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Original Research Article

Formulation and Evaluation of Vitamin C Peel-off Masks Using Different Skin Penetration Enhancers

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ABSTRACT

Vitamin C is widely used as antioxidant in various cosmetic products; however, its effectiveness is limited by poor skin permeability. *Aloe vera*, vitamin E, Tween-20, and Tween-80 have been reported as skin penetration enhancers (SPEs). Nevertheless, their effectiveness as vitamin C enhancers has not been extensively studied. This study aimed to compare the effectiveness of the aforementioned SPEs in enhancing the permeation of vitamin C in peel-off mask formulations using Franz diffusion method. Several peel-off mask formulations containing vitamin C and different SPEs at concentrations of 0.5% and 2% were prepared and evaluated for their characteristics, stability, and vitamin C permeation using porcine ear skin. All formulations exhibited a pH range of 4.19 - 4.33, a viscosity range of 18802 - 113094 cps, and a drying time of approximately 18 minutes. After 45 days of storage at 4 °C, room temperature and 44 °C, vitamin C concentrations and viscosity values generally decreased, while pH remained relatively stable. All formulations demonstrated higher permeation compared to the control group, with the greatest permeation-enhancing effect observed in the 2% vitamin E formulation (J_{ss} = 21.06 ± 2.17 μg/cm^2/min; Kp = 5 ± 0.51 x 10^{-4} cm/min; ER = 1.749). This *in vitro* study concludes that 2% vitamin E was the most effective SPE for enhancing vitamin C permeation in peel-off mask formulations.

Keywords: Vitamin C, Skin penetration enhancers, Peel-off mask, *In vitro* permeation study, Stability

Introduction

Cosmetics have increasingly become everyday necessities, not only for older adults but also for the younger generation. They are used not only to enhance appearance but also to improve, protect, cleanse, and maintain various parts of the body. Cosmetics can boost self-esteem, particularly in social situations where physical appearance plays a dominant role.[1] Among the wide range of cosmetics available in the Asian market, skincare products—particularly anti-aging and skin-whitening items—have seen a notable increase in demand.[2,3] This trend may be influenced by media-driven beauty standards, where pale, flawless-skinned models frequently dominate the media.[4]

One of the compounds known to promote skin fairness is L-ascorbic acid, commonly referred to vitamin C. Vitamin C is widely used as an anti-pigmentation agent by interacting with copper ions at the active sites of tyrosinase, thus inhibiting the conversion of tyrosine into melanin, and resulting in a fairer skin tone.[5,6] In addition, vitamin C also demonstrates anti-aging properties by inhibiting free-radical damage to the skin,[5] increasing collagen synthesis, stabilizing collagen fibers, and reducing collagen degradation, which helps to prevent wrinkles.[7,8]

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Unfortunately, the human body is incapable of synthesizing vitamin C due to mutations in the *L-gulono-γ-lactone oxidase* gene, which encodes the enzyme responsible for catalyzing the final step of vitamin C biosynthesis.[9,10] Therefore, vitamin C must be obtained from external sources such as fruits, vegetables, or dietary supplements.

Despite its potential as an anti-aging and skin-whitening agent, vitamin C is limited by its hydrophilic properties. Hydrophilic vitamin C has a reduced ability to penetrate deeply into the skin and is unstable when exposed to light, oxidation, and elevated temperatures.[11,12] Therefore, to enhance the delivery of vitamin C to deeper layers of the skin, various methods and formulations have been developed. One practical approach is the subcutaneous injection of vitamin C, although this method still lacks sufficient evidence and produced contradictory findings.[13] Direct injection into the dermis is believed to provide optimal delivery to the target site in a short time. However, this method is relatively expensive, invasive, less comfortable, requires the assistance of a healthcare practitioner, and may trigger hypersensitivity reactions.[13]

On the other hand, topical formulations of vitamin C are more practical, although they exhibit poor skin penetration.[12] Lowering the formulation pH below 3.5 has been reported to improve vitamin C stability and permeability.[6] However, formulations with too low pH levels may lead to skin irritation.[14] A review by Sasidharan *et al.* on ascorbic acid derivatives discussed several modified forms of ascorbic acid, including sodium ascorbyl phosphate (SAP), magnesium ascorbyl phosphate (MAP), ascorbyl palmitate, ascorbyl tetraisopalmitate, ascorbyl glucoside, tetrahexyldecyl ascorbate, sodium ascorbate, and 3-O-ethyl ascorbic acid.[15] While most of these derivatives exhibit improved stability compared to the parent form, their transdermal penetration, conversion into active ascorbic acid, and clinical efficacy have yet to be fully established. Another study by Lee *et al.* demonstrated enhanced topical delivery of vitamin C using laser and microdermabrasion techniques.[16] Nevertheless, both derivatization processes and advanced

delivery techniques are often complex, costly, and may not guarantee the effective release of the parent L-ascorbic acid at the target site.

Since the introduction of skin penetration enhancers (SPEs) to facilitate drug delivery through the skin, numerous topical formulations containing SPEs have been studied and developed.[17,18] Their mechanisms are reported to involve alterations in the structure of stratum corneum lipids, interactions with intercellular proteins that induce relaxation of the cytoplasmic matrix, or modulation of the partitioning of the drug, solvent, or co-enhancer into the stratum corneum.[19-21] Several substances that have been reported to enhance the penetration of active compounds into the skin include *Aloe vera* gel,[22] vitamin E,[23] Tween-20, and Tween-80.[17,24]

A recent review by Liston *et al.* revealed that various types of SPEs have been used in the topical delivery of vitamin C, including amino acids classes, colloids, esters, fatty acids, non-ionic surfactants, phospholipids, polyols, polymers, and terpenes.[12] However, the data were collected from independent studies, which complicates the comparison of the results due to variations in experimental conditions and formulations. Furthermore, to the best of the authors' knowledge, there is currently no literature addressing the permeation-enhancing effects of vitamin E and *Aloe vera* in the topical delivery of vitamin C.

In this study, several vitamin C peel-off mask formulations incorporating different SPEs—including *Aloe vera* gel, vitamin E, Tween-20, and Tween-80—will be evaluated for their effectiveness in improving the penetration of vitamin C. Peel-off masks serve as a cosmetic delivery system that allows prolonged contact between the active ingredient and the skin, while also enhancing penetration through skin hydration.[25,26] The final formulations will be characterized based on drying time, pH, viscosity, and stability. Most importantly, the ability of *Aloe vera* gel, vitamin E, Tween-20, and Tween-80 to enhance vitamin C penetration will be evaluated *in vitro* using Franz diffusion methods. The objective of this study is to provide valuable insights into the most effective and practical peel-off mask formulation for delivering vitamin C to the deeper layers of the skin, with potential applications as a skin-whitening product.

Materials and Methods

Materials

L-ascorbic acid (pharmaceutical grade) was purchased from Shandong Luwei Pharmaceutical Co. Ltd. (Shandong, China). Polyvinyl alcohol BP 24 was obtained from Chang Chun Petrochemical Co., Ltd. (Taiwan). Xanthan gum (food grade) was acquired from Qingdao Rich Trading Co., Ltd. (Qingdao, China). Sodium hyaluronate (cosmetic grade) with a molecular weight of 1.25 x 10^6 Da was obtained from Shandong Topscience Biotech Co., Ltd. (Shandong, China). Glycerin and propylene glycol were purchased from Shandong Baovi Energy Technology Co., Ltd. (Shandong, China) and Dongying City Longxing Chemical Co., Ltd (Shandong, China). PEG 40 hydrogenated oil was obtained from Haihang Industry Co., Ltd. (Shandong, China). Citric acid, sodium citrate, and ethyl alcohol were sourced from Merck (Germany). DL-α-tocopherol acetate (vitamin E) and phosphate buffer saline (pH 7.4) were purchased from Sigma Aldrich (US). Methyl paraben was purchased from Gujarat Organics (Mumbai, India). The green tea fragrance (cosmetic grade) was acquired from Java Soap (Indonesia). Tween-20 and Tween-80 was purchased from Merck (Germany).

Plant collection

Aloe vera was purchased from Farmers Market, North Jakarta, Indonesia, and was identified by Herbarium Bandungense SITH ITB from Bandung Institute of Technology, Indonesia (voucher number: 4877).

Preparation of Aloe vera gel freeze-dried powder:

Aloe vera gel freeze-dried powder was prepared by extracting the gel from *Aloe vera* leaves, which were then cut into small pieces and freeze-dried. The freeze-dried *Aloe vera* gel was pulverized using a hand blender for 30 seconds and stored in the dark inside a desiccator until further use.

Preparation of Vitamin C peel-off masks:

The peel-off masks were prepared according to previously published methods, with some modifications.[27] Polyvinyl alcohol (PVA) was soaked in water one day prior to use to ensure complete swelling. The swollen PVA was then heated to 80–90°C with constant stirring. Meanwhile, citric acid and sodium citrate were dissolved in glycerin. L-ascorbic acid, sodium hyaluronate, propylene glycol, and PEG 40 hydrogenated oil were gradually added to the PVA solution with continuous stirring. Various types of skin penetration enhancers (SPEs) at different concentrations were then incorporated into the mixture. Subsequently, the mixture containing citric acid, sodium citrate in glycerin, xanthan gum, and methyl paraben was added consecutively, and the mixture was stirred until homogeneous. Finally, alcohol and green tea fragrance were added to the final mixture. The components of the formulation are presented in Table 1.

Table 1: Formulation of Vitamin C Peel-Off Masks with Different Types of SPEs

Component	F1a (w/w)	F1b (w/w)	F2a (w/w)	F2b (w/w)	F3a (w/w)	F3b (w/w)	F4a (w/w)	F4b (w/w)
PVA	12%	12%	12%	12%	12%	12%	12%	12%
Sodium hyaluronate	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%
Propylene glycol	2%	2%	2%	2%	2%	2%	2%	2%
Glycerin	4%	4%	4%	4%	4%	4%	4%	4%
PEG 40 hydrogenated oil	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%
Ethyl alcohol	10%	10%	10%	10%	10%	10%	10%	10%
L-ascorbic acid	5%	5%	5%	5%	5%	5%	5%	5%
Citric acid	0.34%	0.34%	0.34%	0.34%	0.34%	0.34%	0.34%	0.34%
Sodium citrate	3%	3%	3%	3%	3%	3%	3%	3%
SPEs	*Aloe vera* 2%	*Aloe vera* 5%	Vit E 2%	Vit E 5%	Tween-20 2%	Tween-20 5%	Tween-80 2%	Tween-80 5%
Fragrance	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
Xanthan gum	0.3%	0.3%	0.3%	0.3%	0.3%	0.3%	0.3%	0.3%
Methyl paraben	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%
Water	Up to 100%							

Notes: F1a: formula containing *Aloe vera* 2%; F1b: formula containing *Aloe vera* 5%; F2a: formula containing Vit E 2%; F2b: formula containing Vit E 5%; F3a: formula containing Tween-20 2%; F3b: formula containing Tween-20 5%; F4a: formula containing Tween-80 2%; F4b: formula containing Tween-20 5%

Physical properties evaluation of peel-off masks:
Drying time
The drying time was assessed by applying 1 gram of each peel-off mask formulation onto the back of the subject's hand, covering a surface area of 37.5 cm². Drying time is defined as the duration required for the formulation to dry completely and be easily peeled off the skin.

Viscosity
The viscosity of each formulation was measured using a B-One Touch LR viscometer (Lamy Rheology Instruments, France). Spindle L-3 was used for all formulations, except for formula 1b, which was measured using spindle L-4. The spindle speed was set to 20 rpm for 60 seconds. Measurements were performed in triplicate, and the results are expressed as the mean value ± SD.

pH
One gram of each formulation was diluted with 10 mL of deionized water, and the pH was measured using an OHAUS pH meter.

Organoleptic testing
The colour and scent of each formulation were observed by three assessors and recorded immediately after preparation.

Stability testing
Approximately 150 g of each formulation was stored in plastic clips covered with aluminum foil under three conditions: 4 °C, room temperature (RT), and 44 °C in an oven, for 45 days. Changes in physical properties were observed and recorded. The viscosity of the stored samples was measured after a 1-hour equilibrium period at RT. Additionally, the concentration of vitamin C before and after storage was determined using a UV spectrophotometer model UV-1280 (Shimadzu, Japan) at a wavelength of 264 nm. A 100 mg sample of each formulation was dissolved in 30 mL of deionized water and then centrifuged at 2,000 rpm for 5 minutes. The resulting supernatant was diluted 20 times before measurement. Vitamin C concentration was determined by plotting the absorbance against a standard calibration curve. The percentage of vitamin C deterioration was calculated using the following equation 1:

$$\% \text{ deterioration of vitamin C} = \frac{\text{concentration of vitamin C after 45 days' storage}}{\text{initial concentration}} \times 100\% \quad \ldots\ldots\ldots\ldots (1)$$

In vitro permeation study:
Skin preparation
The permeation study was conducted according to previously reported methods, with some modifications.[28,29] Porcine ears from four different donors were obtained from freshly slaughtered animals at a local slaughterhouse (Jakarta, Indonesia) and were immediately washed with tap water and soap to remove any dirt. The hair was then shaved, and the skin was carefully separated from the cartilage using a scalpel. The obtained skin was cut into 25 mm-diameter discs using surgical scissors and immersed in deionized water heated to 75°C for 90 seconds. Subcutaneous fat was removed with a scalpel, ensuring that the skin thickness was maintained at approximately 2 mm. The skin discs were gently dried with clean cotton and inspected for any physical damage before being transferred to a Petri dish containing phosphate-buffered saline (PBS, pH 7.4) for 1 hour prior to measurement.

Donor compartment
The permeation study was conducted using Franz diffusion cells with a surface area of 2.5 cm². A 100 mg sample of each peel-off mask formulation was applied to the skin in the donor compartment, which was then sealed with Parafilm to protect it from atmospheric exposure. The receptor chamber was filled with PBS (pH 7.4) and equipped with a magnetic stirring bar. The system temperature was maintained at 32±1 °C using water circulation to simulate skin surface temperature.[30] The donor chamber was placed on top of the receptor chamber, and the interface was sealed with Parafilm.

Kinetic studies
The receptor solution was stirred at approximately 600 rpm. At predetermined time intervals (15, 20, 25, 30, and 35 minutes), 0.5 mL of the receptor solution was collected and immediately replaced with fresh PBS (pH 7.4) maintained at 32 ± 1°C. Each sample was transferred into aluminum foil-covered test tubes and diluted with PBS (pH 7.4) for drug analysis.

Evaluation
The concentration of vitamin C that permeated the skin was immediately measured using a UV spectrophotometer model UV-1280 (Shimadzu, Japan) at a wavelength of 264 nm. Absorbance values were plotted against a standard curve to determine the vitamin C concentration in each sample. The cumulative amount of vitamin C permeated (Q, µg/cm²), the steady-state flux (J_{ss}, µg/cm²/min), and the permeability coefficient (K_p, cm/min) for each formulation were calculated as described elsewhere.[31]
The cumulative amount of vitamin C (Q) was expressed as the mean ± SD and plotted as a function of time (t, min). The steady-state flux (J_{ss}) was derived from the slope of the linear portion of the permeation curve, while the K_p was calculated by dividing J_{ss} by the initial concentration of vitamin C in the donor compartment. The enhancement ratio (ER) for each SPE was calculated by dividing the flux (J_{ss}) of the respective formulation by the flux of the control formulation.[32]

Statistical analysis
The data obtained were analyzed using Excel software. Values are expressed as the mean ± SD from a minimum of three replicated measurements. Differences between groups were statistically analyzed using repeated measures ANOVA followed by the Bonferroni *post-hoc* test. A *p*-value <0.05 was considered statistically significant.

Results and Discussion

In this study, several vitamin C peel-off mask formulations were prepared using four different types of SPEs at two concentrations: 0.5% and 2%. The amount of PVA in each formula was set at 12%, based on preliminary studies that evaluated the effect of PVA on the drying time of the masks. This polymer forms a transparent gel that dries quickly, produces a strong film layer that adheres well to the skin, and is easily removed.[33] Peel-off masks containing 12% PVA exhibited good consistency, acceptable spreadability, and a drying time of less than 25 minutes. This concentration was also consistent with findings from other reported studies using PVA as a film-forming agent.[27]
To form a homogeneous PVA gel system, the PVA was soaked in water overnight and subsequently heated to 80-90 °C while stirring. The resulting vitamin C peel-off masks were clear, light yellow in colour, and had a green tea scent due to the added fragrance. Exceptions were formulas 2a and 2b, which appeared opaque due to the presence of vitamin E oil in the water emulsion (Figure 1). A blank vitamin C peel-off mask, containing no SPEs, was also prepared to serve as a control.
The drying time for each formulation was initially observed to be approximately 18 minutes (Table 2). After this period, all the masks were completely dry and could be easily peeled off the skin. These results fell within the acceptable range for peel-off masks, which typically ranges from 10 to 30 minutes.[34]
It was observed that different SPEs had varying effects on the viscosity of the final peel-off mask formulations. Formula F1b, which contained 2% dried *Aloe vera* gel, exhibited the highest viscosity (113094 ± 9291 cps), whereas F4b, which contained 2% Tween-80, demonstrated the lowest viscosity (18802 ± 506 cps) (Table 2). Chandira *et al*. stated that the optimal viscosity range for peel-off mask formulations is between 7100 and 83144 cps.[35] Almost all prepared formulations met this criterion, with the exception of F1b. The high viscosity of F1b was attributed to the high concentration of dried *Aloe vera* gel, which had

an extraction yield of 1.03%. Therefore, the addition of 2% dried *Aloe vera* gel was anticipated to significantly increase the final viscosity.
The pH values of the prepared vitamin C peel-off masks ranged from 4.19 to 4.33 (Table 2). This slightly acidic pH can be attributed to the presence of vitamin C and the citrate buffer. These values were in good agreement with the natural pH range of the stratum corneum, which is between 4.1 and 5.8.[36] It is recommended to formulate peel-off masks with a pH that closely matches the skin's natural pH to minimize the risk of irritation.[37]

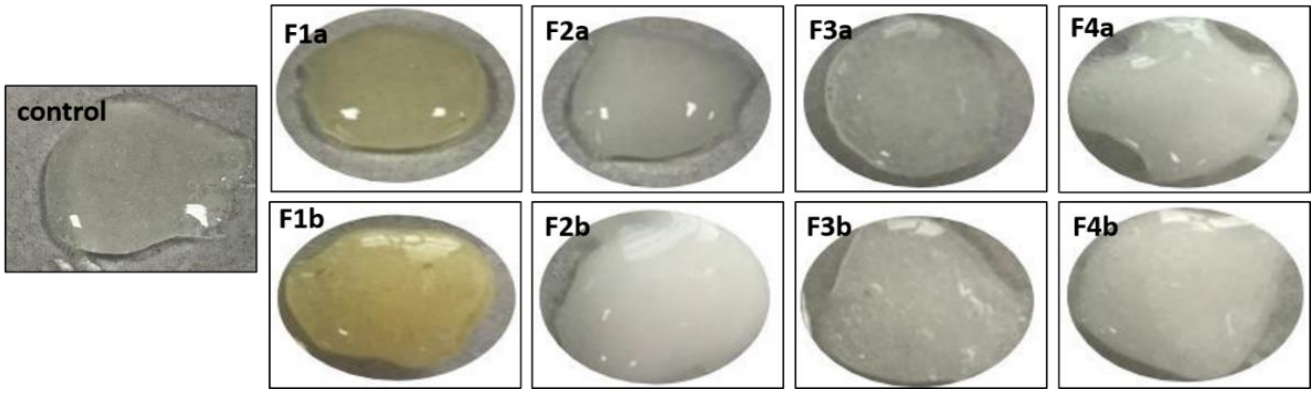

Figure 1: Initial physical appearance of vitamin C peel-off masks

Table 2: Evaluation of The Drying Time, Viscosity, and pH of Vitamin C Peel-Off Mask Formulations Initially and After 45 Days Storage

Formula	Storage Temperature (ºC)	Initial drying time (min, sec)	Final drying time (min, sec)	Initial viscosity (cps)	Final viscosity (cps)	Initial pH	Final pH
	4		18'15"		49427 ± 198		4.29
Control	20~35 (RT)	16'45"	16'45"	53462 ± 1879	27858 ± 511*	4.29	4.33
	44		16'		24143 ± 867*		4.46
	4		18'		65754 ± 1985		4.33
1a	20~35 (RT)	18'	18'	62607 ± 1340	43087 ± 2044*	4.24	4.41
	44		17'		31219 ± 1201*		4.52
	4		20'		126613 ± 9303*		4.21
1b	20~35 (RT)	18'45"	20'30"	113094 ± 9291	89127 ± 6952*	4.33	4.16
	44		17'30"		73944 ± 7607*		4.4
	4		21'		41147 ± 1152*		4.17
2a	20~35 (RT)	18'	18'	52013 ± 353	26555 ± 1162*	4.19	4.22
	44		19'		19046 ± 959*		4.41
	4		18'		42840 ± 899*		4.27
2b	20~35 (RT)	18'	19'	54268 ± 4142	30681 ± 1462*	4.25	4.35
	44		19'		16844 ± 1048*		4.56
	4		20'		39836 ± 895		4.37
3a	20~35 (RT)	18'	20'	40522 ± 1497	22864 ± 1800*	4.28	4.32
	44		17'		16340 ± 321*		4.5
	4		18'		36734 ± 1472*		4.25
3b	20~35 (RT)	18'	18'	43925 ± 1069	24157 ± 1272*	4.24	4.35
	44		17'		17871 ± 2387*		4.52
	4		20'		35053 ± 3354		4.13
4a	20~35 (RT)	18'	18'	24522 ± 195	19022 ± 1243	4.23	4.18
	44		18'		8145 ± 601*		4.39
	4		18'		20474 ± 3283		4.35
4b	20~35 (RT)	17'45"	17'	18802 ± 506	14406 ± 2379	4.29	4.32
	44		17'		10208 ± 1806		4.49

* Statistically different compared to the initial value (p <0.05); RT: Room Temperature

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Stability studies were conducted by storing each peel-off mask at 4 °C, room temperature (20–35 °C), and in an oven at 44 °C for 45 days. At the end of the storage period, each formulation was evaluated for drying time, viscosity, pH, and organoleptic properties. The final drying times exhibited slight variations of 1 to 2 minutes compared to the initial measurements (Table 2) but remained within the acceptable limit of under 30 minutes.[34] In contrast, the viscosity of masks stored at room temperature and 44 °C significantly decreased, while those stored at 4 °C showed no major changes (Table 2). Despite these reductions, the final viscosity values remained within the acceptable viscosity range,[35] except for F1b, which initially exhibited a high viscosity.

Additionally, peel-off masks stored at 44 °C exhibited increased pH values, while those stored at 4 °C and room temperature remained relatively stable (Table 2). The increase in pH at elevated temperatures was likely due to the oxidation of vitamin C into dehydro-L-ascorbic acid,[38] which reduces the acidity of the formulation. This explanation is further supported by a significant reduction in vitamin C concentration in the peel-off masks stored at 44 °C (Figure 2).

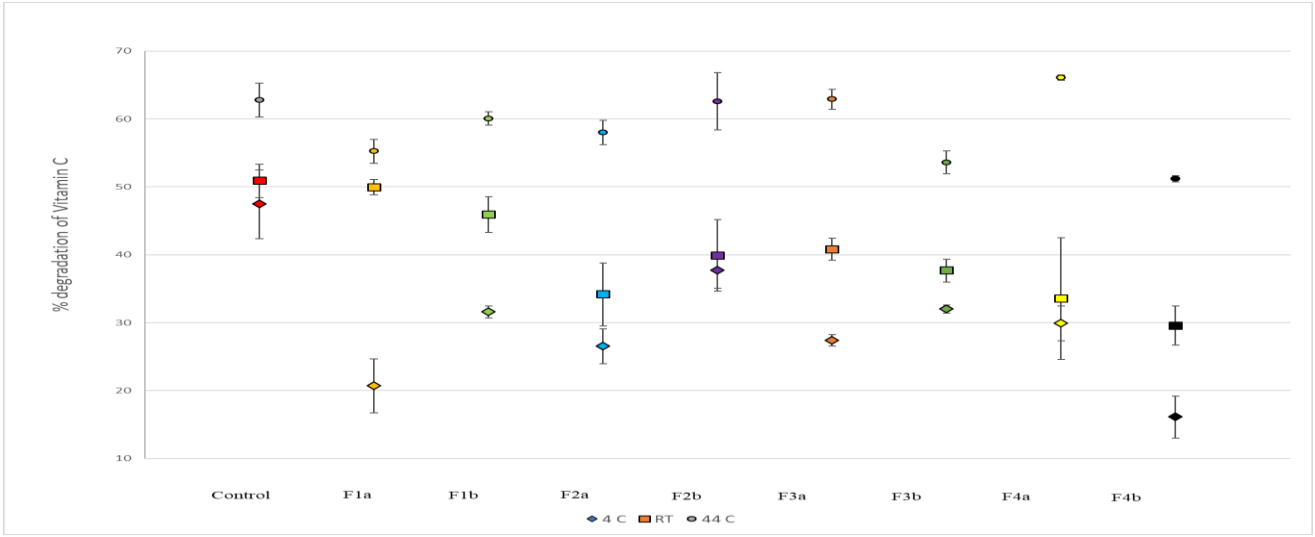

Figure 2: Percentage of vitamin C degradation of the peel-off masks after storage at 4 ˚C (◊); room temperature (□); and 44 ˚C (○)

Vitamin C is highly unstable after 45 days of storage, even at low temperatures (4 °C). The percentage of vitamin C deterioration in each peel-off mask formulation is illustrated in Figure 2. It was observed that an increase in storage temperature resulted in greater degradation of vitamin C, as indicated by the higher percentage of deterioration at 44 °C. In fact, the stability of vitamin C can be affected by several factors, including oxidation in aqueous solutions, high pH levels, elevated temperatures, the presence of dissolved oxygen, and trace amounts of metal ions.[39] Thus, strategies to protect vitamin C from oxidation should be considered in future formulations.

Surprisingly, peel-off masks containing 2% Tween-80 demonstrated the most stable levels of vitamin C. This finding somewhat contradicts other research that reports the oxidation of Tween-20 and Tween-80, which produces hydroperoxides that can decrease vitamin C stability.[40] However, the stabilizing effect of Tween-80 and Tween-20 is likely attributed to their surfactant properties, which allow them to form micelle structures that encapsulate vitamin C and protect it from environmental oxidation.

Changes in the physical appearance of the peel-off masks were also observed after storage at room temperature and 44 °C for 45 days (Figure 3). The formulations turned yellow-brown and developed a burnt caramel and acidic odour (Table 3). These observations provide the evidence of vitamin C oxidation, as its degradation is often accompanied by yellowish discolouration.[41]

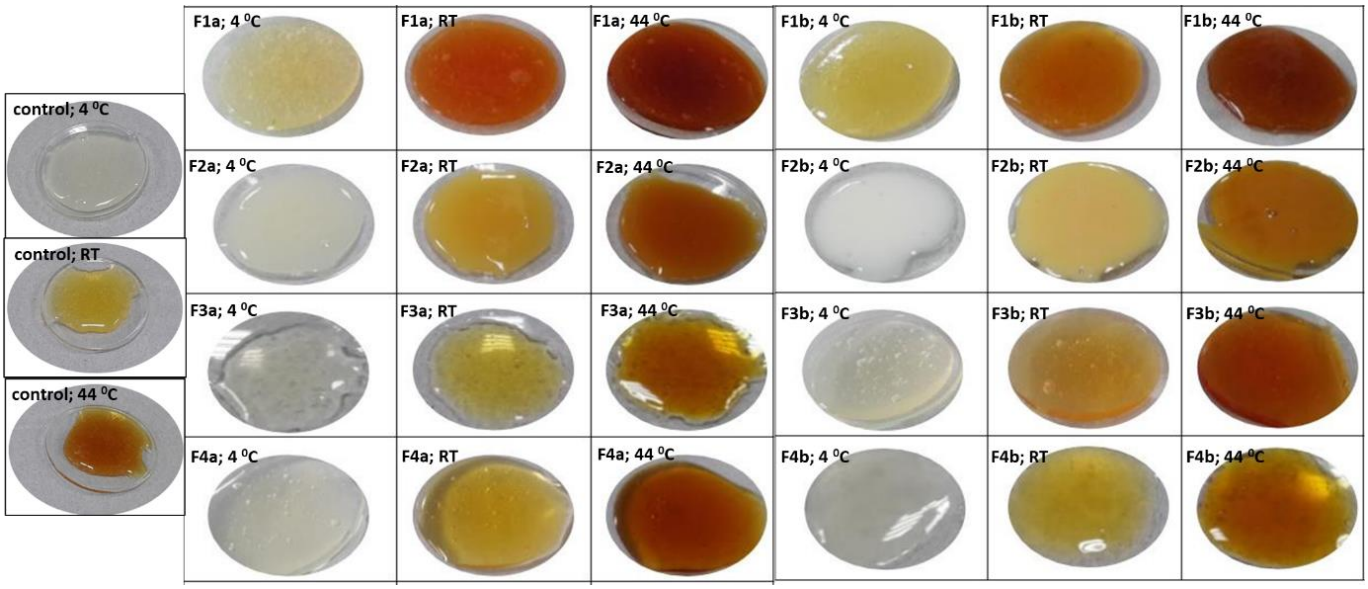

Figure 3: Physical appearance of vitamin C peel-off masks after 45 days storage

Table 3: Organoleptic Evaluation of Vitamin C Peel-Off Mask Formulations Initially and After 45 Days Storage

Formula	Temperature (ºC)	Initial		After storage	
		Colour	Odour	Colour	Odour
Control	4	Clear, translucent	Scent of green tea	Clear, slight yellow	Scent of green tea
	20-35 (RT)			Light orange	Scent of green tea
	44			Brown	Caramel odour
F1a	4	Light yellow	Scent of green tea	Yellow translucent	Green tea
	20-35 (RT)			Brownish orange	Green tea
	44			Burnt brown	Hint of green tea and caramel
F1b	4	Clear yellow	Scent of green tea	Dark yellow	Green tea
	20-35 (RT)			Brownish orange	Green tea
	44			Burnt brown	Hint of green tea, caramel odour
F2a	4	White opaque	Scent of green tea	White opaque	Green tea
	20-35 (RT)			Dark yellow	Green tea
	44			Brown	A hint of green tea, acidic odour
F2b	4	White opaque	Scent of green tea	White opaque	Green tea
	20-35 (RT)			Dark yellow	Green tea
	44			Brown	Green tea
F3a	4	Translucent	Scent of green tea	Translucent yellowish	Green tea
	20-35 (RT)			Orange (honey-like colour)	Hint of green tea
	44			Brown	Hint of green tea, caramel odour
F3b	4	Translucent	Scent of green tea	Translucent yellow	Green tea
	20-35 (RT)			Orange (honey-like colour)	Green tea
	44			Brown	Green tea, acidic smell
F4a	4	Translucent	Scent of green tea	Translucent yellow	Green tea
	20-35 (RT)			Orange (honey-like colour)	Green tea
	44			Brown	A hint of green tea, caramel odour
F4b	4	Translucent	Scent of green tea	Translucent yellow	Hint of green tea
	20-35 (RT)			Orange (honey-like colour)	Green tea
	44			Brown	Hint of green tea, caramel odour

Notes: RT: Room Temperature

The *in vitro* penetration of vitamin C through the skin was evaluated using Franz diffusion cells and fresh full-thickness skin samples obtained from porcine ears. In this study, full-thickness pig ear skin was selected due to its availability, ease of handling, and common use in cosmetic research.[31] The skin samples were used immediately after excision to prevent degradation. PBS (pH 7.4) was employed as the receptor fluid because it mimics the physiological pH of body fluids and can completely dissolve vitamin C.

Figure 4 illustrates the cumulative amount of vitamin C (µg/cm²) penetrated the skin samples from various peel-off mask formulations over time (in minutes). The results are presented as the mean ± SD. It was confirmed that peel-off mask formulations containing SPEs exhibited higher vitamin C penetration profiles compared to the control, consistent with findings from previous studies.[12,23,42] Among the tested formulations, the 2% vitamin E formulation (F2b) demonstrated the highest level of penetration, followed by the 2% Tween-80 (F4b) and 2% Tween-20 formulations (F3b). The steady-state flux (J_{ss}), permeability coefficient (K_p), and enhancement ratio (ER) of vitamin C for the 2% vitamin E formulation (F2b) were 21.06 ± 2.17 µg/cm²/min, 5 ± 0.51 × 10^{-4} cm/min, and 1.749, respectively (Table 4). In comparison, the J_{ss} and K_p values for the control peel-off mask were 12.04 ± 1.47 µg/cm²/min and 2.15 ± 0.26 × 10^{-4} cm/min, respectively.

There have been many attempts to enhance the penetration of vitamin C through dermal applications.[12,16,24] Nevertheless, none of these studies have incorporated vitamin E and *Aloe vera* as SPEs. In this study, vitamin C was delivered through a peel-off mask gel system containing SPEs and several other ingredients designed to improve the penetration of vitamin C. Since the objective of this study was to compare the effectiveness of four different SPEs in enhancing the permeation of vitamin C in the peel-off mask formula, the permeation data obtained from both the control peel-off mask and the peel-off masks containing SPEs were sufficient to determine the most effective SPE among the four tested.

Previously, vitamin E has been reported to enhance the permeation of hydrocortisone in excised cadaver skin.[23] It is hypothesized that vitamin E might intercalate into the lipid bilayer region of the stratum corneum, disrupting the gel-phase lipids, thereby increasing the permeability of the membrane. This mechanism is similar to that of Tween-20 and Tween-80, which also modify the physicochemical properties of membranes.[40] However, the distinction may lie in the micelle formation of Tween-20 and Tween-80, which likely requires more time to interact with the skin membrane. Meanwhile, *Aloe vera* gel has been reported to enhance the partitioning effect of drugs across the skin membrane.[22,43] It is worth mentioning that *Aloe vera* improved the permeation of higher molecular weight (Mw) drugs (e.g., colchicine, Mw 399.44 g/mol), but was less effective in enhancing the permeation of lower Mw drugs (e.g., caffeine, Mw 194.19 g/mol).[43] This phenomenon, referred to as the 'pull effect,' is explained by the ability

of larger Mw drugs to block permeation routes and interact with *Aloe vera* before being transported across the skin. Vitamin C, with a Mw of 176.12 g/mol, is considered a small Mw drug; therefore, its permeation might not be significantly affected by *Aloe vera* gel.
Moreover, the combination of vitamin C and vitamin E for topical application may offer an additional advantage for their antioxidant activity. Caruso *et al*. reported synergistic effect of vitamin C and vitamin E combination in scavenging superoxide radicals.[44] Vitamin C able to regenerates oxidized vitamin E by donating a hydrogen atom to the semiquinone radical, thereby restoring antioxidant capability of vitamin E and allow for continuous scavenging.

Table 4: Permeability Parameters of Vitamin C Peel-Off Masks Formulations

Formula	J_{ss} (µg/cm^2/min)	K_p (cm/min)x10^{-4}	ER
control	12.04 ± 1.47	2.15 ± 0.26	1
1a	11.58 ± 2.39	2.19 ± 0.45	0.962
1b	13.09 ± 2.21	2.64 ± 0.45	1.085
2a	12.33 ± 3.91	2.59 ± 0.82	1.024
2b	21.06 ± 2.17*	5 ± 0.51*	1.749
3a	16.99 ± 2.48	3.83 ± 0.56*	1.411
3b	14.27 ± 2.38	3.11 ± 0.52	1.185
4a	10.98 ± 2.08	2.33 ± 0.44	0.912
4b	12.57 ± 2.94	2.82 ± 0.66	1.04

* Statistically different compared to the control (p <0.05)

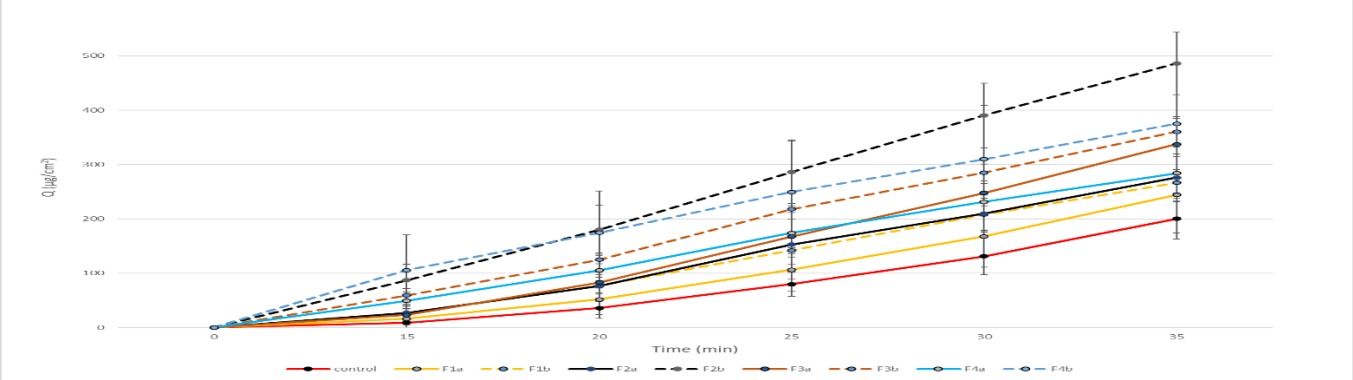

Figure 4: Permeation profile of vitamin C from peel-off masks formulations

Conclusion

In this study, vitamin C peel-off masks were successfully formulated using four different skin penetration enhancers (SPEs)—namely *Aloe vera*, vitamin E, Tween-20 and Tween-80—each at concentrations of 0.5% and 2%. All formulations exhibited acceptable drying times, viscosities and pH for topical application. However, stability studies conducted at 4 ˚C, room temperature and 44 ˚C over 45 days indicated significant vitamin C degradation.
Based on the *in vitro* studies conducted using Franz diffusion method on porcine ear skin, 2% vitamin E demonstrated the highest flux (J_{ss}), permeability coefficient (K_p) and enhancement ratio (ER). This enhancement likely due to its ability to disrupt the lipid bilayer structure of the stratum corneum, thus facilitating greater penetration of vitamin C into the skin. These findings support the use of 2% vitamin E as effective enhancer for vitamin C in peel-off mask formulations. Further optimization is necessary to improve vitamin C stability during storage in order to maximize its skin-whitening effects.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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