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화장품 효능성분 피부흡수 증진을 위한 지질나노베지클 연구

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A Study on Lipid Nanovesicles for Enhanced Skin Absorption of Cosmetic Active Ingredients

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요 약: 본 연구에서는 레시틴, 지방산(palmitic acid) 및 계면활성제(polysorbate 60)를 변화시켜 니아신아마이드를 함유한 지질나노베지클(lipid nanovesicles, LNV)을 제조하여 물리화학적 특성 분석과 피부투과성에 대한 평가를 수행하였다. 제조된 LNV는 레시틴과 polysorbate 60의 함량에 따라 평균직경 77 ~ 160 nm, 제타 전위 −63 ~ −31 mV로 측정되었다. 12 주 간의 경시 안정도 관찰 결과, LNV1이 가장 우수한 안정도를 보여주었다. 또한 이렇게 만들어진 LNV에 대해 프란츠셀을 이용하여 *in vitro* 피부 흡수를 평가하였다. 이 때 모델 약물은 니아신아마이드가 선정되었다. 피부 흡수 평가 결과, 지방산 함유 LNV1의 피부 흡수가 대조군 대비 우수한 것을 확인할 수 있었다. 이러한 결과로부터 레시틴, 지방산 및 계면활성제가 함유된 LNV는 화장품의 효능 성분 피부 흡수에 도움을 줄 수 있음을 확인하였다.

Abstract: In this work, lipid nanovesicles (LNV) containing niacinamide were prepared by changing the concentration of lecithin, fatty acids (palmitic acid), and surfactant (polysorbate 60), and physicochemical characteristics analysis and skin permeability evaluation were performed. The present LNVs were measured with an average diameter of 77 ~ 160 nm, zeta potential -63 ~ -31 mV depending on the concentration of lecithin and polysorbate 60. As a result of observing the stability over time for 12 weeks, LNV1 showed much better colloidal stability than others. In addition, *in vitro* skin penetration using Franz cell was evaluated for LNV. In this work, niacinamide was selected as the model drug. As a result of skin penetration, it was confirmed that the skin penetration of fatty acid containing LNV1 was much superior than control. These results confirm that LNVs containing lecithin, fatty acids, and surfactants can support the skin absorption of active ingredients in cosmetics.

Keywords: *lipid nanovesicle, lecithin, palmitic acid, polysorbate 60*

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1. Introduction

Nanotechnology possesses the capability to bring about progress and breakthroughs in formulations and delivery systems, presenting innovative solutions to challenges in the medical and pharmaceutical sectors. In the realm of cosmetics, the integration of nanotechnology is a relatively recent but immensely promising and extensively studied field[1-4]. This cutting-edge approach offers various advantages, including enhanced control and targeted delivery, improved texture and transparency, increased penetration, superior adhesion, and heightened stability and efficacy of formulations[5-7]. The multitude of benefits stems from the smaller size characteristic of nanotechnology, enabling the acquisition of new properties such as improved solubility, transparency, chemical reactivity, and stability.

In contrast to other macromolecules, lipid-based nanovesicles have demonstrated themselves as outstanding vesicles and delivery systems. They showcase remarkable attributes, including low toxicity, cost-effective scale-up production, strong biocompatibility, and high efficiency in loading active substances[8]. Utilizing lipid nano-vesicles to encapsulate active ingredients in cosmetics provides increasing benefits, including improved skin penetration into deeper layers, as well as sustained and targeted release[9].

In the production of lipid nanovesicles, the most frequently employed lipid monomers include phospholipids, cholesterol, and triglycerides[8]. Among various lipid-based systems, those based on phospholipids (specifically lecithin) have emerged as particularly intriguing[10]. Phospholipids, being natural and biocompatible molecules, exhibit an affinity for cellular membranes, enhancing the absorption of various active compounds[11]. Due to their similarity to biomembrane composition, they are acknowledged as non-allergenic and bio-friendly permeation enhancers. When in the presence of water, phospholipids can create diverse supramolecular structures that may be altered using polymeric substances, solvents, or other methods to modulate a topical delivery system. However, it is now recommended to incorporate stabilizers, such as non-ionic surfactants and other lipids, to impart steric stabilization effects, as the standalone use of lecithin lacks the desired level of stability and is insufficient for effectively modifying the size of the nano vehicle.

To overcome this limitation, a significant research effort has been undertaken, introducing additional components—specifically, palmitic acid (PA) and polysorbate 60 (PS 60)—into the formulation of the nanovehicle. Fatty acids stand as one of the most commonly employed enhancers for formulating transdermal drug delivery systems[12]. They are recognized as safe chemical penetration enhancers for transdermal use[13]. Among saturated fatty acids with carbon chains ranging from 12 to 20, we choose PA with its 16 carbon chain. Increasing the carbon chain length generally increases lipophilicity, but fatty acids with too long carbon chain will show a stronger affinity for the lipids in the stratum corneum, which slows down permeation[14]. On the other hand, the non-ionic surfactant PS 60 is acknowledged for its capability to stabilize the nano system through a robust steric repulsion mechanism, improving the physicochemical stability of the formulation and reducing interfacial energy, consequently leading to a reduction in particle size[15].

In this study, we aimed to use PA and PS 60 in our lecithin based nanovesicles, as penetration enhancer for active ingredient. We prepared LNV containing of lecithin, ceramide, PA and PS 60. The effect of the fatty acid and surfactant on colloidal stability was observe by measured the particle size and zeta potential using DLS. In addition, we also analyzed the structure of LNV using Cryo-TEM. The ability of this LNV as the skin penetration enhancer was tested using *in vitro* Franz diffusion cell. This approach aims to identify the most stable LNV and promising potential for enhancing the effectiveness of cosmetic products.

2. Experimental

2.1. Materials

Ceramide NP (Solus Biotech, Korea), hydrogenated lecithin (Lipoid Kosmetik, Germany), polysorbate 60 (Croda, USA), dipropylene glycol (SK PICGLOBAL, Korea), palmitic acid (Acid Chem, Malaysia), sodium phytate (Evonik, Germany), niacinamide (DSM, China), 1,2-hexanediol (Symrise, USA), pentylene glycol (M.I.Pharm, Korea), and for all experiments, deionized double-distilled water was used.

2.2. Preparation of LNV Formulations

Figure 1 shows a schematic illustration of the process of LNV. In detail, for the preparation of LNV formulations, lipid phase which include palmitic acid, hydrogenated lecithin, polysorbate 60, and ceramide NP were dissolved in dipropylene glycol at 80 ~ 90 ℃. Water phase which include active ingredient and additives was dissolved in distilled water at 75 ~ 80 ℃. Then the lipid phase was poured to the water phase and mixed with the agi-mixer for 5 min. The detailed compositions of each sample are listed in Table 1. The manufacturing method of LNV was based on our previous work[16].

2.3. Physicochemical Characterization of LNV

The droplet size and zeta potential of the LNV were acquired using dynamic light scattering (DLS, SZ-100, HORIBA, Japan) measurements. The excitation light source was a 10 mW He—Ne laser at 632.8 nm, and the intensity of the scattered light was measured at 90°. All measurements were triplicates, and the average value was reported. The size and the structure of LNV were analyzed using cryogenic transmission electron microscopy (Cryo-TEM, Tecnai F20 electron microscope, FEI, USA). Each sample was loaded onto a Lacey Formvar/Carbon on a 200 mesh Copper thick grid and immersed in liquid ethane to rapidly freeze them. The transmission electron micrographs of the frozen samples were acquired at an acceleration voltage of 200 kV.

Table 1. Composition of LNV Formulations Containing Active Ingredient (wt%)

Ingredients	LNV 1	LNV 2	LNV 3	LNV 4
Palmitic acid	0.05	0.05	0.05	0.05
Polysorbate 60	0.30	0.30	1.50	1.50
Hydrogenated lecithin	0.20	0.60	0.20	0.60
Ceramide NP	0.02	0.02	0.02	0.02
Dipropylene glycol	10.00	10.00	10.00	10.00
Niacinamide	2.00	2.00	2.00	2.00
Additives	q.s.	q.s.	q.s.	q.s.
Water	To 100	To 100	To 100	To 100

Additives are disodium EDTA and preservatives.

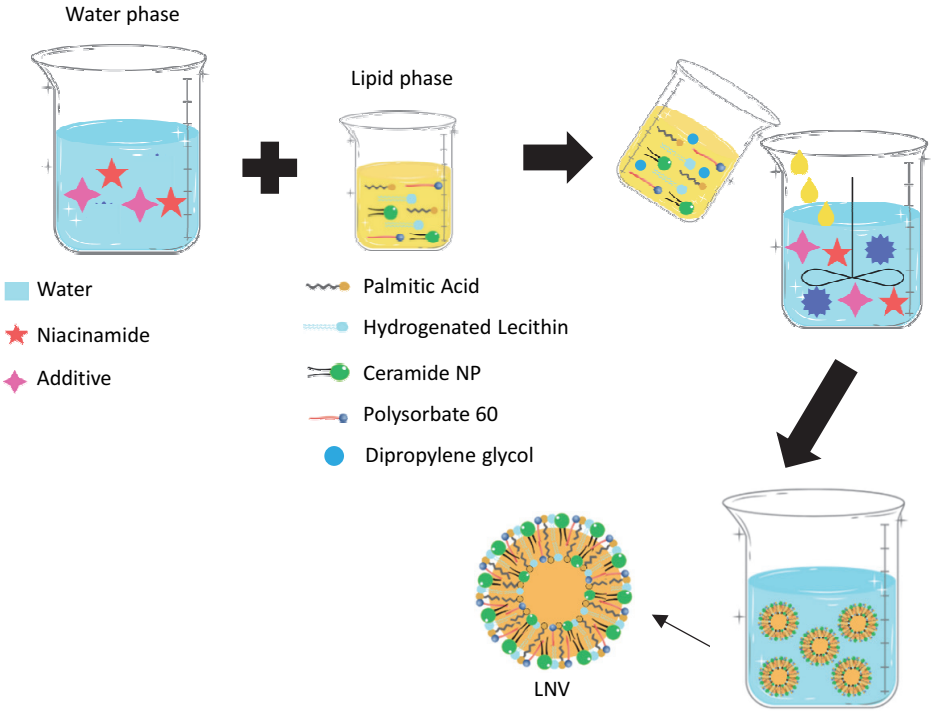

Figure 1. Schematic illustration of the process of LNV with varying compositions.

2.4 *In Vitro* Skin Penetration Study

To evaluate the penetrating ability of LNV in dermal delivery, an *in vitro* Franz diffusion cell study was conducted. 2 wt% niacinamide (NA) served as the model drug, and a synthetic membrane (Strat-M membrane, Transdermal diffusion test model, 25 mm, Merck, USA) was used as the skin equivalent. The skin equivalent was positioned between the donor and receptor chambers with the stratum corneum facing the donor compartment. The receptor compartment was filled with 7 mL of phosphate buffered solution (pH 7.4), maintained at 36 ± 0.5 °C by circulating water through a water jacket, and continuously stirred at 350 rpm for 24 h. 200 μL of the sample was applied to the surface of the stratum corneum in the donor compartment. After 2, 4, and 24 h of LNV application, 1 mL of the receptor phase was withdrawn through the sampling port of the receptor compartment, and the receptor compartment was refilled with fresh receptor phase to maintain a constant volume. The amount of NA that permeated through the skin equivalent was analyzed by HPLC (Ultimate 3000, Dionex, USA) using a reversed-phase column (Jupiter® 5 μm C_{18} 300 Å, 250 × 4.6 mm, Phenomenex, USA). The mobile phase consisted of 10 mM KH_2PO_4 and acetonitrile in a 93 : 7 ratio, with a flow rate of 1 mL/min, and UV detection was performed at a wavelength of 263 nm.

2.5. Statistics

All experimental results were analyzed more than three times, and the significance difference of each experimental group was tested at the 95% significance level by student's *t*-test.

3. Result and Discussion

3.1. Preparation and Characterization of Lipid Nanovesicle (LNV)

Several combinations of LNV 1 ~ 4 are determined in

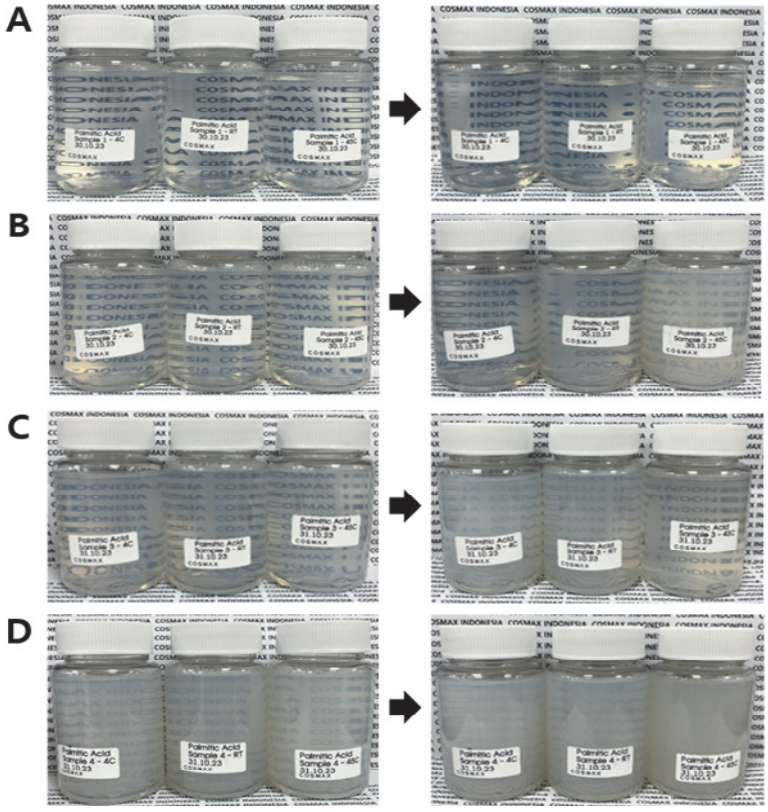

Figure 2. Visual inspection of (A) LNV 1 (B) LNV 2 (C) LNV 3 (D) LNV 4 in initial day and after 12 weeks at three different temperatures: 4 °C, room temperature, and 45 °C.

order to obtain optimize condition of the colloidal system. The formula is consisted by lecithin as the main component to create LNV system, since LNV need to be stabilized by a layer consisting of a single surfactant or through a mixture of surfactant which result in smaller particle size and improved storage stability, hence PS 60 and PA are added to the system. Briefly, dropwise addition of dipropylene glycol (DPG) solution containing PA, PS 60, lecithin and ceramide in water phase that already contains niacinamide and additives, followed by rigorous stirring with a mixer for 5 min. Visual inspection of the different LNV is observed at initial time and after 12 weeks (Figure 2). Among all combination that using PA, it was confirmed that LNV 1 has the most stable

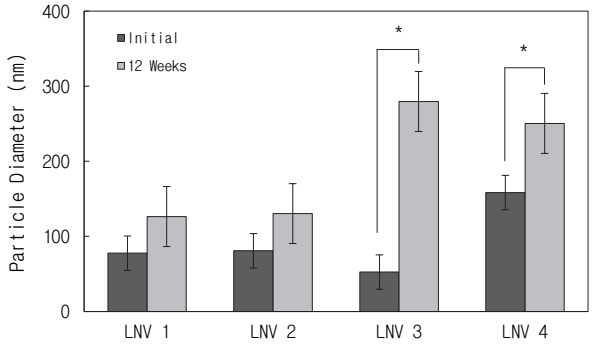

Figure 3. Comparison of the particle size for each LNV after 12 weeks. After 12 weeks of observation, all LNV experience changes in particle size. However, LNV 1 showed the least change in particle size compared to other LNV.

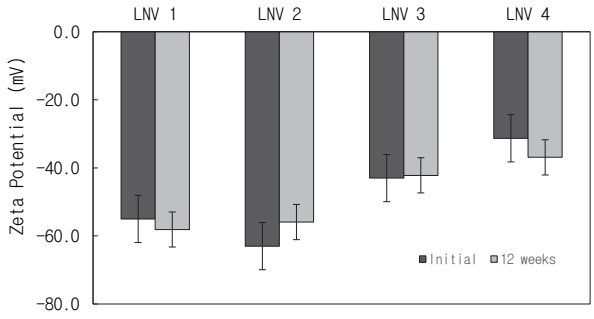

Figure 4. Comparison of zeta potential for each LNV after 12 weeks. All LNV experience changes in zeta potential, with some increasing and others decreasing. Among all LNV, LNV 1 proved to have the smallest zeta potential which is likely to indicate greater stability compared to the others.

appearance due to its resistance in maintaining transparent system while some of the LNV was found to have poor stability. Additionally, we also tried to replace PA with linoleic acid at the same concentration. However, the results indicated that the formulation with linoleic acid showed poor stability which lead us to use PA as the fatty acid in our LNV (data not shown).

Meanwhile, polysorbate 60 (PS 60) might show a negative effect on colloidal stability. Actually, LNV 3 and LNV 4, where PS 60 was used at 1.5%, they could be seen that LNV size distribution increased markedly after 12 weeks. On the other hand, this did not appear in LNV 1 and LNV 2, where PS 60 was used at 0.3%. This might be due to the lack of sufficient lipids and surfactants at the interface of LNV by the bulky head group of PS 60. Thus, it is thought that PS 60 will be difficult to use excessively in preparation of LNV.

Based on the visual observation, LNV 1 exhibited the highest transparency both initially and after 12 weeks, indicating the greatest stability in terms of transparency and color. LNV 2 showed a change in transparency at 45 ℃. LNV 3 displayed changes in transparency at both 4 ℃ and room temperature. LNV 4, which had the poorest initial transparency, became even less transparent at all temperatures after 12 weeks and also showed a slight color change at 45 ℃.

To more directly assess the stability of the LNV, we also monitor the particle size distribution and zeta potential from initial and 12 weeks result. It is confirmed that particle size of each LNV initially and after 12 weeks reveals different trends. LNV 1 and LNV 2 shows no statistical significance difference at 95% confidence level. While LNV 3 and LNV 4 shows statistical significance difference at 95% confidence level. It means that LNV 3 and LNV 4 has weak colloidal stability for long-term period (Figure 3). However, the zeta potential of LNVs before and after a 12 weeks period reveals that there is no statistical significance difference at 95% confidence level (Figure 4).

3.2. TEM Image

Transmission Electron Microscopy (TEM) is a powerful tool that plays a pivotal role in the characterization of nanoparticles. It provides accurate information about the size

and shape of nanoparticles, which is essential for understanding their behavior and optimizing their performance in applications like drug delivery and materials science. Additionally, TEM enables the observation of the distribution of nanoparticles within a sample, allowing researchers to assess how uniformly they are dispersed. This uniform dispersion is crucial for the effective utilization of nanoparticles in diverse fields. Furthermore, TEM assists in identifying defects in the nanoparticle structure and provides insights into whether they tend to agglomerate. This information is invaluable for quality control and refining the production processes. Overall, TEM offers a detailed and comprehensive view of nanoparticles, offering insights into their size, shape, distribution, and surface characteristics. Such comprehensive information is fundamental for researchers and scientists working with nanoparticles, guiding advancements across various scientific disciplines. For this study, we selected LNV 1, characterized by the smallest particle size and the most stable during 12 weeks period, to undergo examination via TEM imaging. The image in Figure 5 reveals both the size and shape of the particles.

TEM image of nanoemulsions normally exhibits uniform bright shade throughout the droplet and liposome typically shows single-layer or multilayer structure. Interestingly, in this Figure 5, we could observe a dark band encircling the surface of the nano-sized droplets. This dark band might be due to the addition of fatty acids for LNV. This result was similar to our previous work[16]. Although we did not perform the small-angle X-ray scattering (SAXS) analysis on this sample, fatty acid introduced at the interface of LNV are highly likely to form lamellar structure. And, this lamellar structure could lead to the colloidal stabilization of LNV[17].

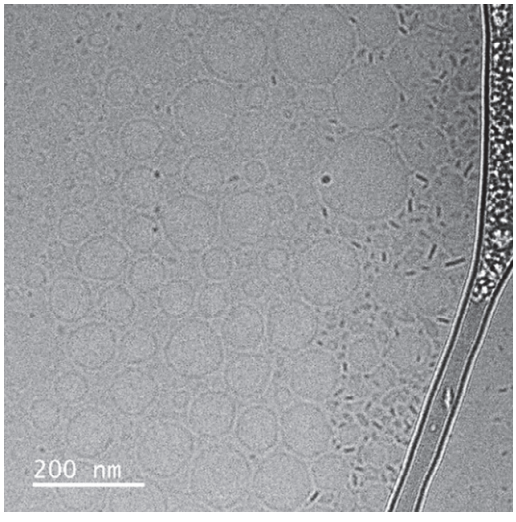

Figure 5. TEM Image of LNV 1.

3.3. *In Vitro* Skin Penetration Study

To check the effect of the LNV in dermal delivery, we performed *in vitro* Franz diffusion cell. In the Franz diffusion cell test, the amount of NA permeated through the artificial synthetic membrane was monitored 2, 4, and 24 h after application of the LNV. The result showed that the LNV formulation which containing PA and PS 60 was able to permeate NA through the artificial synthetic membrane, compared to the control sample which not containing PA. As shown in Figure 6, the percentage of NA permeating through the artificial synthetic membrane using the LNV formulation was higher and began to increase after 4 h compared to the control. The graph shows that using LNV increased the transfer of NA to the receptor cell, indicating enhanced dermal delivery. This improved ability to deliver active ingredients to the skin can significantly improve the efficacy of the formulation. According to our *in vitro* study, PA, with its 16-carbon chain, demonstrates an optimal balance between two key factors: the partition coefficient (or solubility parameter) and its affinity for the skin, resulting in enhanced penetration into the skin[12]. Although LNV 1 shows much

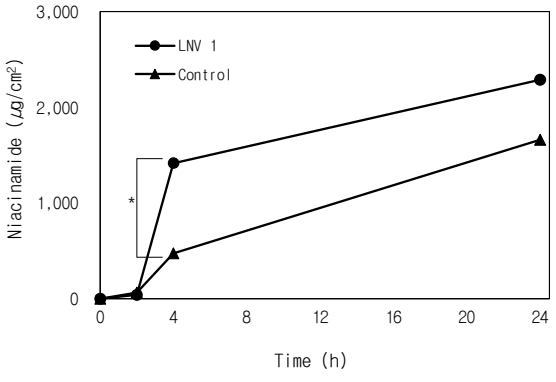

Figure 6. NA released profiles of LNV under *in vitro* Franz diffusion cell system using artificial synthetic membranes.

more improved dermal delivery than control, we have no direct evidence of how niacinamide was released from LNV. Interestingly, the surface layer of artificial membrane has more than 1 μm of lipid domains[18]. From the consideration of this structure, we can assume that LNV can pass through the artificial membrane and be penetrated well into the receptor.

4. Conclusion

In this study, we found that adding PA and PS 60 to the formulation of LNV plays a crucial role in determining the particle size and zeta potential. The TEM images we captured show that fatty acid, surfactant, and some lipids such as lecithin and ceramide may not exist in inner structure but the the interface of LNV. Additionally, our *in vitro* test revealed that this LNV allows for better diffusion, suggesting it could enhance dermal delivery. These promising results indicate that our LNV could be highly beneficial for future cosmetic applications, contributing to the advancement of nanovesicle technology in this field.

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