

RESEARCH ARTICLE

Development of Solid Lipid Microparticles From Vitamin C Incorporated Zinc-Aminoclay for Cosmetic Applications

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ABSTRACT

This study focused on creating solid lipid microparticles (SLMs) that combine vitamin C (VC) and zinc-aminoclay (ZnAC) incorporation to improve skincare effectiveness. VC is known for its antioxidant and skin-brightening benefits, but it can be unstable and is poorly absorbed by the skin. ZnAC, a two-dimensional material with antimicrobial properties, helps enhance delivery. By encapsulating VC in ZnAC with SLMs, we aimed to boost its stability and skin absorption. We evaluated the physical properties, entrapment efficiency, and stability of these formulations. The results showed that VC-ZnAC-SLMs had better skin permeability and stability compared to VC alone and VC combined with ZnAC and SLM. The transdermal absorption of the VC-ZnAC-SLM achieved the highest transdermal absorption (2.42%), followed by VC-ZnAC and pure VC, with the transdermal absorption rates of 1.25% and 1.04%. The unique structure of ZnAC played a key role in these improvements. Our findings suggest that SLMs with ZnAC and VC could be an effective solution for cosmetic products.

Practical Applications: The developed VC-ZnAC-SLMs offer a practical solution for formulating more effective skincare products, especially those aimed at brightening and protecting the skin. By enhancing vitamin C's stability and absorption, these formulations can provide prolonged antioxidant benefits and improved delivery of skin-brightening effects. The inclusion of ZnAC further adds antimicrobial protection, which is beneficial for sensitive or acne-prone skin types. This combination could be applied in serums, creams, and masks, helping consumers achieve better skin health with more reliable, long-lasting results compared to conventional vitamin C products.

1 | Introduction

Vitamin C (VC) is an important ingredient in skincare products owing to its numerous benefits for skin health and aesthetics. It acts as an antioxidant, protecting the skin from the oxidative stress caused by harmful factors (e.g., UV radiation and pollution), stimulates collagen production, and brightens the skin tone [1, 2]. When applied to the skin, VC can penetrate the outer layer through intercellular lipid and transcellular pathways,

although it faces challenges owing to its chemical properties and the nature of the skin barrier [3, 4]. The topmost layer of the skin, the stratum corneum, is mainly composed of lipids, creating a barrier to water-soluble substances, such as VC [5]. VC is chemically unstable and is easily oxidized when exposed to light, air, or specific metal ions [1]. This instability can make topical VC less effective because it may degrade before or during application to the skin. Moreover, VC is most stable at an acidic pH (approximately 3.5), but the natural pH of the skin is slightly

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acidic (approximately 4.7–5.5), making it challenging to maintain VC in its active form [6]. Innovations in nanotechnology, such as encapsulating VC in delivery systems, including nanoparticles, liposomes, and solid lipid microparticles (SLMs), have helped address these challenges and enhance the effectiveness of VC upon application.

Zinc-aminoclay (ZnAC) is a two-dimensional (2D) layered material with aminopropyl functional groups, created through a sol–gel process involving 3-aminopropyltriethoxysilane (APTES) and cationic zinc ions [7]. ZnAC has shown antimicrobial effects and biocompatibility [8, 9]. When dispersed in water, the aminopropyl groups in ZnAC adopt a cationic state, facilitating the quick loading of negatively charged drug molecules and providing antimicrobial properties [8]. Similar to other 2D materials, ZnAC can be used as a carrier of active ingredients in various biomedical and cosmetic formulations [8, 9]. SLMs, composed of solid lipids, offer controlled release, improved bioavailability, the protection of VC, and the ability to encapsulate hydrophilic and lipophilic substances, making them ideal for cosmetic applications [10, 11].

In this study, we aimed to develop an SLM system that incorporates both ZnAC and pure VC to harness their combined benefits in skincare formulations. By utilizing the structural stability and antimicrobial properties of ZnAC alongside the enhanced skin permeability and controlled release capabilities of SLMs, we hypothesized that this formulation will improve VC stability, facilitate deeper transdermal delivery, and provide a sustained release effect for prolonged skin benefits. This work builds on the potential of using advanced delivery systems to address common issues with VC, such as instability and limited skin absorption, thereby contributing to more effective and reliable cosmetic products. Our research thus focuses on evaluating the physical characteristics, entrapment efficiency (EE), and stability of these VC-ZnAC-SLMs, as well as their performance in skin permeability and release studies. The findings from this work are anticipated to provide valuable insights for the formulation of next-generation skincare products with enhanced efficacy and longevity.

2 | Materials and Methods

2.1 | Materials

Zinc nitrate ($\text{Zn}(\text{NO}_3)_2$), hydrochloric acid, and ethanol (EtOH) were sourced from Daejung Chemicals and Metals, Republic of Korea. L-Ascorbic acid, stearic acid, lauric acid, myristic acid, palmitic acid, Tween 80, citric acid, glycerin, and triethanolamine were supplied by Sigma-Aldrich. APTES was provided by the Tokyo Chemical Industry (Japan). Potassium bromide (KBr) was obtained from PIKE Technologies, USA. Distilled (DI) water was obtained using a Milli-Q Millipore filter system (Millipore, USA). The Nivea Cream (No. 80104) was purchased from Nivea brand shop in the Republic of Korea. The cadaver skin for research (epidermis and dermis 2 cm × 2 cm) was purchased from UMC Korea.

2.2 | Synthesis of VC-ZnAC and VC-ZnAC-SLM

2.2.1 | Preparation of VC-ZnAC

VC-ZnAC was prepared by utilizing a previously reported sol–gel method [12]. Briefly, 1.68 g of $\text{Zn}(\text{NO}_3)_2$ and 0.8 g of L-Ascorbic acid (VC) were dispersed in 40 mL of EtOH and stirred for about 10 min. Next, 2.6 mL of APTES was added, and the mixture was stirred for 3 h at 200–250 rpm. Afterwards, the blend was centrifuged at 3500 rpm for 5 min, and the solution was carefully removed. The residue was dried in a conical tube at 60°C for 24 h. The obtained VC-ZnAC was dried at 60°C for 24 h and then gently ground with a pestle to obtain a fine VC-ZnAC powder for further use.

2.2.2 | Preparation of VC-ZnAC-SLMs

VC-ZnAC-SLM was synthesized using four different types of lipids: stearic acid, lauric acid, myristic acid, and palmitic acid, through a sol–gel transition process [13]. Briefly, the oil phases were prepared by melting 15 g of the lipid (stearic acid, lauric acid, myristic acid, or palmitic acid) with 90 mL of glycerin at 75°C. An aqueous phase was prepared by dispersing 0.1 g of VC-ZnAC and 0.5 g of Tween 80 in 10 mL of DI water. The aqueous phase was sonicated for 5 min at 60% amplitude to completely disperse VC-ZnAC and the surfactant Tween 80. VC-ZnAC-SLMs were prepared by homogenizing the lipid mixture (oil and aqueous phases) while maintaining the temperature at 75°C in a water bath (10000 rpm for 15 min). The molten lipid dispersion phase of the SLMs was solidified by lowering the temperature to room temperature (25°C) while gently stirring the dispersion at 50 rpm. In this case, natural cooling promotes crystal growth. The crystal VC-ZnAC-SLMs were freeze-dried at –48°C for 24 h to form an SLM powder.

2.3 | Characterizations of VC-ZnAC and VC-ZnAC-SLM

The morphologies of the VC-ZnAC and VC-ZnAC-SLM samples were studied using scanning electron microscopy (SEM) (Hitachi S-4700 FE-SEM, Japan). Transmission electron microscopy (TEM) was performed using a JEM 3010 microscope (JEOL, Tokyo, Japan) at 300 kV. Fourier-transform infrared (FT-IR) spectra were recorded in the range 400–4000 cm^{-1} on a JASCO-4600 spectrometer using KBr pellets to detect the functional groups.

2.4 | EE (%) of VC in VC-ZnAC

The EE (%) of VC in VC-ZnAC was measured using an UV–vis spectrophotometry [14]. After synthesizing the VC-ZnAC solution, the untrapped VC in the supernatant was separated by centrifugation (2000 rpm for 5 min). The amount of untrapped VC was analyzed using UV–vis spectrophotometry and VC standard curve. The EE (%) of VC in VC-ZnAC was calculated

using the following equation:

$$EE(\%) = \frac{\text{Total VC} - \text{Untrapped VC}}{\text{Total VC}} \times 100 \quad (1)$$

2.5 | EE (%) of VC in VC-ZnAC-SLMs

The EE (%) of VC in the VC-ZnAC-SLMs was measured using high-performance liquid chromatography (HPLC) analysis [15]. First, the VC-ZnAC-SLM solution was dispersed in HCl to dissolve the untrapped VC. The VC-ZnAC-SLM solution was centrifuged at 4000 rpm for 10 min to separate the SLM from the untrapped VC. The supernatant was then filtered using a 0.2 µm filter to remove the remaining SLMs. The filtered solution was collected, and the VC concentration was measured using HPLC analysis. The EE (%) of VC in the VC-ZnAC-SLM was measured using Equation (1).

2.6 | Franz Diffusion Cells

First, a 10000 ppm solution of pure VC, VC-ZnAC, and VC-ZnAC-SLMs in DI water was prepared. The pH of the VC, VC-ZnAC, and VC-ZnAC-SLM samples was adjusted to three and five using citric acid and triethanolamine, respectively. The gasket was placed on the isolated human skin ($2 \times 2 \text{ cm}^2$), and each sample was applied according to a previously described protocol [16]. The isolated human skin was then placed on the receptor chamber with the stratum corneum facing upward, and the donor chamber was placed on the skin (Logan Instruments DHC-6TD Transdermal Diffusion Franz Cell System, USA). The temperature of the receptor compartment was maintained at 32°C using a water jacket. Then, 12 mL of EtOH solution (30% v/v ethanol in DI water) was injected into the receptor chamber using a syringe. Stirring was performed at 600 rpm using a magnetic bar to ensure that the transmitted samples were well mixed. The transmittance was measured by UV-vis spectroscopy at the intervals of 1, 3, 6, and 9 h.

2.7 | Long-Term Stability in Hand Cream

The long-term stability of the VC-SLM, VC-ZnAC-SLM, VC-ZnAC, and pure VC samples was evaluated by thoroughly mixing each prepared sample (1 g) with 50 g of Nivea hand cream in a brown bottle. These mixtures were stored at 50°C for 10 days in a hot-air oven. After 10 days, 30 mg of each sample was collected and dissolved in 50 mL of hot ethanol (60°C) through sonication. The stability of the samples in the hand cream was confirmed by measuring the absorbance in the range of 200–500 nm using UV-vis spectroscopy in a 3 mL quartz cuvette.

2.8 | Statistics

2.8.1 | Statistical Analysis

One-way ANOVA and Duncan's multiple range test were used in SPSS Statistics 27.0 for statistical assessment of the data. The experiments were done in triplicate, and the results are displayed

as mean $\pm$ standard deviation, with a 95% confidence level ($p < 0.05$).

3 | Results

3.1 | Characterizations of VC-ZnAC and VC-ZnAC-SLMs

The FT-IR spectrum showed that surface functional groups were formed owing to the coprecipitation of VC, ZnAC, and the oil phases (Figure 1a). The two peaks at 3418 and 2936 cm^{-1} were associated with N–H and C–H stretching, suggesting the presence of amino groups on the surface of the VC-ZnAC-SLM. The broad band at 3131 cm^{-1} confirmed the presence of the OH group. The prominent peaks near 1620 and 989 cm^{-1} correspond to the stretching of aromatic rings or C=C double bonds. The small band at 1100 cm^{-1} resulted from alkoxy C–O–C stretching, and the band at 789 cm^{-1} indicates the presence of the aliphatic CH group. Photographs of the prepared VC-ZnAC and VC-ZnAC-SLM are displayed in Figure 1b,c.

The exterior morphology of the prepared samples was analyzed using FE-SEM, as shown in Figure 2. These images illustrate the structures of VC-ZnAC and VC-ZnAC-SLMs. VC-ZnAC exhibited a solid structure characterized by a disordered morphology (Figure 2a–c). In contrast, after the addition of SLMs, VC-ZnAC transformed into a spherical shape with a rougher texture, exhibiting an average particle size of 30 µm (Figure 2d–f).

3.2 | Immobilization of VC in VC-ZnAC and VC-ZnAC SLMs

The fixation rate is a crucial criterion for coprecipitation. The fixation rate of VC in VC-ZnAC was measured to be approximately 22.34%.

The EE (%) of VC in the VC-ZnAC-SLM was determined using HPLC analysis. The measured amounts of untrapped VC are presented in Table 1. The VC-ZnAC-SLM produced from stearic acid achieved the highest EE (59.58%) among the four types of lipids, followed by myristic acid, lauric acid, and palmitic acid, with the EE values of 35.69%, 27.58%, and 24.71%, respectively. On the basis of their high EE, the VC-ZnAC-SLM produced from stearic acid was selected for further evaluation.

3.3 | Transdermal Absorptions of As-Prepared Samples

A Franz diffusion cell experiment was used to study the transdermal absorptions of the VC, VC-ZnAC, and VC-ZnAC-SLM through the skin.

First, the transdermal absorptions of pure VC and VC-ZnAC were compared. To ensure relevance, we evaluated VC stability both at pH 3, where it is optimally stable, and at pH 5, which closely mimics the physiological skin environment. This dual-pH approach provides a more comprehensive understanding of the performance of formulation under both ideal storage and

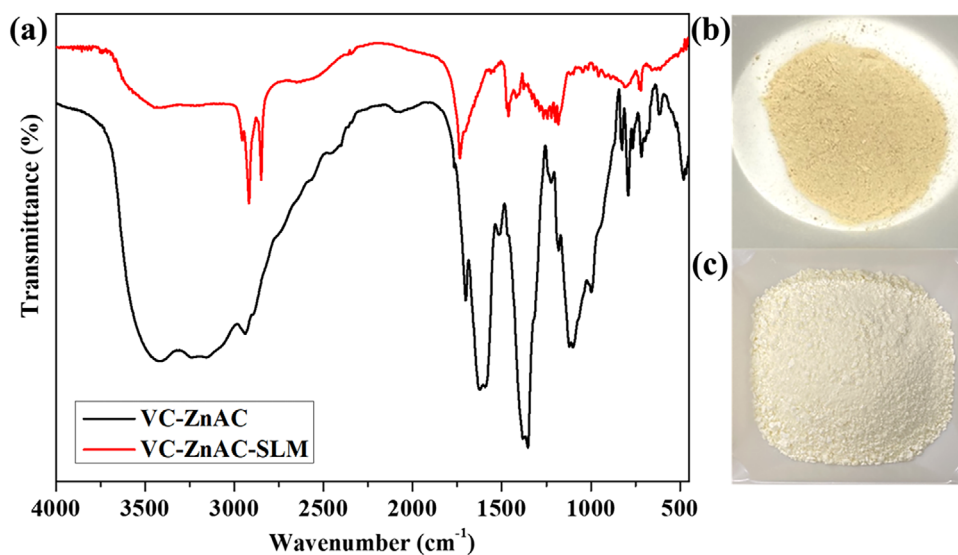


FIGURE 1 | (a) FT-IR spectra of VC-ZnAC and VC-ZnAC-SLM; (b) photograph of VC-ZnAC; and (c) photograph of VC-ZnAC-SLM. SLM, solid lipid microparticles; VC, vitamin C; ZnAC, zinc-aminoclay.

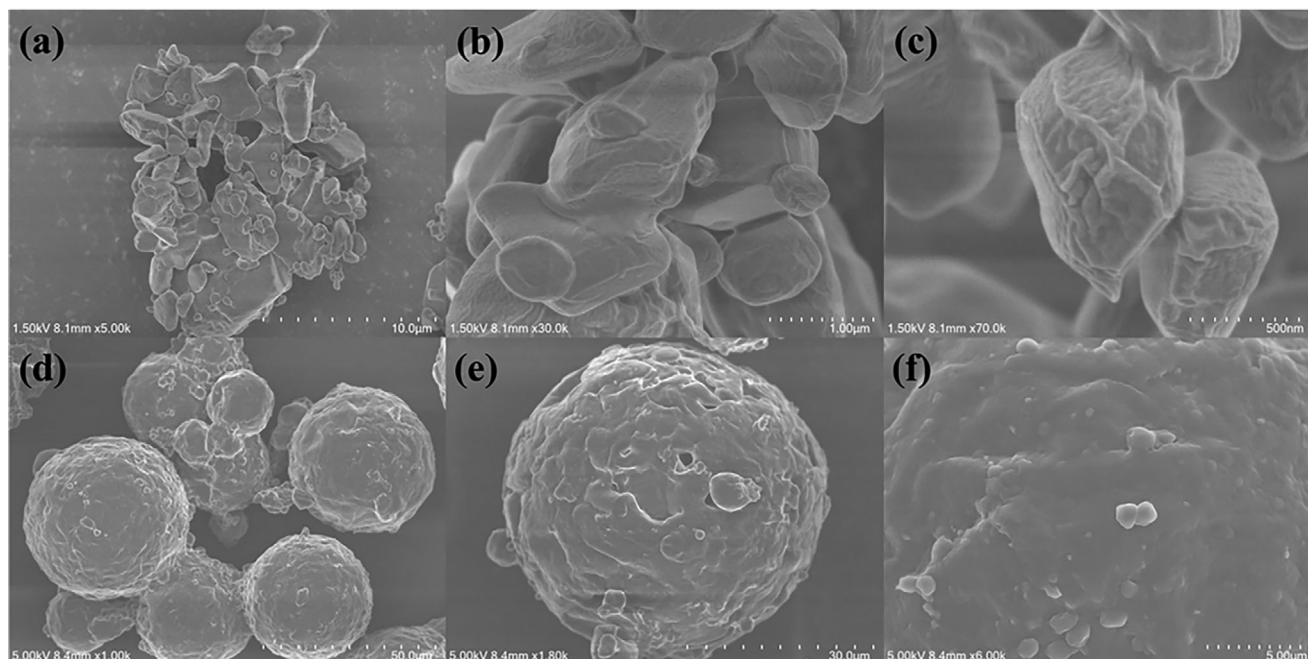


FIGURE 2 | FE-SEM images of the VC-ZnAC (a–c) and VC-ZnAC-SLMs (d–f).

TABLE 1 | Entrapment efficiency (EE, %) of VC-ZnAC-SLM prepared using four different lipids.

Lipid	Total VC (mg/100 mL)	Unentrapped VC (mg/100 mL)	Entrapped efficiency (EE, %)
Stearic acid	22.34	9.03	55.58
Lauric acid	22.34	16.18	27.58
Myristic acid	22.34	14.37	35.69
Palmitic acid	22.34	16.82	24.71

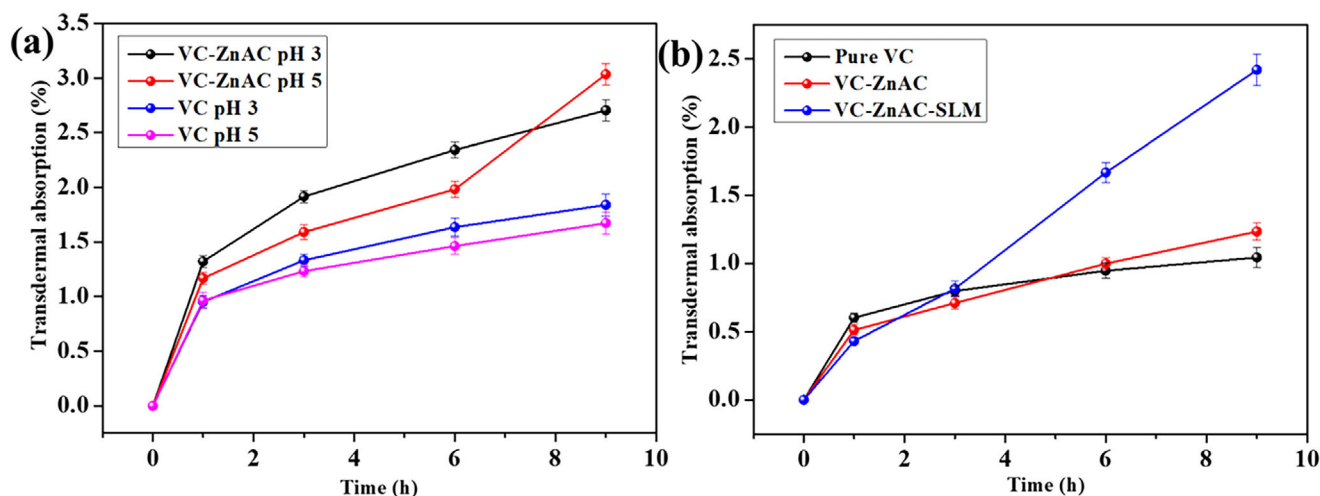


FIGURE 3 | Comparison of the transdermal absorptions of samples: (a) pure VC and VC-ZnAC (pH 3 and 5); (b) pure VC, VC-ZnAC, and VC-ZnAC-SLM at pH 5. SLM, solid lipid microparticles; VC, vitamin C; ZnAC, zinc-aminoclay.

realistic application conditions. The pH values of the sample solutions were adjusted to three and five using citric acid and triethanolamine, respectively. The results demonstrated that the skin permeability of VC and VC-ZnAC at pH 3 was lower than that at pH 5. As the pH of the skin is approximately 5, pure VC and VC-ZnAC prepared at pH 5 could easily penetrate the skin layers. At pH 5, VC-ZnAC penetrated the skin more compared with the pure VC solution. The transdermal absorption of VC-ZnAC was approximately 3.03%, followed by VC-ZnAC at pH 3 (2.70%), pure VC at pH 5 (1.84%), and pure VC at pH 3 (1.67%) (Figure 3a).

Subsequently, the transdermal absorptions of the VC, VC-ZnAC, and VC-ZnAC-SLM were compared at pH 5 (citric acid and triethanolamine). The results showed that the VC-ZnAC-SLM achieved the highest transdermal absorption (2.42%), followed by VC-ZnAC and pure VC with the transdermal absorption rates of 1.25% and 1.04%, respectively (Figure 3b). The comparison showed that the VC-ZnAC-SLM exhibited better skin permeability than pure VC and VC-ZnAC at the same concentration and pH conditions. Thus, the SLM system was shown to enhance the skin permeability of VC.

3.4 | Long-Term Stability in Hand Cream

The UV-vis absorbance spectra in Figure 4a,b demonstrated the stability of four formulations: pure VC (unencapsulated), VC-SLM (VC encapsulated in SLMs), VC-ZnAC (VC complexed with ZnAC), and VC-ZnAC-SLM (VC complexed with ZnAC and then encapsulated in SLM) before and after 10 days of storage at 50°C in a hand cream. The results showed that the absorbance peak shifted to 272 nm for all samples after 10 days, with VC-ZnAC-SLM displaying the highest absorbance, indicating the greatest stability. The VC-ZnAC and VC-SLM formulations exhibited slightly lower absorbance, whereas pure VC showed the lowest absorbance, suggesting significant degradation. These findings highlight that the encapsulation of VC in ZnAC, combined with SLM, effectively enhanced VC's stability, offering better protection against degradation compared to pure VC, VC-SLM, and VC-ZnAC.

4 | Discussion

In this study, VC was encapsulated in the positively charged ZnAC and SLM delivery systems to improve its cosmetic properties (Figure 5). SEM images and FT-IR spectra confirmed the successful synthesis of VC-ZnAC and VC-ZnAC-SLM, which exhibited more spherical morphologies and smaller sizes than pure VC. These changes in the physical properties were considered crucial for improving the cosmetic properties of VC-based formulations.

First, VC was successfully encapsulated in ZnAC at a fixation rate of 22.34%. During the generation of VC-ZnAC, a crosslinking reaction between VC and ZnAC occurred, involving chemical bonds between these two substances to form a composite material with novel properties [12, 17]. The amino groups (—NH_2) of ZnAC reacted with the hydroxyl groups (—OH) of VC to form amide bonds. The ionic bonds were formed by an electrostatic reaction between the zinc ions (Zn^{2+}) and the carboxylate groups (—COOH) of the deprotonated VC can create a stable complex. Moreover, Zn^{2+} can form coordination complexes with the hydroxyl groups (—OH) of VC, potentially creating a more stable and structured network [17]. Through crosslinking reactions, VC is more stable under stress conditions when integrated into VC-ZnAC than in its pure form [18]. Additionally, with the multilayered structure of ZnAC, VC-ZnAC has a core-shell structure, with VC as the core and positively charged ZnAC as the shell (Figure 5a). This core-shell structure helps shield VC from oxidation and degradation, thus preserving its stability and effectiveness [11, 19]. The positive charge, attributed to the NH_2^+ groups, helps VC-ZnAC interact more strongly with the skin surface, enabling better penetration through the dermal layer compared with pure VC [11]. The nanosize (nm) of VC-ZnAC also enabled it to move through the spaces between corneocytes in the stratum corneum, facilitating deeper penetration into the skin layers. The novelty of the physical properties was crucial for explaining these results. With the excellent layer structure and positive surface charges, VC-ZnAC exhibited better transdermal ability (2.70%–3.03%) compared with VC (1.67%–1.84%). Moreover, at a pH similar to that of the skin (pH 5), the skin permeability of VC-ZnAC (3.03%) was higher than that at the

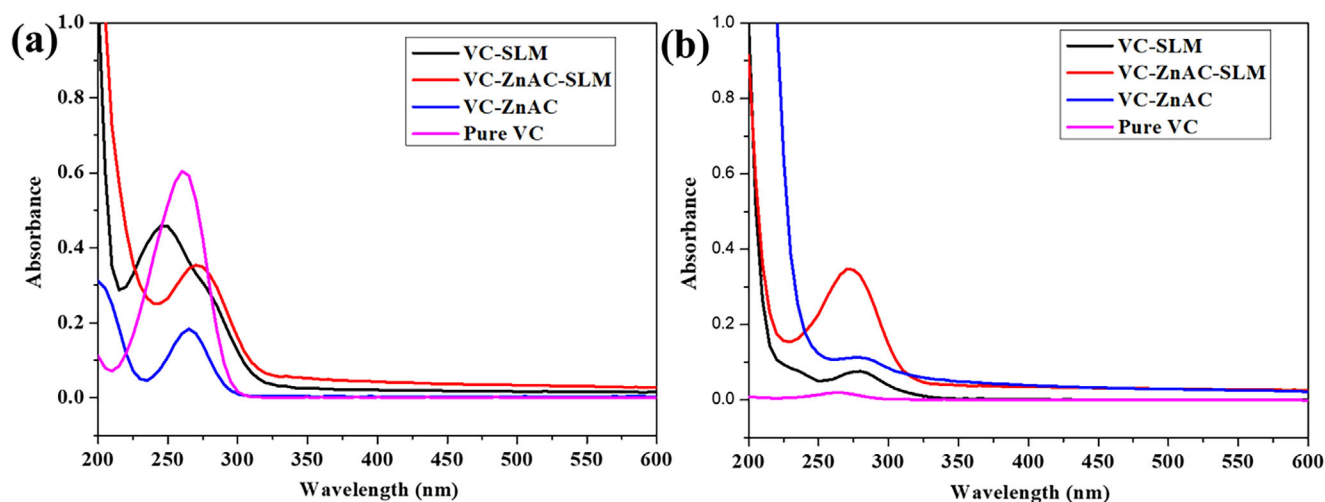


FIGURE 4 | UV-vis absorbance of pure VC, VC-ZnAC, and VC-ZnAC-SLM (a) before and (b) after the storage period. SLM, solid lipid microparticles; VC, vitamin C; ZnAC, zinc-aminoclay.

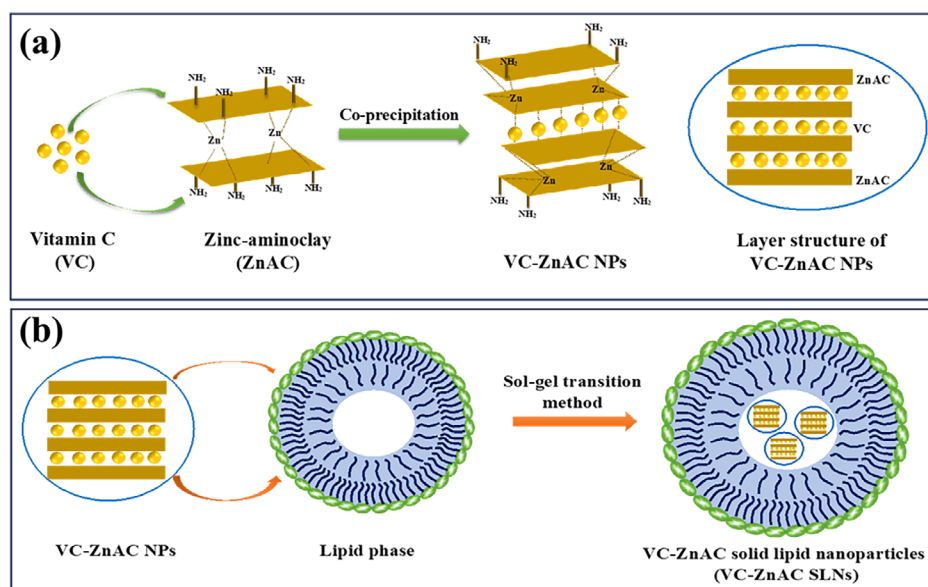


FIGURE 5 | Schematic for the synthesis of (a) VC-ZnAC and (b) VC-ZnAC-SLM.

more acidic pH of 3 (2.70%). This suggested that VC-ZnAC was more stable at pH 5 than at pH 3. At a very low pH, VC-ZnAC may have aggregated or degraded, reducing its ability to penetrate the skin effectively. Proteins and lipids in the stratum corneum were more stable and functioned optimally at the natural pH (5 and 6). At pH 5, these components maintained their structure and function, enabling better interactions and potential penetration of VC-ZnAC. Therefore, VC-ZnAC was prepared at a pH of 5 for further evaluation.

After successfully integrating VC into ZnAC, we encapsulated the prepared material in an SLM delivery system. VC-ZnAC was well dispersed in the lipid phase (stearic acid, myristic acid, lauric acid, and palmitic acid) to form SLMs (Figure 5b). The results indicated that the VC-ZnAC-SLM based on stearic acid achieved the highest EE (~56%) compared with the other lipid phases. Stearic acid, which had a longer carbon chain (C_{18}) than myristic acid (C_{14}), palmitic acid (C_{16}), and lauric acid (C_{12}), yielded the

best results [20, 21]. Longer chain fatty acids, such as stearic acid, generally had higher melting points, resulting in a more stable and less permeable lipid matrix, which aided in the better entrapment of SLMs [20]. The crystallinity and packing density of the lipid matrix could have influenced the EE. Stearic acid can form a highly ordered and tightly packed crystalline structure, reducing the diffusion of the encapsulated material and leading to a higher EE [22]. Thus, stearic-acid-based VC-ZnAC was chosen for further evaluation.

The skin permeability of the VC-ZnAC-SLM was evaluated and compared with that of pure VC and VC-ZnAC. At pH 5, the VC-ZnAC-SLM showed greater penetration into the skin than VC-ZnAC and pure VC, with the transdermal absorptions of 2.42%, 1.25%, and 1.04%, respectively. These results were attributed to the structures of the SLMs. The VC-ZnAC-SLM had occlusive properties, forming a thin lipid film on the skin surface, which reduced the transepidermal water loss, increased skin hydra-

tion, and enhanced skin permeability [11]. Hydrated skin had a more permeable stratum corneum, which facilitated better penetration of VC-ZnAC. In addition, the lipid matrix of SLMs was structurally similar to natural lipids in the skin, enabling better integration and interaction with the skin lipid matrix, and promoting the penetration and absorption of the encapsulated VC-ZnAC [23]. The solid lipid state provided a controlled and sustained release profile for the encapsulated VC-ZnAC. This stable delivery system protects VC against degradation, ensuring stability during storage, handling, and transportation. Long-term stability tests demonstrated that the VC-ZnAC SLMs were more stable in the hand cream formulation than VC-ZnAC and pure VC under the same conditions (10 days and room temperature).

5 | Conclusions

This study developed SLMs incorporating ZnAC and pure VC to enhance their effectiveness in cosmetic products. The physical properties, EE, and stability of the SLMs were analyzed over time. The results demonstrated that the VC-ZnAC-SLM had better skin permeability and stability than VC-ZnAC and pure VC. The addition of ZnAC provided a stabilizing effect and improved the transdermal absorption owing to its unique layered structure and positive surface charge. These findings suggest that SLMs loaded with ZnAC and VC are promising delivery systems in the cosmetic industry. Future studies should focus on in vitro and in vivo evaluations to confirm the cosmetic properties of VC-ZnAC-SLM for real-world applications.

Author Contributions

Ga-Min Kim: writing – original draft, visualization, formal analysis, conceptualization. **Le Thi Nhu Ngoc:** writing – original draft, investigation, methodology, writing – review and editing. **Eun-Chae Shin:** formal analysis. **Ju-Young Moon:** formal analysis, data curation. **Young-Chul Lee:** supervision, writing – review and editing, project administration, funding acquisition.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data supporting the findings of this study are available from the corresponding author upon request.

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