IFSCC 2025 full paper (IFSCC2025-1133)

Whitening promotion of Ascorbyl Tetraisopalmitate (VCIP) nano-emulsion through surfactin encapsulation

Xiping LUO[1], Xin SUN[1*], Kuan CHANG[2], Ruiyan WANG[1]

[1]Bloomage Biotechnology Corporation Limited, Floor 1-4, Building 3, No. 26 Yongwang West Road, Daxing Biomedical Base, Zhongguancun Science Park, Daxing District, Beijing, China.
[2]School of Chemical & Material Engineering, Jiangnan University, Wuxi, 214122, P. R. China.
Contact：sunxin-bj@bloomagebiotech.com

Abstract

Ascorbyl Tetraisopalmitate (VCIP) is a derivative of Vitamin C that has gained attention for its skin whitening and brightening efficacy, primarily due to its antioxidant properties and ability to inhibit melanin production. However, its whitening effectiveness is often considered less robust than that of traditional Vitamin C, particularly in cases of severe hyperpigmentation.

In this study, we employed cutting-edge surfactant nano-emulsification technology to encapsulate VCIP, significantly enhancing its water solubility, thermal stability (an increase of 23%), ultraviolet (UV) stability (an increase of 37%).

Testing on melanin skin models (Skinovo®-Mela) exposed to UV light demonstrated that the VCIP nano-emulsion resulted in lighter skin (higher L*) and reduced melanin content compared to free VCIP. Moreover, human trials indicated that the VCIP nano-emulsion achieved significantly better L* and ITA° at 28 days compared to free VCIP, with participants noting a noticeable improvement in brightness and radiance on the treated side.

In addition to its whitening effects, the use of the nano-emulsion led to a significant reduction in crow's feet, outperforming free VCIP after 1 day. Furthermore, improvements were observed in skin barrier function, with higher water content in the stratum corneum and lower trans-epidermal water loss (TEWL) in the VCIP nano-emulsion group compared to the free VCIP group.

These findings highlight the potential of VCIP nano-emulsion as a superior option for skin whitening and overall skin quality enhancement.

Keywords：Surfactin, ascorbyl tetraisopalmitate, skin whitening, nano-emulsion

Introduction

Vitamin C is a multifunctional antioxidant that plays a crucial role in promoting collagen synthesis, photoprotection, and inhibiting melanogenesis in skincare [1,2]. It functions by scavenging free radicals, thereby preventing ultraviolet (UV)-induced dermal damage [3]. However, the chemical instability of Vitamin C severely limits its practical use in cosmetic formulations. Ascorbyl tetraisopalmitate (VCIP), a more stable derivative of Vitamin C [4], has gained attention for its strong antioxidant properties and ability to inhibit melanogenesis [5]. Despite these advantages, the fully esterified structure of VCIP reduces its whitening effectiveness and other potential benefits.

To overcome these limitations, various advanced delivery technologies have been developed, including liposomes, nano-emulsions, and microencapsulation [6,7]. This study introduces a novel sodium surfactin-based nanoencapsulation approach for VCIP. This encapsulation technology not only significantly enhances the stability of VCIP but also improves its transdermal absorption, increasing bioavailability and enabling more potent effects at lower concentrations.

As consumer awareness of skin health and aesthetics continues to rise, the demand for effective whitening products is expected to grow [8]. This innovative technology provides new

perspectives on optimizing VCIP formulations and supported the development of more efficient and stable whitening products in the cosmetic industry.

Materials and Methods

Materials and Instruments

UV lamp (ZF-1，Shanghai Xiniulaibo), UV-spectrophotometer (UV5 nano, Mettler toledo), high-performance liquid chromatography (HPLC, Thermo Fisher Vanquish), ultrasonic cleaning machine (KUDOS), CO_2 incubator (Thermo, 160i), biosafety cabinet (ESCO, LA2-6A1), inverted fluorescence microscope (Keens BZ-X810), enzyme labeling instrument (Tecan, Spark), color chromatograph (Konica Minolta, CR400), corneometer CM825 (Courage&Khazaka), tewameter TM Hex (Courage&Khazaka), skin-colorimeter CL 400 (Courage&Khazaka), mexameter® MX18 (Courage&Khazaka), VISIA-CR (Canfield Scientific), antera 3D® CS (Miravex), 3D whole layer skin model culture solution (Shanghai S.A.B.N. Biotechnology), maintenance culture solution (Skinovo ®), PBS (VivaCell), anhydrous ether (Kokusaika), kojic acid (Kokusaika), NaOH (Kokusaika), anhydrous ethanol (Kokusaika).

Methods

Preparation of VCIP nano-emulsion and free VCIP solution

The formulas of VCIP nano-emulsion and free VCIP solution are presented in Table I.

Table I Formula of VCIP nano-emulsion and free VCIP oil solution

Phase	Ingredient	Content (%)	
		VCIP nano-emulsion (Formula 1)	Free VCIP (Formula 2)
A	Sodium surfactin	0.75	-
	Glycerin	6.75	-
B	VCIP	2	2
	Caprylic/Capric Triglyceride (GTCC)	28	98
	Aqua	62.5	-

VCIP nano-emulsion was prepared by adding 0.75 g sodium surfactin to 6.75 g glycerin, stirring at 80°C and 800 rpm for 30 minutes to form Phase A. Phase B (28 g GTCC + 2 g VCIP) was mixed at 50°C and 500 rpm until clear. Phase B was slowly added to Phase A at 50°C and 1200 rpm to form a gel, then 62.5 g deionized water was added at room temperature and 500 rpm. After 10 minutes, a milky emulsion (Formula 1) was obtained.

For the free VCIP solution (Formula 2), 2 g VCIP was dissolved in 98 g GTCC at 50°C and 500 rpm until clear.

Preparation of formula for human trial

The test formulas for human trial are shown in Table II.

Table II Formula of human trial

Ingredient	**Content (%)**		
	VCIP nano-emulsion test formula (Formula 3)	VCIP nano-emulsion test formula (Formula 4)	Free VCIP test formula (Formula 5)

Aqua	80.5	89.5	90.3
Acrylates/c10-30 alkyl acrylate crosspolymer	0.35	0.35	0.35
Glycerin	2.5	2.5	2.5
Butylene glycol	5	5	5
Disodium edta	0.1	0.1	0.1
1,2-hexanediol	0.5	0.5	0.5
Hydroxyacetophenone	0.5	0.5	0.5
VCIP nano-emulsion	10	1	-
VCIP	-	-	0.2
PPG-26-buteth-26&PEG-40 hydrogenated castor oil&aqua (solubilisant LRI)	0.5	0.5	0.5
Sodium hydroxide	0.05	0.05	0.05

Encapsulation efficiency measurement

The encapsulation efficiency of Formula 1 was initially determined, followed by stability assessment after storage at 45 °C for 24 hours, 7 days, and 30 days.

To quantify total VCIP content ($C_{total\ VCIP}$), 0.5 g of nano-emulsion was mixed with 5 mL methanol, sonicated for 15 minutes, diluted to 50 mL with methanol, and filtered through a 0.22 μm membrane. For free VCIP content ($C_{free\ VCIP}$), 0.5 g of nano-emulsion was mixed with 5 mL water, diluted to 50 mL with water, and filtered using a 0.22 μm aqueous filter. Both samples were analyzed via HPLC.

Encapsulation efficiency was calculated using the equation:

Encapsulation Efficiency (%) = $(C_{total\ VCIP} - C_{free\ VCIP}) / C_{total\ VCIP} \times 100$

Thermal and UV stability

Formula 1 and Formula 2 were subjected to thermal and UV stability tests. For the thermal stability test, both samples were incubated at 50°C for 28 days, with VCIP content (C) measured on days 0, 7, 14, and 28 (d0, d7, d14 and d28). The retention rate was calculated using the formula:

Retention rate (%) = $C_{dx}/C_{d0} \times 100$

For UV stability, both samples were exposed to UV light for 8 hours, with VCIP content measured at 1, 4, 6, and 8 hours. The retention rate was calculated alike.

Transdermal absorption

Transdermal absorption was tested using a 3D full-thickness human skin model. Formula 1 and Formula 2 were each diluted 10-fold, and 80 μL was applied to the model surface, followed by incubation in a CO_2 incubator. Subcutaneous samples were collected by aspirating 1 mL of culture solution at 2, 4, 6, and 24 hours. After 24 hours, the skin model was minced, placed in PBS, and ultrasonicated for 30 minutes to extract intradermal samples. VCIP levels were quantified by HPLC.

Evaluation of Whitening Efficacy on the 3D Melanin Skin Model

3D melanin skin models were incubated in 6-well plates with maintenance medium at 37 °C, 5% CO_2 for 24 h. Formula 1 and Formula 2 were diluted 20-fold, and 100 μL was applied per model. After application, the negative control (NC) and sample groups were exposed to UVB (50 mJ/cm²), while the blank control (BC) remained unexposed. Treatments

were repeated every 48 h (twice total). At the endpoint, models were rinsed and dried for analysis.

Brightness (L) was measured using a colorimeter with three readings per model. For melanin content, models were lysed, extracted with ethanol and ethyl ether, then treated with NaOH/DMSO, heated at 80 °C, and OD measured at 405 nm. Each sample was tested in duplicate.

Human trial for skin whitening efficacy evaluation

To compare the whitening efficacy of Formula 3 and Formula 5 (both with 0.2% VCIP), 20 Chinese female participants (aged 18–55, prone to sunburn and tanning, with 3–4 hours of daily sun exposure) were enrolled (Trial 1). For Formula 4 (0.02% VCIP) versus Formula 5, 14 Chinese female participants (same criteria) were enrolled (Trial 2).

In both trials, participants applied 1 g of Formula 3 or 4 to one side of the face and Formula 5 to the other side twice daily after cleansing for 28 days. The study followed the Declaration of Helsinki, with informed consent obtained.

Skin color parameters (L*, b*, ITA°) were assessed using a Skin-Colorimeter CL 400, and the melanin index (MI) was measured with a Mexameter® MX 18. Visual skin tone grading (scale 1–24; lower scores indicate whiter skin) was performed by dermatologists. Stratum corneum hydration was evaluated with a Corneometer CM825, and trans-epidermal water loss (TEWL) with a Tewameter TM Hex. Anti-wrinkle effects were analyzed through forehead imaging using Antera 3D® CS 3.0.

Percentage changes were calculated as follows:

Percentage change (%) = (post-treatment value – baseline value)/ baseline value × 100

Statistical analysis was performed via t-test using SPSS version 23.0.

Results

Stability of VCIP nano-emulsion

After 30 days at 45 °C, the VCIP nano-emulsion (Formula 1) maintained 99.9% encapsulation efficiency, indicating excellent thermal stability. At 50 °C for 28 days, its retention rate was 81%, compared to 66% for free VCIP (Figure 1). Under 8 hours of UV exposure, the nano-emulsion retained 37% more active compound than free VCIP, confirming that nanoencapsulation significantly improves VCIP's thermal and photostability.

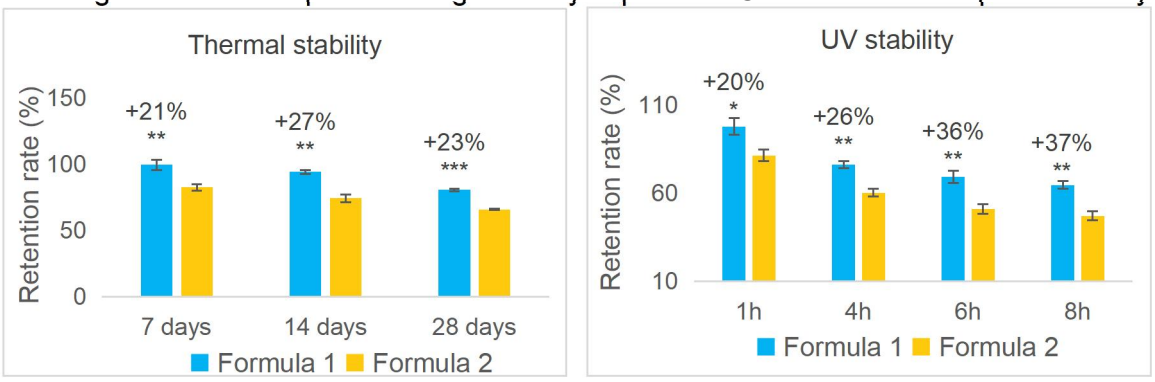

Figure 1 The VCIP nano-emulsion (Formula 1) showed significantly greater thermal and UV stability than free VCIP (Formula 2) (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

Transdermal absorption

In a 3D skin model, the VCIP nano-emulsion (Formula 1) penetrated the subcutaneous layer within 6 hours, while free VCIP (Formula 2) was detectable only after 24 hours. After 24 hours, the nano-emulsion achieved 30% dermal and 7% subcutaneous permeation, with total absorption 10.5 times higher than free VCIP, indicating significantly enhanced transdermal delivery (Figure 2).

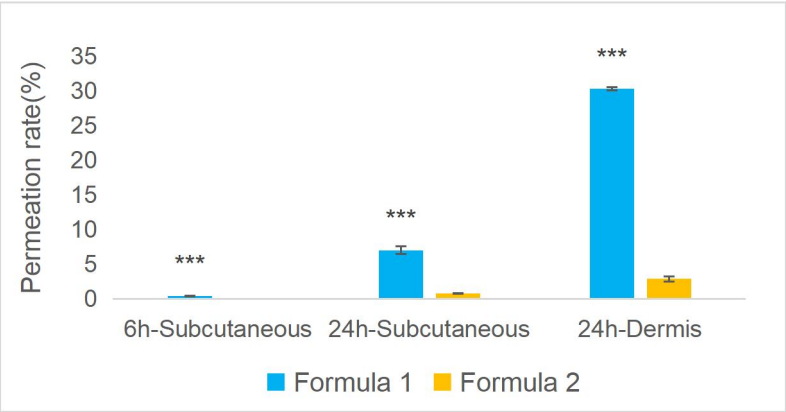

Figure 2 VCIP nano-emulsion (Formula 1) showed significantly higher transdermal absorption than free VCIP (Formula 2) (***$p < 0.001$).

Whitening efficacy testing on the 3D melanin skin model

Quantitative analyses are shown in Figure 3. Formula 1 (VCIP nano emulsion) increased the L-value by 36% and reduced relative melanin content by 48%, significantly outperforming Formula 2 (free VCIP). These findings demonstrate the enhanced efficacy of VCIP nano-emulsion in mitigating UV-induced hyperpigmentation and improving overall skin tone.

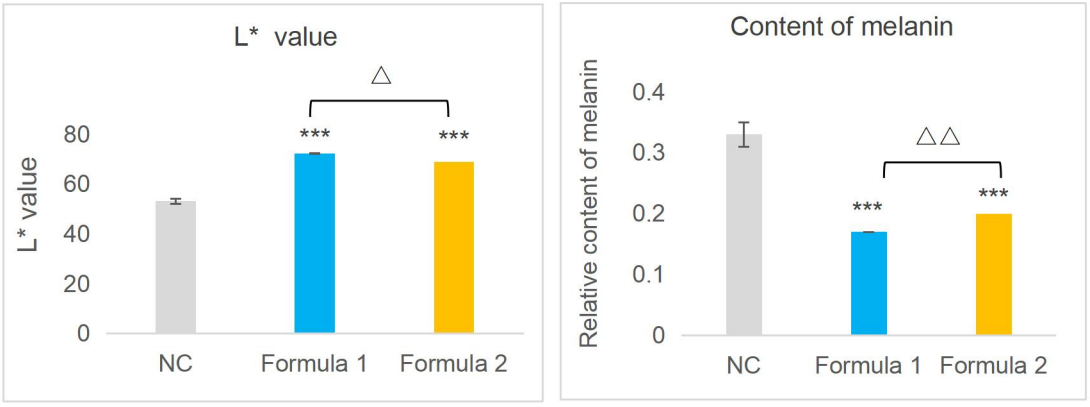

Figure 3 The VCIP nano-emulsion (Formula 1) showed significantly higher L* and lower melanin content than NC (***$p < 0.001$). Compared to free VCIP (Formula 2), differences were significant (△$p < 0.05$, △△$p < 0.01$).

Human Trials on Multifunctional Efficacy

In Trial 1, compared to free VCIP (Formula 5), the VCIP nano-emulsion (Formula 3) showed improved L* and ITA°, indicating enhanced whitening efficacy. After 28 days, Formula 3 demonstrated better L* and ITA° than baseline. In addition to whitening, the nano-emulsion significantly reduced crow's feet (up to 37% compared to baseline) and outperformed free VCIP after 1 day (Figure 4). Furthermore, improvements in skin barrier function were observed, with up to a 41% increase in stratum corneum water content and a 14% reduction in TEWL compared to baseline. The nano-emulsion group consistently showed higher water content and lower TEWL than the free VCIP group.

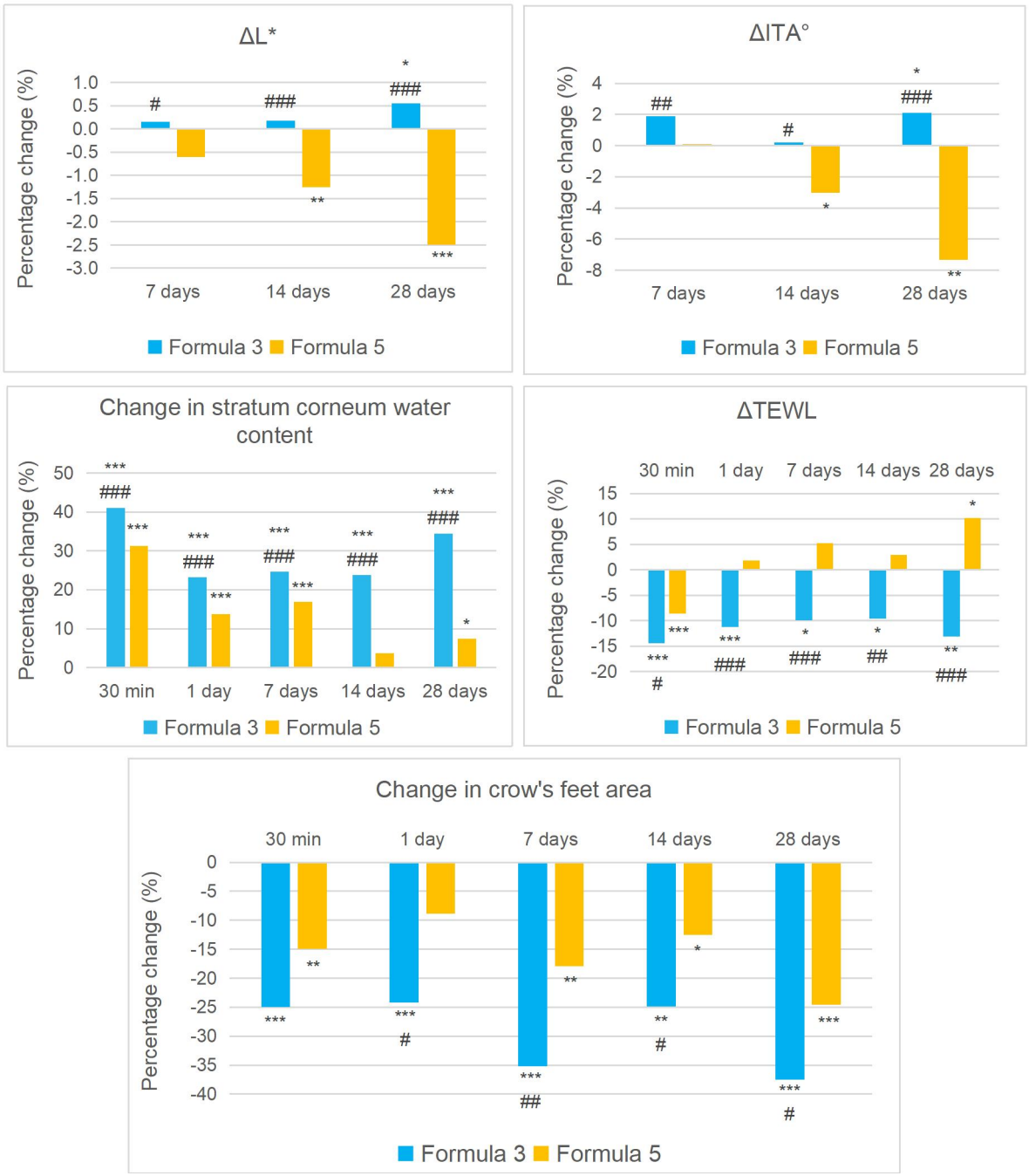

Figure 4 ΔL*, ΔITA°, change in stratum corneum water content, ΔTEWL, and change in crow's feet area (Trial 1). For L*, ITA°, water content in the stratum corneum, TEWL, and crow's feet area: Formula 3 (or Formula 5) vs baseline, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Formula 3 vs Formula 5: #$p < 0.05$, ##$p < 0.01$, ###$p < 0.001$.

Since the VCIP nano-emulsion has 10× higher transdermal absorption than free VCIP, Formula 4 (1/10th the VCIP of Formula 5) was compared to Formula 5 in Trial 2. After 14 days, Formula 4 showed significant improvements over Formula 5: b* decreased by 11%, ITA° increased by 11%, and the Melanin Index dropped by 13% (Figure 5). At 28 days, visual skin tone grading decreased by 9%.

Formula 4 also enhanced skin barrier function and reduced wrinkles: stratum corneum hydration rose by 39–63%, TEWL dropped by 11–18%, and forehead wrinkles decreased by 26% at 14 days, while free VCIP (Formula 5) showed no significant wrinkle change. Participants reported better skin tone with the nano-emulsion.

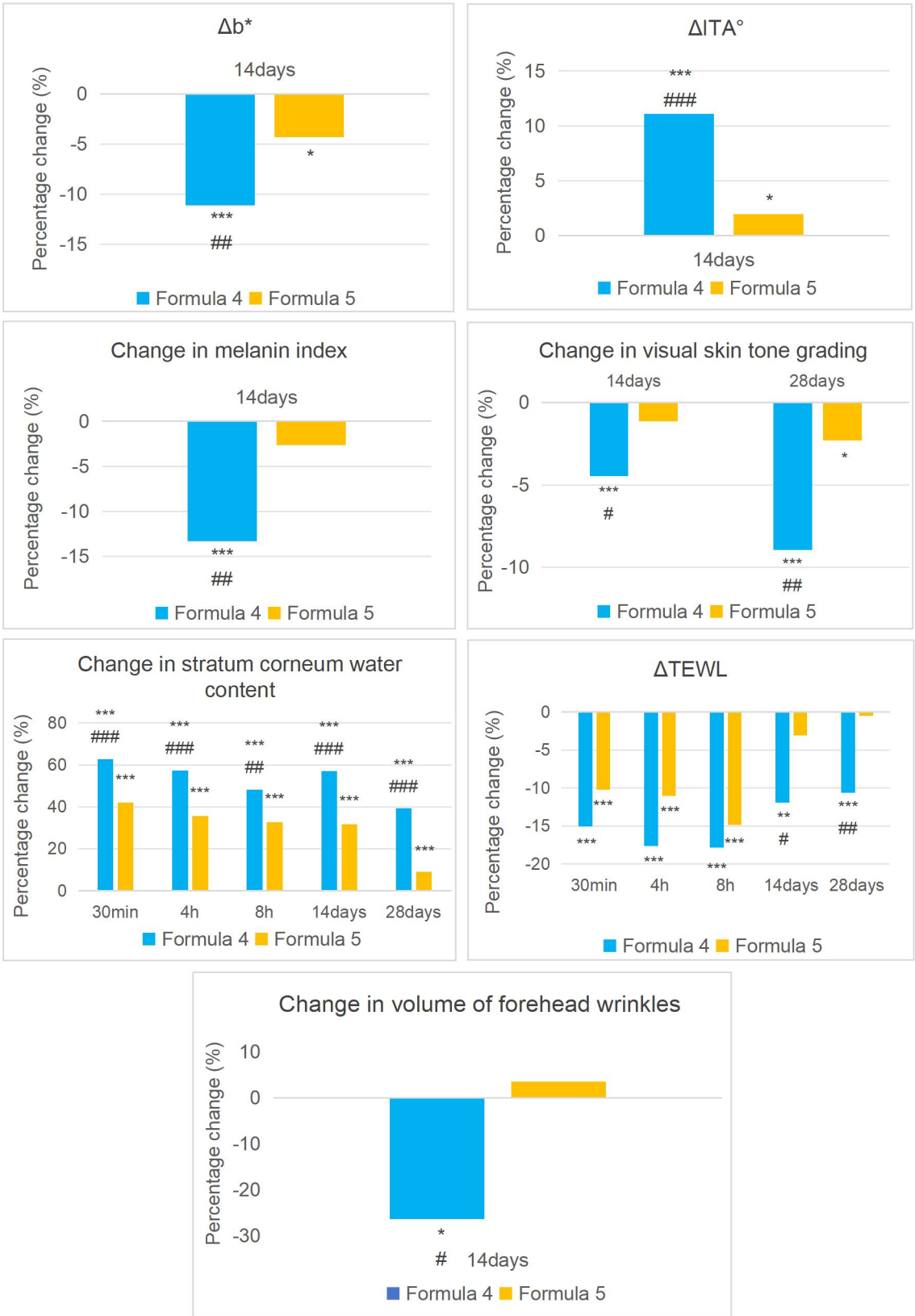

Figure 5 Δb*, ΔITA°, change in melanin index, change in visual skin tone grading, change in stratum corneum water content, ΔTEWL, and change in forehead wrinkle volume (Trial 2). For b*, ITA°, melanin index, visual skin tone grading, water content in the stratum corneum, TEWL, and forehead wrinkle volume: Formula 4 (or Formula 5) vs baseline, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Formula 4 vs Formula 5: #$p < 0.05$, ##$p < 0.01$, ###$p < 0.001$.

Discussion

VCIP, a derivative of Vitamin C, has attracted attention for its skin whitening and brightening effects. It is also known to promote skin barrier function, stimulate collagen synthesis, and exhibit anti-aging properties [9,10]. However, its efficacy is often considered less robust than that of pure Vitamin C [11]. To enhance its performance, we employed advanced surfactant-based nano-emulsification technology to encapsulate VCIP.

Encapsulated VCIP in sodium surfactin-based nanocarriers significantly improves its thermal and UV stability, with a 23% increase under 50°C conditions and 37% under UV exposure compared to free VCIP. This protective matrix shields VCIP from heat, UV radiation, and oxidation, extending shelf-life and enhancing bioactivity in cosmetic formulations.

Encapsulation also boosts transdermal absorption, achieving a 10.5× higher 24-hour penetration rate than free VCIP, for the encapsulation matrix could partially modify the structure of the stratum corneum, potentially facilitating the penetration of the active ingredient [12].

In *in vitro* melanin models, VCIP nano-emulsion outperforms free VCIP in increasing skin luminance (L*) and inhibiting melanin production, suggesting improved bioavailability and greater UV stability.

Human trials further validate the superior whitening efficacy of the VCIP nano-emulsion. After 14 days, treated areas show significant reductions in b* and MI values, increased ITA°, and improved visual skin tone grading, outperforming free VCIP even at 10× the concentration, which shows minimal or no whitening effects.

Additionally, the VCIP nano-emulsion enhances skin barrier function and reduces wrinkles, significantly improving stratum corneum hydration, lowering TEWL, and reducing crow's feet for up to 28 days — with more sustained and pronounced benefits compared to free VCIP. These effects are likely attributed to the nano-emulsion's controlled release, enhanced bioavailability, and improved active stability [13].

Conclusion

In conclusion, the encapsulation of VCIP using surfactant-based nano-emulsification technology significantly enhances its stability, transdermal absorption, and bioactivity compared to free VCIP. The nano-emulsion form demonstrates superior whitening, moisturizing, and anti-aging effects in both *in vitro* models and human trials, even at lower active concentrations. These improvements are possibly attributed to better protection against environmental stress, controlled release, and increased skin penetration. Overall, encapsulated VCIP offers a highly effective and multifunctional solution for cosmetic formulations targeting whitening applications.

Conflict of Interest Statement

NONE.

References

1. Taylor, M. B.; Yanaki, J. S.; Draper, D. O.; Shurtz, J. C.; Coglianese, M. Successful Short-Term and Long-Term Treatment of Melasma and Postinflammatory Hyperpigmentation Using Vitamin C with a Full-Face Iontophoresis Mask and a Mandelic/Malic Acid Skin Care Regimen. *J. Drugs Dermatol.* 2013, *12* (1), 45–50.
2. Huh, C.-H.; Seo, K.-I.; Park, J.-Y.; Lim, J.-G.; Eun, H.-C.; Park, K.-C. A Randomized, Double-Blind, Placebo-Controlled Trial of Vitamin C Iontophoresis in Melasma. *Dermatology* 2003, *206* (4), 316–320.
3. D'Angelo Costa, G. M.; Maia Campos, P. M. B. G. Efficacy of Topical Antioxidants in the Skin Hyperpigmentation Control: A Clinical Study by Reflectance Confocal Microscopy. *J. Cosmet. Dermatol.* 2021, *20* (2), 538–545.
4. Ochiai, Y.; Kaburagi, S.; Obayashi, K.; Ujiie, N.; Hashimoto, S.; Okano, Y.; Masaki, H.; Ichihashi, M.; Sakurai, H. A New Lipophilic Pro-Vitamin C, Tetra-Isopalmitoyl Ascorbic Acid (VC-IP), Prevents UV-Induced Skin Pigmentation through Its Anti-Oxidative Properties. *J. Dermatol. Sci.* 2006, *44* (1), 37–44.
5. Maia Campos, P. M. B. G.; Gianeti, M. D.; Camargo, F. B.; Gaspar, L. R. Application of Tetra-Isopalmitoyl Ascorbic Acid in Cosmetic Formulations: Stability Studies and in Vivo Efficacy. *Eur. J. Pharm. Biopharm.* 2012, *82* (3), 580–586.
6. Han, X.; Yan, Q.; Gao, N.; Kang, Y.; Li, N.; Zou, A. Biocompatible Lyotropic Nanocarriers for Improved Delivery of Ascorbyl Tetraisopalmitate in Skincare. *Langmuir* 2025, *41* (5), 3278–3290.
7. Rathnasinghe, N. L.; Kaushani, K. G.; Rajapakshe, P. S.; De Silva, A.; Jayasinghe, R. A.; Liyanage, R. N.; Tissera, N. D.; Wijesena, R. N.; Priyadarshana, G. Current Trends on Unique Features and Role of Nanomaterials in Personal Care Products. *Cosmetics* 2024, *11* (5), 152.
8. Rosen, T.; Givens, J. Public Interest in Skin Lightening across the United States, January 2015–December 2020. *J. Cosmet. Dermatol.* 2022, *21* (5), 1931–1935.
9. Yokota, M.; Yahagi, S. Evaluation of the Anti‐wrinkle Effect of a Lipophilic Pro‐vitamin C Derivative, Tetra‐isopalmitoyl Ascorbic Acid. *J. Cosmet. Dermatol.* 2022, *21* (8), 3503–3514.
10. Fossa Shirata, M. M.; Maia Campos, P. M. B. G. Sunscreens and Cosmetic Formulations Containing Ascorbyl Tetraisopalmitate and Rice Peptides for the Improvement of Skin Photoaging: A Double‐blind, Randomized Placebo‐controlled Clinical Study. *Photochem. Photobiol.* 2021, *97* (4), 805–815.
11. Maia Campos, P. M. B. G.; Gonçalves, G. M. S.; Gaspar, L. R. *In Vitro* Antioxidant Activity and *in Vivo* Efficacy of Topical Formulations Containing Vitamin C and Its Derivatives Studied by Non‐invasive Methods. *Skin Res. Technol.* 2008, *14* (3), 376–380.
12. Fluhr, J. W.; Feingold, K. R.; Elias, P. M. Transepidermal Water Loss Reflects Permeability Barrier Status: Validation in Human and Rodent *in Vivo* and *Ex Vivo* Models. *Exp. Dermatol.* 2006, *15* (7), 483–492.
13. Shi, S.; Zhang, J.; Quan, S.; Yang, Y.; Yao, L.; Xiao, J. A Highly Biocompatible and Bioactive Transdermal Nano Collagen for Enhanced Healing of UV-Damaged Skin. *Int. J. Biol. Macromol.* 2024, *272*, 132857.