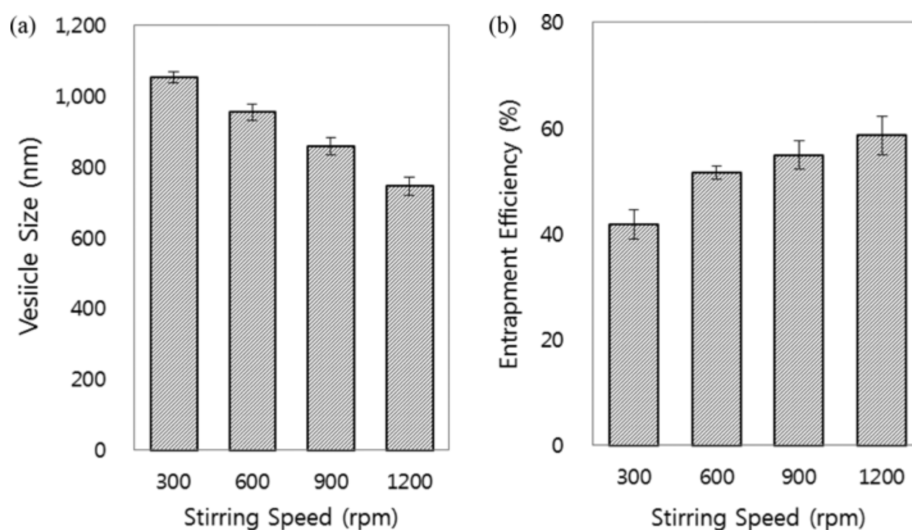

Fig. 5. Effect of stirring speed on size of Rh-B-loaded ethosomes (a) and entrapment efficiency of Rh-B within ethosomes (b) (n=3-5).

Table 1. Ethosome size and Rh-B entrapment efficiency of Rh-B-loaded ethosomes (n=3-5)

Samples	P1	P2	P3	E1	E2	E3
Ethosome size (nm)	442.9 (±6.7)	717.5 (±5.5)	956.1 (±9.1)	836.0 (±3.3)	831.1 (±5.4)	834.9 (±2.7)
Rh-B entrapment efficiency (%)	52.9 (±0.6)	52.7 (±1.1)	53.1 (±0.9)	45.3 (±1.2)	56.5 (±3.7)	69.6 (±3.4)

ciency of ingredients loaded within ethosomes on skin permeation of ingredients, *in vitro* skin permeation tests were conducted using Rh-B-loaded ethosomes. Since we wanted to see the effect of ethosome size and entrapment efficiency on skin permeation separately, we prepared two types of samples: one sample type had the same entrapment efficiency and different sizes (P1, P2, and P3) and the other sample type had the same size and different entrapment efficiencies (E1, E2, and E3). The ethosome size and entrapment efficiency of Rh-B are summarized in Table 1. Fig. 6 presents the effect of ethosome size and entrapment efficiency of Rh-B within the ethosomes on the skin permeability of Rh-B. As we assumed, the skin permeability of Rh-B increased as the size decreased and the entrapment efficiency increased. This was because the smaller ethosomes could better penetrate through the lamellae structure in the stratum corneum, and the ethosomes having more Rh-B could deliver more Rh-B.

3. *In Vitro* Skin Permeation of Niacinamide-loaded Ethosomes

Since we verified our assumption that ethosomes having smaller size and higher entrapment efficiency of ingredients showed higher skin permeability of ingredients, we prepared ethosomes containing Rh-B or niacinamide using synthesis conditions where ethosomes having the smallest size and highest entrapment efficiency

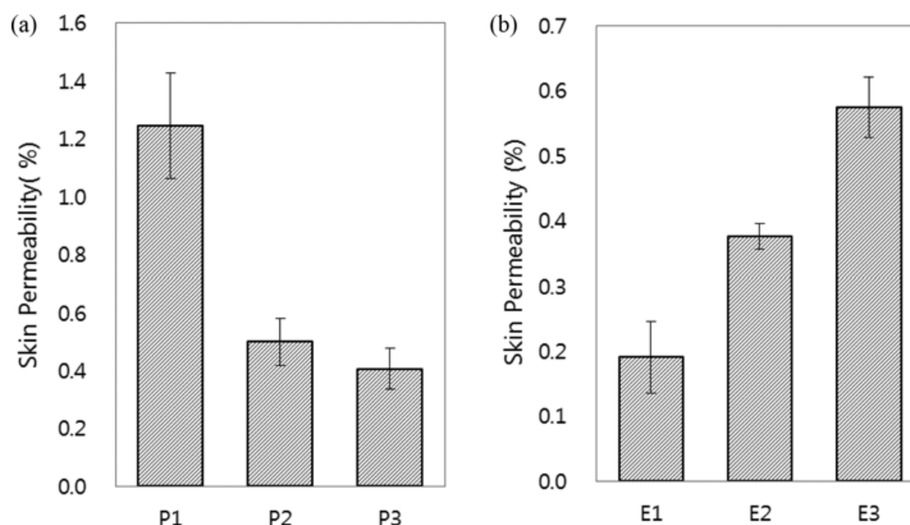

Fig. 6. Effect of size of Rh-B-loaded ethosomes (a) and entrapment efficiency of Rh-B within ethosomes (b) on the skin permeability of Rh-B. The sizes of P1, P2, and P3 are 442.9, 717.5, and 956.1 nm, respectively. The entrapment efficiencies of Rh-B of E1, E2, and E3 are 45.3, 56.5, and 69.6%, respectively (n=3-5).

could be produced, and then carried out *in vitro* skin permeation experiments. The ethosome size and entrapment efficiency of Rh-B or niacinamide used in the skin permeation experiments are listed in Table 2. The ethosome suspensions containing Rh-B or niacinamide were placed on the porcine skin in the diffusion cell to determine the amount of Rh-B or niacinamide transported through the skin. For control experiments (skin permeability of Rh-B or niacinamide without ethosomes), Rh-B or niacinamide aqueous solutions having the same amount of Rh-B or niacinamide were placed on the surface of the skin without ethosomes. Fig. 7 shows the permeation of Rh-B or niacinamide through porcine skin with and without ethosomes. When the Rh-B or niacinamide was loaded within ethosomes, significantly higher skin permeability was observed. The results indicated that using ethosomes can improve the skin permeability of niacinamide, and we can expect the same effect for other cosmetic ingredients. Based on the correlation obtained in this study, using synthesis conditions, ethosome size and entrapment efficiency of ingredients within ethosomes, we can control the skin permeation of cosmetic ingredients.

Table 2. Ethosome size and entrapment efficiency of ethosomes containing Rh-B or niacinamide (n=3-5)

	Rh-B-loaded ethosomes	Niacinamide-loaded ethosomes
Ethosome size (nm)	530.1 (±13.5)	616.0 (±27.0)
Entrapment efficiency (%)	84.0 (±2.3)	10.8 (±0.4)

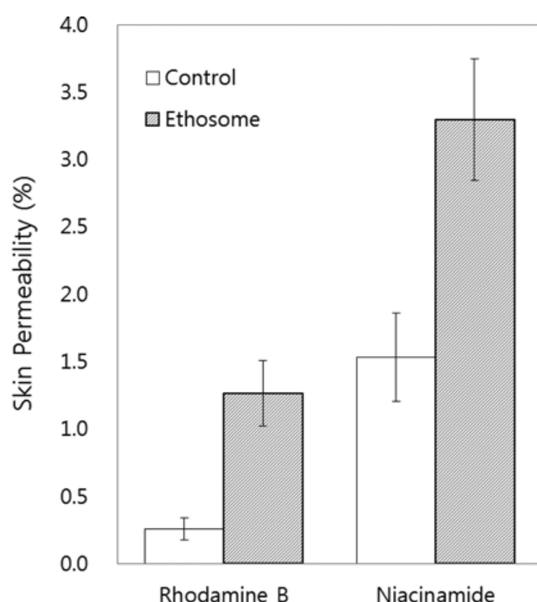

Fig. 7. Skin permeability of Rh-B and niacinamide through porcine skin with and without the use of ethosomes (n=3-5).

CONCLUSIONS

Ethosomes containing Rh-B were prepared and the correlation between synthesis conditions of ethosomes, ethosome size and entrapment efficiency of Rh-B within the ethosome was determined. Based on the correlation, we investigated the effect of ethosome size and entrapment efficiency of Rh-B within ethosomes on the skin permeation of Rh-B. The skin permeability of Rh-B increased as ethosome size decreased and entrapment efficiency of Rh-B increased. When Rh-B or niacinamide were loaded within ethosomes, significantly higher skin permeability was observed. From these results, we can conclude that using ethosomes can improve the skin permeability of niacinamide and the same effect can be expected for other cosmetic ingredients. In addition, we can control the transport of cosmetic ingredients through the skin using the correlations obtained in this study.

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