FABAD J. Pharm. Sci., 51, 2, 933-950, 2026
Doi: 10.55262/fabadeczacilik.1892600
RESEARCH ARTICLE

Retinol and Vitamin C Encapsulation in Liposome for Topical Delivery Using Microfluidization

Yasemin YAĞAN UZUNER[*°], Bilge DEMİRCİ[**], Neval Sevinç ÖZDEMİR[***]

Retinol and Vitamin C Encapsulation in Liposomes for Topical Delivery Using Microfluidization

SUMMARY

The concurrent topical delivery of Retinol and Vitamin C (Vit C) poses notable formulation challenges due to their contrasting solubility characteristics and chemical stability. In this study, liposomal nanocarriers were engineered via high-pressure microfluidization to achieve co-encapsulation of Retinol and Vit C within a single vesicular system. The formulations were characterized in terms of physicochemical stability (particle size distribution, polydispersity index, zeta potential, scanning electron microscopy (SEM)) and release profiles of the actives by Franz cell diffusion test. Liposomes exhibited nanoscale particle sizes and maintained electrostatic stability over a 60-day period. These results indicated that microfluidization was an easy and appropriate method for producing liposomes. Franz diffusion cell studies revealed sustained release of Vit C from liposomes compared to its simple gel and solutions, while retinol did not show considerable difference, probably due to its limited diffusion ability through the membranes. Collectively, the findings support the feasibility of utilizing phospholipid-based vesicular systems for sustained delivery of chemically distinct bioactives. The developed liposomes offer a promising platform for dermal anti-ageing and antioxidant delivery, warranting further investigation in vivo to substantiate long-term efficacy and better cutaneous compatibility.

Keywords: *Retinol, vit C, liposomes, microfluidization, anti-ageing*

Mikrofluidizasyon Kullanılarak Topikal Kullanım için Lipozom İçinde Retinol ve Vitamin C Enkapsülasyonu

ÖZ

Retinol ve Vitamin C'nin (Vit C) birlikte eşzamanlı topikal uygulanması, karşıt çözünürlük özellikleri ve kimyasal kararlılıkları nedeniyle dikkate değer formülasyon zorlukları içerir. Bu çalışmada, Retinol ve Vit C'nin tek bir veziküler sistem içinde birlikte kapsüllenmesini hedefleyen lipozomal nano taşıyıcılar, yüksek basınçlı mikroakışkanlık (mikrofluidizasyon) yöntemiyle tasarlanmıştır. Formülasyonlar, fizikokimyasal kararlılık (parçacık boyutu dağılımı, polidispersite indeksi, zeta potansiyeli, taramalı elektron mikroskobu (SEM)), biyoaktif maddelerin Franz difüzyon hücresi testi ile salım profilleri açısından karakterize edilmiştir. Lipozomlar nano parçacık boyutlarına sahip olmuş ve 60 gün boyunca elektrostatik kararlılığını sürdürmüştür. Bu bulgular, mikrofluidizasyon yönteminin lipozom üretimi için kolay ve uygun bir yöntem olduğunu göstermiştir. Franz difüzyon hücresi çalışmalarında Vit C'nin lipozomlardan basit çözeltilerine ve jel yapılı ürünlerine göre daha yavaş bir salım sağladığı gözlemlenmişken, retinol membranlardan geçiş yeteneğinin kısıtlayıcılığına bağlı olarak bu konuda önemli bir fark göstermemiştir. Birlikte değerlendirildiğinde, bu bulgular kimyasal olarak farklı biyolojik aktiflerin kademeli salımı için fosfolipit tabanlı veziküler sistemlerin kullanılabilirliğini desteklemektedir. Geliştirilen lipozomlar, dermal yaşlanma karşıtı ve antioksidan taşıyıcılar için umut vadeden bir platform sunmakta olup, uzun vadeli etkinliği ve daha iyi dermal uyumu doğrulamak amacıyla in vivo olarak daha fazla incelemeyi gerektirmektedir.

Anahtar Kelimeler: *Retinol, vit C, lipozom, mikroakışkanlastirma, yaşlanma karşıtı*

Received: 18.02.2026
Revised: 25.06.2026
Accepted: 30.07.2026

[*] ORCID: 0000-0001-6586-4158, Pharmaceutical Technology Department, Faculty of Pharmacy, Acıbadem Mehmet Ali Aydınlar University, İstanbul, Turkey
[**] ORCID: 0009-0009-5787-2970, Faculty of Pharmacy, Acıbadem Mehmet Ali Aydınlar University, İstanbul, Turkey
[***] ORCID: 000-0003-3416-9755, Pharmaceutical Biotechnology Department, Faculty of Pharmacy, Acıbadem Mehmet Ali Aydınlar University, İstanbul, Turkey

° Corresponding Author; Yasemin YAĞAN UZUNER
E-mail: yasemin.uzuner@acibadem.edu.tr

INTRODUCTION

Topical antioxidants are widely employed in dermatology and cosmetology to reduce oxidative damage, promote epidermal and dermal renewal, and activate and support skin barrier function. Among antioxidants, Vit C (ascorbic acid) and Retinol (a vitamin A derivative) are particularly valued for their synergistic actions in stimulating fibroblasts to produce collagen and other extracellular matrix (ECM) proteins, therefore reducing signs of photoaging and enhancing skin tone and texture (Pullar, Carr, & Vissers, 2017; Wohlrab & Kreft, 2014; Zasada & Budzisz, 2019). Despite their individual benefits, the combined use of these two actives in a single topical formulation remains technically challenging due to their markedly different physicochemical properties.

Vitamin C is a water-soluble, pH-sensitive antioxidant with a high tendency towards oxidation when exposed to light, oxygen, and heat, leading to a rapid loss of efficacy. Retinol, conversely, is a lipophilic compound prone to photoisomerization and oxidative degradation under similar environmental conditions (Caritá et al., 2020). Retinoic acid (RA) signals regulate key biological pathways by binding to nuclear receptors that control gene expression. The structure of retinol was first explained by Paul Karrer in the 1930s, who received the Nobel Prize in 1937 for this discovery. Due to its importance in various signaling pathways, Retinoids have been used extensively since the 1960s and 1980s for many reasons; hence, there are a number of clinical trials listed to investigate the involvement of retinoids in many diseases (Vašková, Stupák, Vidová Ugurbaş, Židzik, & Micková, 2025). Retinol is used extensively for skin rejuvenation effects (Pradhan, Kuchekar, Gawade, & Kore, 2024). Although its irritant effects on the skin upon sun exposure are mentioned (Zhong, Zhao, Song, Du, & Shu, 2024), according to the current regulations, retinol is not classified as a photosensitizer.

In the first Scientific Committee for Cosmetics (SCCS) opinion published on Vitamin A in cosmetic products (SCCS/1576/16, finalized 6 October 2016, corrigendum 23 December 2016), the SCCS evaluated the safety of retinol, retinyl acetate and retinyl palmitate at the then - typical use concentrations up to 0.3% RE in face/hand products and up to 0.05% RE in body lotions (SCCS, 2016). The Committee's primary concern was the cumulative systemic exposure to vitamin A from all sources (diet, food supplements and cosmetics) relative to the Tolerable Upper Intake Level (UL) of 3000 μg RE/day established by EFSA/SCF. Importantly, this opinion did not identify retinol as a phototoxic or photosensitizing ingredient, and did not recommend any restriction to night time application. The revised Opinion SCCS/1639/21 (final version adopted 2 / 3 December 2022; published in 2023) reached the same qualitative conclusion (SCCS, 2022). The SCCS stated that vitamin A in cosmetics *"at the concentrations of 0.05% Retinol Equivalent (RE) in body lotion, and 0.3% RE for other leave-on and rinse-off products is safe"*. The probabilistic exposure assessment confirmed that cosmetic use alone does not exceed the UL; the issue is the additional contribution from food and supplements for the 95th-percentile consumer. Again, no photo-irritation, phototoxicity, or nighttime-only restriction was imposed. On 3 April 2024, the European Commission decided to translate the SCCS conclusions into a binding law through the Commission Regulation (EU) 2024/996-, and first-time added retinol, retinyl acetate and retinyl palmitate to Annex III of Regulation (EC) No 1223/2009 (entry no 321–323). The maximum permitted concentrations are 0.05% RE in body lotion and 0.3% RE in other leave-on and rinse-off products, applicable from 1 November 2025, with full market withdrawal of non-compliant products by 1 May 2027. In Annex III, the only mandated warning is: "Contains vitamin A. Consider your daily intake before use". This warning explicitly addresses cumulative dietary intake of vitamin A — not the time of day of cosmetic application. There is no EU or US regulation which prohibits, restricts or even warns against the daytime or daily use of retinol within the Annex III limits.

Furthermore, neither SCCS/1576/16 nor SCCS/1639/21 classifies retinol as a phototoxic, photoallergenic or photogenotoxic substance. The U.S. Cosmetic Ingredient Review (CIR) Expert Panel reached the same conclusion in its safety assessments of *Retinol and Retinyl Palmitate* (originally 1987; re-reviewed 2005 and 2017 (Johnson, 2017). The CIR Panel also concluded that retinol and retinyl palmitate are *"safe as used in cosmetic products"* in current practices of use and concentration, are neither mutagenic nor carcinogenic, and — following a detailed review of the 2012 NTP photocarcinogenicity study - that the methodological flaws of that study did not support an additional photo-related risk for these ingredients.

The clinical phenomenon often colloquially described as "retinol-induced sun sensitivity" is more accurately characterised as transient photo-irritation, secondary to accelerated corneocyte turnover and a temporarily thinner stratum corneum during the retinisation phase. It is a barrier-mediated effect, not a chemical photosensitisation reaction, and it is well addressed by the standard cosmetic instruction to use a broad-spectrum sunscreen. This is independent of the time of day at which retinol is used. Vitamin C contributes photoprotection because it reacts synergistically, not antagonistically, with retinol. It is one of the most potent endogenous and topically applicable antioxidants in human skin. A substantial body of peer-reviewed work is available to explain its effects. Pullar et al. (2017), Telang (2013) and many other researchers demonstrated that topical L-ascorbic acid and stabilised derivatives such as tetrahexyldecyl ascorbate, sodium ascorbyl phosphate and ascorbyl glucoside quenches UVA- and UVB-generated reactive oxygen species (ROS), including superoxide, hydroxyl and peroxyl radicals (Kaźmierczak-Barańska, Boguszewska, Adamus-Grabicka, & Karwowski, 2020), reduces UV-induced erythema, thymine-dimer formation and apoptotic sunburn-cell formation (Darr, Dunston, Faust, & Pinnell, 1996; Lin et al., 2005). Vit C down-regulates UV-driven pro-inflammatory cytokines (TNF-α, IL-1, IL-6) and matrix-metalloproteinase expression (Cárcamo, Pedraza, Bórquez-Ojeda, & Golde, 2002) and regenerates oxidised α-tocopherol and stabilises the cutaneous antioxidant network (Burke, 2007). In fact, Vit C is not a UV filter but a post-absorption antioxidant; its photoprotective role is additive to the keratinocyte-differentiation and dermal remodelling effects of retinol. When the two actives are present in the same daytime application, vitamin C reduces the ROS-driven oxidative stress to which the slightly thinner, retinised stratum corneum would otherwise be more susceptible to light. Therefore, co-formulation can work like an antioxidant-pairing system similar to the concept of the well-established ascorbic acid / α-tocopherol / ferulic acid combinations (Lin et al., 2005; Murray et al., 2008). Clinical evidence supports the tolerability and efficacy of retinol + vitamin C, and co-application or co-formulation is not contraindicated. An open-label clinical study demonstrated the Efficacy and Tolerance of a Retinol and Vitamin C Facial Regimen in Women With Mild-to-Moderate Hyperpigmentation and Photodamaged Facial Skin (Herndon, Jiang, Kononov, & Fox, 2016). In this 12-week study, 0.5% encapsulated retinol was used together with a 30% tetrahexyldecyl-ascorbate that demonstrated statistically significant improvements in fine lines, tone, brightness, and pigmentation, with good tolerability and no photo-irritation signal. Another study, reviewed the additive clinical benefit of pairing ascorbic acid with retinoids (Farris, 2005), and Sorg, Antille, Kaya and Saurat, discussed concurrent use of retinoids with antioxidants (Sorg et al., 2006).

There are retinol + vitamin C serums and creams formulated and commercially available, particularly those employing stabilised retinol forms (microencapsulated retinol, retinyl propionate, hydroxypinacolone retinoate) together with oil-soluble ascorbate derivatives (THD ascorbate, ascorbyl tetraisopalmitate) at near-neutral pH. The properties of both actives complicate co-formulation efforts, mostly resulting in chemical instability, phase separation, or fast reduction of their bioactivity (Elsayed, Abdallah, Naggar,

& Khalafallah, 2007; Pando, Matos, Gutiérrez, & Pazos, 2015; Touitou, Dayan, Bergelson, Godin, & Eliaz, 2000). The formulation of a stable system which effectively delivers both compounds to the skin is still a formulation challenge. To overcome these limitations, nanoscale vesicular systems, particularly liposomes and ethosomes, have been explored for their ability to protect sensitive molecules and enhance dermal penetration (Dragicevic-Curic, Scheglmann, Albrecht, & Fahr, 2008; Touitou et al, 2000). Liposomes are structures that have a bilayer membrane structure composed of microscopic phospholipid bubbles. They are composed of cholesterol and non-toxic natural phospholipids, and their shell consists of a double layer of lecithin building blocks (Lombardo & Kiselev, 2022). Liposomes are biocompatible, phospholipid-based bilayers that can encapsulate both hydrophilic and lipophilic molecules within their aqueous core and lipid membrane shell, respectively. Liposomes are the most suitable method for encapsulating hydrophilic and lipophilic drugs, and they are appropriate for ligand binding and biocompatibility (De Leo Maurelli, A. M., Giotta, L., & Catucci, 2022; Dymek & Sikora, 2022). Additionally, among the types of nano carriers, the structures that most resemble live skin cells are liposomes, and their sizes range from 0.025 to 2.5 μm (Nsairat et al., 2022). Although the delivery of Vitamin C or Retinol through liposomes has been studied by many researchers, their combined encapsulation in liposomes and their release profiles have not been studied extensively. In this study, we developed dual-loaded liposomal formulations using high-pressure microfluidization to co-encapsulate Retinol and Vitamin C, and conducted a systematic evaluation of their physicochemical properties, encapsulation efficiency, *in vitro* release behavior, and antioxidant activity. Release profiles of liposomal Retinol and Vit C were compared with simple dispersion of the same ingredients to investigate the sustained release opportunity of the liposomal actives. The slower release of these anti-aging actives will provide not only a longer duration of activity, but also a better cutaneous compatibility due to lower exposure of the skin. Retinol together with Vit C will offer many benefits as anti-ageing actives due to this co-delivery by the same nano-delivery system.

MATERIALS AND METHODS

Materials

Retinol (Retinol 50 C, BASF SE, 67056 Ludwigshafen, Germany), Vitamin C (3-O-Ethyl Ascorbic Acid; Et-VC®, Corum, Chiayl Country 621017, Taiwan), Lipoid P75 (75% soybean phosphatidylcholine, "Lipoid" GmbH, Ludwigshafen, Germany), Vitamin E (Tocopheryl Acetate, BASF SE, 67056 Ludwigshafen, Germany), Ultrez 20 (Acrylates/C10-30 alkyl acrylate crosspolymer; Lubrizol, USA) were used as received from the suppliers. The phosphatidylcholine sources screened for selection were Phospholipon 85G (>85% phosphatidylcholine; Phospholipid, USA), Epikuron 170 (soybean phosphatidylcholine; Cargill, USA), and Lipoid P75 (>70% phosphatidylcholine; Lipoid GmbH, Germany). Phosphate-buffered saline (PBS, pH 7.4) was used as the receptor phase in diffusion studies. Strat-M® (Merck Millipore, USA) and cellulose acetate membranes (Spectra/Pore, MWCO: 12–14 kDa) were utilized for *in vitro* diffusion experiments.

Preparation of liposomal formulations

A liposomal formulation was designed to contain phosphatidylcholine to form the shell of the liposome. The conventional methods for preparing liposomes, such as thin-film hydration, injection of solvent, and emulsification are all based on the spontaneous self-assembly of lipids. These methods generally yield multilamellar vesicles and heterogeneous size distribution, and have poor reproducibility (Arangüren, Torres, Muñoz-Camargo, Osma, & Cruz, 2020). Microfluidics is a relatively new technology used for the production of nanoparticles and liposomes due to its features, which help to overcome the limitations of other methods. By changing the parameters like flow rates, phospholipid concentration, temperature, and the geometry of the microfluidic reactor, controllable sizes can be obtained (Arangüren et al., 2020).

Liposomes were prepared to select the most suitable PC source. Three PC sources of different origin were compared: animal-based (egg yolk, Phospholipon 85G), plant-based (soybean, Epikuron 170), and synthetic (Lipoid P75). Liposomes were prepared using the "cold method", in which all formulation components were mixed in water with a mechanical stirrer at room temperature (Uzuner, Sevinç, & Kanlidere, 2026). Liposomal formulations contained 10% (w/v) phosphatidylcholine and 0.5% (w/v) vitamin E, and water made up the mixture to 100%. The mixtures were stirred with a mechanical stirrer at 300 rpm, and then passed through a microfluidisation device. In total, twenty-seven liposome formulations were prepared using different sources of PC, under different pressures, and cycled 1,3 or 5 times through the microfluidiser (Microfluidics M110-P, Newton, MA, USA), as shown in Table 1.

Table 1. Formulations of liposomes prepared with different sources of lecithin (Sevinç, 2020)

Brand	CODE	Deionized Water (%)	Lecithin (%)	Vitamin E (%l)	Pressure(bar)	Cycle
Lipoid P75	F1	89.5	10	0.5	500	1
	F2	89.5	10	0.5	500	3
	F3	89.5	10	0.5	500	5
	F4	89.5	10	0.5	750	1
	F5	89.5	10	0.5	750	3
	F6	89.5	10	0.5	750	5
	F7	89.5	10	0.5	1000	1
	F8	89.5	10	0.5	1000	3
	F9	89.5	10	0.5	1000	5
Epikron 170	F10	89.5	10	0.5	500	1
	F11	89.5	10	0.5	500	3
	F12	89.5	10	0.5	500	5
	F13	89.5	10	0.5	750	1
	F14	89.5	10	0.5	750	3
	F15	89.5	10	0.5	750	5
	F16	89.5	10	0.5	1000	1
	F17	89.5	10	0.5	1000	3
	F18	89.5	10	0.5	1000	5
Phospholipon 85 G	F19	89.5	10	0.5	500	1
	F20	89.5	10	0.5	500	3
	F21	89.5	10	0.5	500	5
	F22	89.5	10	0.5	750	1
	F23	89.5	10	0.5	750	3
	F24	89.5	10	0.5	750	5
	F25	89.5	10	0.5	1000	1
	F26	89.5	10	0.5	1000	3
	F27	89.5	10	0.5	1000	5

The stability of the empty liposome formulations that were kept at 25±2 °C was monitored for 14 days for mean particle size, and uniformity changes. Based on these preliminary results as presented in Table 2, Lipoid P75 was selected as the best PC, and formula F9 produced the most stable liposome formula; the pressure used was 1000 bars, and the number of cycles (number of times recirculated through the microfluidics device) was 5.

Table 2. Particle size and uniformity analysis of empty liposomes (Sevinç, 2020)

	1 day	**5 day**	**7 day**	**14 day**		**1 day**	**5 day**	**7 day**	**14 day**
CODE	**d (0.5) (μm)**				**CODE**	**Uniformity**			
F1	The stability was not recorded.				F1	The stability was not recorded.			
F2	The stability was not recorded.				F2	The stability was not recorded.			
F3	0.199	0.195	0.202	0.199	0.195	0.202	0.199	0.195	0.202
F4	The stability was not recorded.				F4	The stability was not recorded.			
F5	The stability was not recorded.				F5	The stability was not recorded.			
F6	0.208	0.211	0.200	0.208	0.211	0.200	0.208	0.211	0.200
F7	The stability was not recorded.				F7	The stability was not recorded.			
F8	The stability was not recorded.				F8	The stability was not recorded.			
F9	0.205	0.212	0.200	0.205	0.212	0.200	0.205	0.212	0.200
F10	The stability was not recorded.				F10	The stability was not recorded.			
F11	The stability was not recorded.				F11	The stability was not recorded.			
F12	0.279	0.277	0.256	0.279	0.277	0.256	0.279	0.277	0.256
F13	The stability was not recorded.				F13	The stability was not recorded.			
F14	The stability was not recorded.				F14	The stability was not recorded.			
F15	0.253	0.277	0.252	0.253	0.277	0.252	0.253	0.277	0.252
F16	The stability was not recorded.				F16	The stability was not recorded.			
F17	The stability was not recorded.				F17	The stability was not recorded.			
F18	0.225	0.253	0.301	0.225	0.253	0.301	0.225	0.253	0.301
F19	The stability was not recorded.				F19	The stability was not recorded.			
F20	The stability was not recorded.				F20	The stability was not recorded.			
F21	0.233	0.253	0.285	0.233	0.253	0.285	0.233	0.253	0.285
F22	The stability was not recorded.				F22	The stability was not recorded.			
F23	The stability was not recorded.				F23	The stability was not recorded.			
F24	0.229	0.257	0.255	0.229	0.257	0.255	0.229	0.257	0.255
F25	The stability was not recorded.				F25	The stability was not recorded.			
F26	The stability was not recorded.				F26	The stability was not recorded.			
F27	0.224	0.280	0.283	0.224	0.280	0.283	0.224	0.280	0.283

After the preliminary studies, the base formulation to encapsulate different active ingredients in either ethosomal or liposomal carrier systems was selected. The formulations of the empty and loaded liposomes prepared by the cold method (Uzuner et al., 2026) were given in Table 3 and Table 4, respectively. Processing temperature was maintained at 25 °C to minimize thermal effects. After the preparation of the

liposomal formulation, it was incorporated with the base gel formulation in a 1:1 ratio. The same gel formulation (Table 5.) also served as a base for the retinol and Vit C gel solution used for comparison of the in-vitro release profiles by the Franz Cell Diffusion studies (Table 6.).

Table 3. Formulation of empty liposomes formulation

Formula: Ingredient	Quantity %
Phosphatidylcholine	10.0
Water	88.7
Vitamin E	0.3
Retinol	-
Vitamin C	-
Phenoxyethanol	1.0

Table 4. Formulation of liposomes encapsulating Vit C and Retinol

Formula: Ingredient	Quantity %
Phosphatidylcholine	10.0
Water	87.65
Vitamin E	0.3
Retinol	0.05
Vitamin C	1.0
Phenoxyethanol	1.0

Table 5. Gel- base formulation

Ingredients	Quantity %
Water	96.43
Ultrez 20 (Acrylates/C10-30 Alkyl Acrylate Crosspolymer)	3.57

Table 6. Gel formulation of Retinol and Vit C

Ingredients	Quantity %
Water	97.040
Ultrez 20 (Acrylates/C10-30 Alkyl Acrylate Crosspolymer)	1.785
Vitamin E	0.150
Retinol	0.025
Vitamin C	0.500
Phenoxyethanol	0.500

Final formulations with empty and loaded liposomes were stored at 2–8 °C, 25 °C, 30 °C, and 40 °C for stability and characterization studies.

Characterization of liposomal formulations

The physicochemical properties and stability of liposomal formulations were characterized by evaluating pH, zeta potential, average particle size, particle size distribution, and physical appearance. The pH of the liposomal dispersions was measured using a tabletop pH meter (pH 3110, Xylem/WTW, Weilheim, Germany). All measurements were performed in triplicate.

Particle size analysis

The mean particle size and particle size distribution of empty and loaded vesicular systems were determined using a dynamic light scattering (DLS) analyzer (Litesizer™ 500, Anton Paar, Germany) (Verma, Verma, Blume, & Fahr, 2003). The measurements were performed for samples kept at 25 °C, 30 °C, 40 °C, and 2-8 °C on the 14th, 30th, 45th, and 60th days. The measurements were repeated three times. All measurements were performed at 25 ± 2 °C after appropriate dilution with purified water. In DLS measurements, vesicle homogeneity is expressed as the polydispersity index (PDI), which ranges from 0 for highly uniform populations to 1 for highly polydisperse systems.

Zeta potential analysis

The zeta potential (ζ) of both empty and loaded liposomal formulations was determined using a Litesizer™ 500 (Anton Paar, Germany), which utilizes dynamic light scattering principles to determine electrophoretic mobility (Ekmekcioglu et al., 2024). The dispersions were diluted as directed with purified water and transferred into 100 Ω-type zeta cuvettes. Measurements were conducted at 25 ± 2 °C, and the mean zeta potential values represent the average of three replicate readings for each sample, reflecting the surface charge and stability of the vesicular systems. Zeta potential measurements were made for all samples kept at 25 °C, 30 °C, 40 °C, and 2-8 °C on the 14th, 30th, 45th, and 60th days.

Physical stability

The stability of empty liposomes was initially evaluated over 14 days to identify suitable formulations and optimize the methods. Subsequently, the selected liposomal formulations were stored at 25 °C, 30 °C, 40 °C, and 2-8 °C for 60 days. Changes in particle size, size distribution uniformity, zeta potential, and physical properties (color and appearance) at predetermined time points (14th, 30th, 45th, and 60th days) were monitored to assess stability. Any signs of aggregation, sedimentation, or precipitation were visually inspected and recorded.

Encapsulation efficiency (%EE)

Encapsulation efficiency was determined by ultracentrifugation of 0.5 mL liposomal solution samples at 16,000 rpm for 120 min at 4 °C. Supernatants were analyzed for free active compounds after acetone treatment. Amounts of Vitamin C and Retinol were quantified by LC-MS/MS. % Encapsulation efficiency (EE) was calculated as (Formula 1):

$$EE\ (\%) = [(\text{Total drug} - \text{Free drug}) / \text{Total drug}] \times 100 \quad \text{(Formula 1)}$$

Morphological analysis

Morphological properties were examined using Scanning Electron Microscopy (SEM, Zeiss Sigma 300, Germany) and Transmission Electron Microscopy (TEM, JEOL JEM-2100, Japan).

***In vitro* release profiles, Franz diffusion cell studies**

The release profiles of retinol and Vit C from liposomal formulations were evaluated using Franz Diffusion Cells (FDC -6 series, Logan Instruments, USA). This study was conducted using two different synthetic membranes: Cellulose acetate membrane (12-14 kDa MWCO, 25 mm, Spectra/Por®, Carl Roth, USA), used for comparative diffusion analysis; Strat-M® membrane (Merck Millipore, USA), which is considered as mimicking human skin permeability (Arce et al., 2020), although it is not accepted officially by ECVAM to replace animal or human skin. 1 mL liposomal formulation or control was introduced into the donor compartment. The receptor compartment was filled with about 11 mL PBS (pH 7.4) with 20% ethanol to maintain the solubility of retinol. The temperature was maintained at 32 °C to simulate physiological skin conditions. The mixing speed of the receptor side was 300 rpm to ensure homogeneity of the receptor solution. 500 µL aliquot was withdrawn at the sampling times, and fresh pre-heated receptor solution was added to replenish the sample withdrawn. Sampling periods were at 15, 30, 60, 120, 240, 360, 480, 720, and 1440 minutes. Utmost care was taken not to form air bubbles between the membrane and the receptor solution during the entire test period. Spectra/Por® membrane had to be soaked in purified water at least 2 h before it was used, while Strat M® membrane did not require soaking before the experiment. The amounts of Retinol and Vitamin C that diffused through the membrane were determined using Liquid Chromatography-Tandem Mass Spectrometry (Agilent Technologies 6420 Triple Quad LC-MS/MS). The cumulative concentration of active components diffusing across the membrane was calculated and plotted as a function of time. The release profiles of liposomal solution, gel and simple solution systems were compared. The same conditions were used for Strat-M® membrane studies as well.

LC/MS-MS analyses

Measurements of the active material amounts were made using an Agilent Technologies 6420 Triple Quad LC/MS/MS. To prepare the calibration curves, 50 mg/mL Vit C and 5 mg/mL Retinol containing stock solutions were prepared. From this stock solution, different dilutions were prepared, and a calibration curve was prepared. All of the aliquots taken during Franz cell diffusion experiments were brushed with acetone and diluted by 50% or as much as required for LC/MS/MS measurements. The concentrations of retinol and Vit C against the signals were calculated, and the findings were used to analyze % EE; cumulative released amounts of Vit C and Retinol against time were calculated.

Statistical analysis

All experiments were performed in at least three replicates, and results are presented as mean ± standard deviation (SD). No further statistical analysis was performed.

RESULTS AND DISCUSSION

Physicochemical characterization

The average particle sizes of the liposomal (T3) formulation depended very much on the number of cycles the mixture was passed through the microfluidic channels. A consistent trend toward smaller particle sizes was observed for the loaded (T3) liposomes compared with the empty (T1) liposomes. Although the standard deviations largely overlap, so the difference cannot be claimed as statistically significant without formal testing, the trend was clearest at 40 °C on day 14. A plausible explanation is that retinol intercalates into the lipid bilayer, increasing membrane packing density and rigidity and thereby yielding more compact vesicles under microfluidization. (Table 7.). The reported sizes were obtained when pressure was 1000-1500 bars. However, passing the liposomal mixture through fewer cycles produced small enough liposomes, which are still less than the optimal size range (<600 nm) for enhanced dermal penetration (Dragicevic-Curic et al., 2008).

Table 7. Effect of microfluidic pressure homogenization cycles on particle size (PS), nm**.** T1: Empty Liposomes, T3: Vit C and Retinol loaded liposomes

CODE	**PS** (nm) Mean ± SD
Empty liposomes	
T1 1 cycle	1300 ±210
T1 2 cycles	815 ±185
T1 3 cycles	288 ±73
T1 4 cycles	245 ±67
Retinol and Vit C loaded liposomes	
T3 1 cycle	620 ±200
T3 2 cycles	270 ±68
T3 3 cycles	253 ±75
T3 4 cycles	200 ±72

DLS measurements indicated that liposomes produced by this method maintained greater size uniformity and colloidal stability (Table 8.). Dispersity indexes were always less than 0.2%. This could be a result of high-pressure microfluidic homogenization and the particle size reduction process. Although this technology is used and suggested by some other researchers (Ballacchino et al., 2021; Carugo, Bottaro, Owen, Stride, & Nastruzzi, 2016; Yu, Lee, & Lee, 2009), it is not extensively used. We have demonstrated that if the composition of the lipids is in compliance with the actives and correct conditions are used, forcing the mixture through microfluidic channels under correct pressure can produce nanosized and stable liposomes. It is worth noticing that the loaded liposomes were smaller than empty liposomes. As many researchers pointed out earlier, there may be many physicochemical reasons to explain why empty liposomes can have larger sizes. The larger mean size measured for the empty (unloaded) liposomes compared with the retinol and Vit C loaded vesicles can be explained by the different physicochemical nature of the two actives and their sites of localization within the vesicle. Retinol is a lipophilic molecule that does not stay in the aqueous core but partitions into the phospholipid bilayer, locating itself between the acyl chains of the phospholipids. This can increase the ordering and packing density of the hydrocarbon chains and causes a condensing effect on the bilayer, producing a more tightly organized and less hydrated membrane as observed by other researchers (De Mel et al., 2021). In our studies, no specific investigation

was made to uncover the reason; however, a smaller vesicle diameter of loaded liposomes can be explained by this fact. Vitamin C (ascorbic acid), on the other hand, is a strongly hydrophilic and acidic molecule that is accommodated in the internal aqueous compartment facing the polar head-group region of the shell. At the low pH generated by Vit C, the ionization state and hydration of the PC head groups can be modified, and the electrostatic repulsion between adjacent head groups is reduced. This will cause a closer lateral packing of the PC molecules and a shrinking of the vesicles relative to the empty liposomes (Tsubone et al., 2026). In the absence of these actives, the bilayer of the empty liposomes is comparatively more loosely packed and more hydrated, which accounts for their larger measured size.

Zeta potential is one of the properties to predict the stability of colloidal systems. It is the electric potential developed on the solid-liquid interface, and zeta potential values above ±30 mV enhance the electrostatic stability of the carrier systems, preventing the agglomeration of the particles (Rodriguez-Loya, Lerma, & Gardea-Torresdey, 2024). Although some changes in zeta potential values were observed and recorded throughout stability tests (14, 30, 45, and 60 days), the changes were not significant, and liposomes stayed stable during the stability tests, showing no signs of agglomeration, sedimentation, or separation. The results are presented in Table 9. Although natural hydrophobic particles behave differently (Wang & Keller, 2009), for synthetically produced particles, the threshold for static stability is ±30 mV. DLS is also a very powerful method to monitor and characterize nanoparticle aggregation dynamics.

Table 8. Particle size measurements of empty and loaded liposomes, in nm

CODE	14 days	30 days	45 days	60 days	Temperature (°C)
Empty liposomes					
T1	266±37	246.5±42	265.9±38	260.5±41	40 °C
T1	244.9±43	245.8±71	244.4±56	245.1±32	2-8 °C
Retinol and Vitamin C loaded liposomes					
T3	195±35	205.4±42	146.91±32	180.4±45	40 °C
T3	261±34	205.4±37	213.7±41	200±38	2-8 °C

Table 9. Zeta potential values of empty and loaded liposomes

CODE	14 days	30 days	45 days	60 days	Temperature (°C)
Empty liposomes					
T1	-35.6±5	-34.7±	-33.3±5	-33.0±9	25 °C
T1	-45.5±5	-44.2±7	-45.2±6	-44.6±5	2-8 °C
Retinol and Vitamin C loaded liposomes					
T3	-31.3 ±5	-33.2±4	-31.6±5	-39.6±3	40 °C
T3	-41.8±4	-43.7±5	-42.0±4	-42.7±2	2-8 °C

pH values of products ranged between 5.81 and 6.88 for all samples and remained relatively stable across all storage conditions and time points. These values would be considered acceptable for dermal applications without causing any irritation. Morphological analysis using SEM and TEM revealed that both liposomes displayed spherical morphology (Figure 1.).

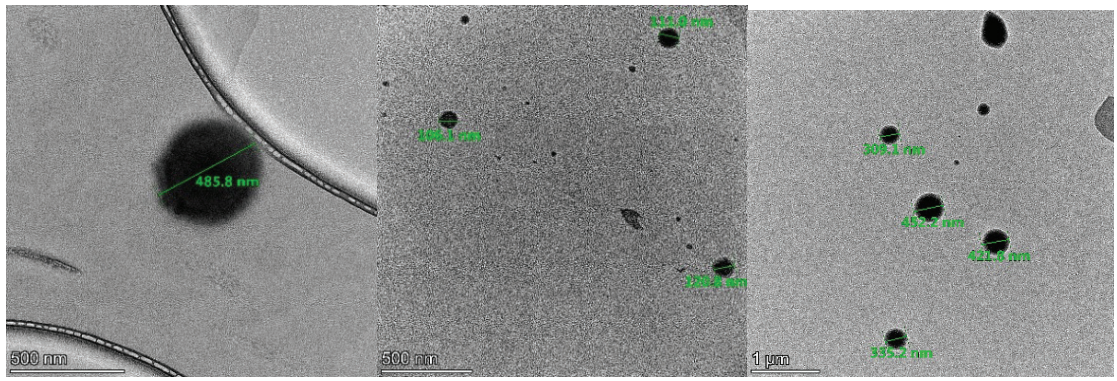

Figure 1. Morphological analysis of vesicles: SEM and TEM images showing spherical liposomes.

% EE analysis showed that Retinol was efficiently encapsulated in liposomal (T3) formulations, with an average entrapment value of 99.47%. In contrast, Vit C demonstrated notably lower encapsulation rates, with 60.55% of Vit C entrapped in liposomes. The higher encapsulation efficiency of Retinol can be attributed to its lipophilic nature, favoring its integration into the lipid bilayers of the vesicular system (Table 10.). The lower % EE of Vit C can be due to the high amount of total Vit C used in the formulation. In this study, there was no effort made for optimization of the Retinol and Vit C amounts used in the formulation. The amounts of the actives used were selected based on industry/supplier recommended amounts. The aim was to estimate how encapsulation of actives in liposomes affected the active material availability when applied on the skin. In future work, optimization of both Retinol and Vit C amounts can be studied in detail. Encapsulated and free amounts of the actives in the product formulation can change the entire availability profile of the actives. An immediate availability of the actives which are not encapsulated, followed by the actives in a reservoir, in liposomes, may create a desirable situation. The depot effect may be very useful for getting a prolonged availability of the active ingredient on the application site. Nanosized delivery systems can deliver the actives like retinol into different layers of the epidermis and dermis. Pena-Rodriguez and his co-workers demonstrated that retinoids can be delivered effectively to different layers of the epidermis by liposomal nano-delivery systems (Pena-Rodriguez, Moreno, Blanco-Fernandez, González, & Fernández-Campos, 2020). For clarity, given the concentrate composition (retinol 0.05%, vitamin C 1.0%) and the measured efficiencies, approximately 0.0497% retinol and 0.6055% vitamin C were encapsulated, leaving about 0.0003% retinol and 0.3945% vitamin C in free (non-encapsulated) form; in the final 1:1 product these correspond to approximately 0.0249%/0.0001% (encapsulated/free) retinol and 0.303%/0.197% vitamin C.

Table 10. Percentage of active ingredients encapsulated in liposomes

Encapsulated Compound	Encapsulation Efficiency (%)
Vitamin C encapsulated in Liposomes (T3)	60.55
Retinol encapsulated in Liposomes (T3)	99.47

***In vitro* release tests**

Franz diffusion cell experiments revealed distinct release profiles for both Retinol and Vitamin C from the liposomal formulation, as well as the control products; gel and simple solutions of the active (Figures 2 to 5.). The selection of membrane type also influenced diffusion characteristics. The cellulose acetate membrane simply acts as a separating barrier between the donor and receptor sides. Based on the cut-off size, it will allow passage of molecules through its pores. On the other hand, Strat-M® is an artificial membrane that was engineered to resemble animal or human skin.

This membrane is supposed to mimic key structural and chemical properties of human skin; for example, it has a very tight top layer coated with a lipid mixture like the stratum corneum. It is stratified, and the lower layers are more porous, like the lower layers of the epidermis and dermis (Haq, Goodyear, Ameen, Joshi, & Michniak-Kohn, 2018). These are the products/combinations used for *in vitro* release testing:

1- "T3" liposome-containing product: 50% of it is the output of the microfluidic processor. This output contains liposomes encapsulating Vit C and retinol together with free Vit C and retinol. The other 50 % is a simple gel product.

2- The simple "gel" product containing the same amount of retinol and Vit C.

3- An aqueous "solution" containing the same quantity of retinol and Vit C.

Figure 2 shows the effect of encapsulation of Vit C in a liposome. The simple solution released the Vit C fastest, but release from the "gel" was slower, and release from T3, the liposome product, was the slowest, as expected. Release from "gel" was slower than the solution probably because of the higher viscosity of the gel on the donor side of the Franz cell. As expected, the slowest release was from T3, because the available Vit C amount ready to diffuse was less than the other donors; % EE of Vit C was 66%. When Strat-M® was used instead of a cellulose acetate membrane (Figure 3.), the general picture did not change; however, this membrane did not reduce the diffusion of Vit C from any of the products tested (T3, gel, sol.). Haq and her co-workers also reported as 'Our data suggest that hydrophilic compounds have higher permeation in Strat-M® membranes compared with lipophilic ones." (Haq et al., 2018). Their observation was like ours.

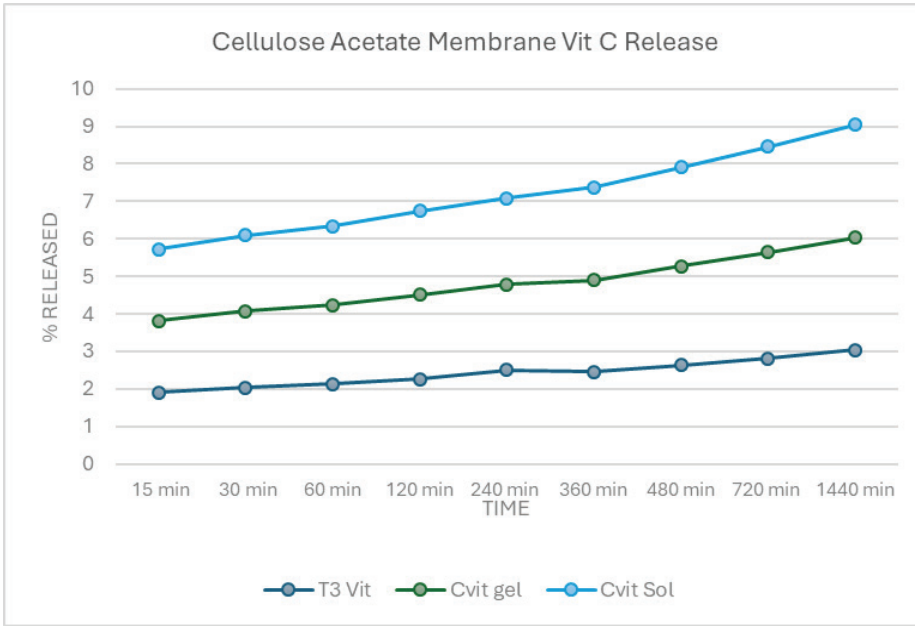

Figure 2. Vitamin C Franz cell diffusion from cellulose acetate membrane

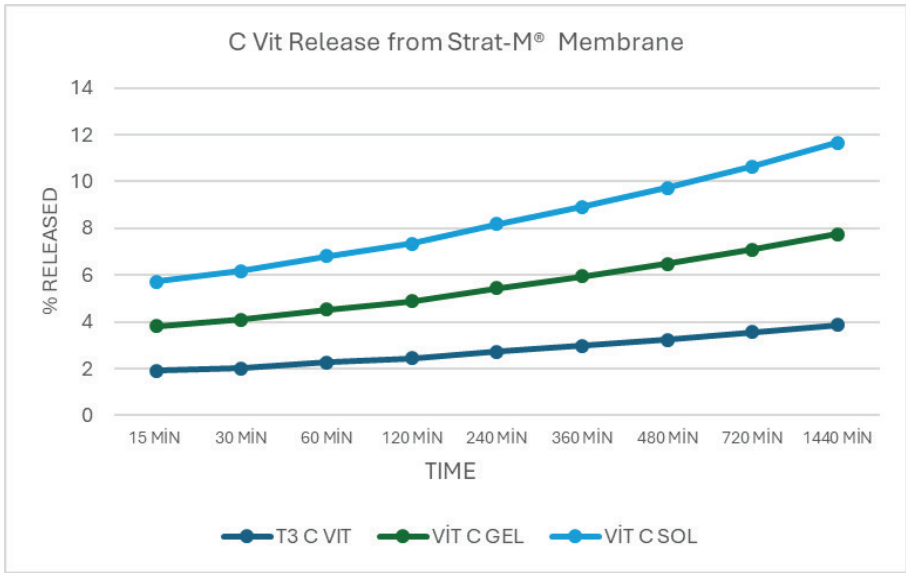

Figure 3. Vit C Franz cell diffusion from Strat M® membrane

When retinol release from cellulose acetate and Strat-M® membranes was investigated, the release profiles seemed different (Figure 4 and Figure 5.). Although some fluctuations were observed, release from liposome, gel, or sol was rather similar. Retinol release from Strat-M® membrane showed less fluctuation, but from the liposomal product, gel, and simple sol showed similar release patterns, suggesting that the rate-limiting factor was the diffusion coefficient of retinol. Retinol is a hydrophobic ingredient, and its solubility in water is almost none. That is the reason that the receptor solution, PBS pH 7.4, contained 20% ethanol to facilitate the solubility of retinol. Although hydrophobic and a small molecule (286.5 mol/gr), retinol is not absorbed very fast from the skin. In one study, Yourick applied formulations containing 0.3% retinol on the skin of rats and measured the amount of retinol that remained on the skin (Yourick, Jung, & Bronaugh, 2008). 24 h after the application, only 23% was absorbed. The allowed cosmetic use amount of retinoids is low. European Scientific Committee on Consumer Safety (SCCS) revised the legally allowable limits in 2022. According to the new regulation, the allowable amounts will be as retinol equivalents: in body lotions, 0.05%; in hand and face creams and all other leave-on products, 0.03%. One can imagine how small and slow will be the penetration is the concentration gradients to facilitate skin absorption (Wang et al., 2024). In Franz cell release studies conducted for retinol, either micelle-forming non-ionics are added, like Tween 80, Tween 20, or ethanol, or another solvent is used to increase the solubility of retinol. Our study aimed to engineer liposomal nanocarriers via high-pressure microfluidization to achieve co-encapsulation of Retinol and Vit C within a single vesicular system. Therefore, to investigate better diffusion conditions was a secondary interest. Adding non-ionics or an excess amount of alcohol was not feasible. Therefore, in a future study we would like to investigate the use of different membranes and receptor solutions to further understand how to best mimic the *in vivo* conditions of skin penetration. We understand that the Strat-M® membrane is also not compatible with high concentrations of ethanol. The results shown in Figure 4 and Figure 5 suggest that the amounts of the released amounts of retinol are low, since the formulation contains a smaller retinol dose than Vit C, due to the regulatory allowable amounts.

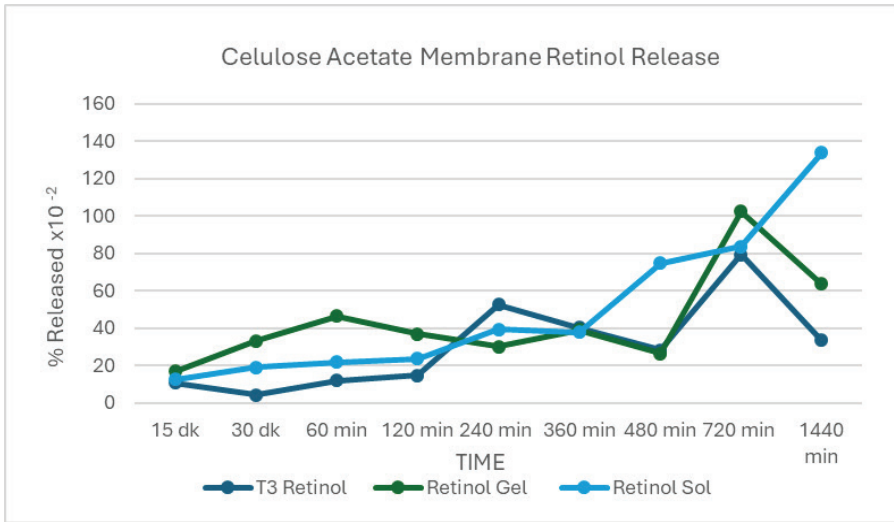

Figure 4. Retinol Franz cell diffusion from cellulose acetate membrane

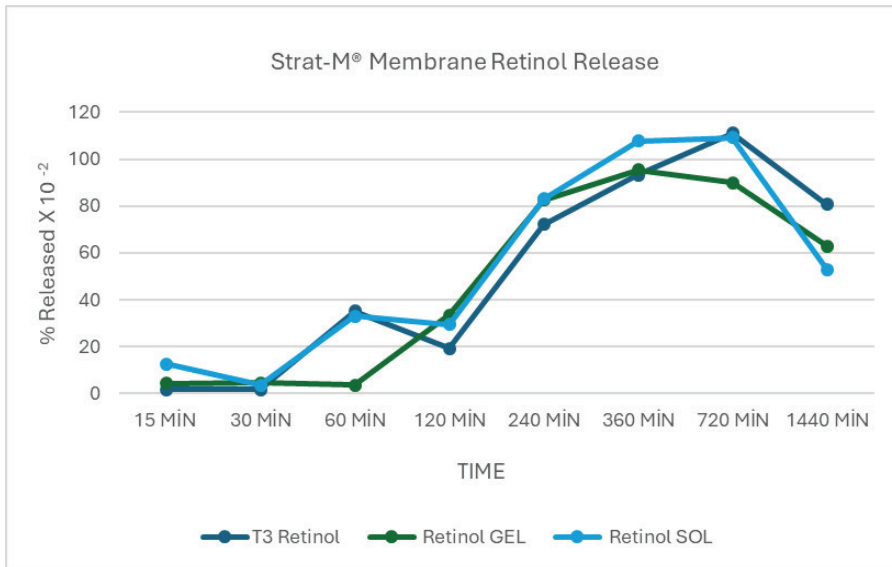

Figure 5. Retinol Franz cell diffusion from the Strat-M® membrane

This study aimed to develop and characterize dual-loaded liposomal delivery systems encapsulating both Retinol and Vitamin C to enhance dermal stability, penetration, and antioxidant efficacy. High-pressure microfluidization was utilized to achieve nanoscale vesicle formation, enabling the successful co-encapsulation of a hydrophobic and a hydrophilic compound within a single carrier system. The resulting formulations exhibited favorable physicochemical characteristics, including nanoscale particle sizes and stable zeta potential values over 60 days. Encapsulation efficiency for Retinol exceeded 98% while Vitamin C showed lower EE, requiring further optimization studies.

Dragicevic-Curic et al. observed low retention of hydrophilic drugs like Vitamin C in ethosomes, which aligns with our findings (Dragicevic-Curic et al., 2008). Elsayed et al. (2007) supported the use of phospholipid bilayers for better encapsulation of hydrophilic compounds (Elsayed et al., 2007). Baspinar and Borchert (2012) reported the benefit of liposomal carriers in enhancing stability and skin delivery.

Furthermore, a recent *in vivo* confocal Raman study showed effective cutaneous delivery of trans-retinol, reinforcing the translational potential of lipid-based carriers (Pudney, Mélot, Caspers, Van Der Pol, & Puppels, 2007). However, studies involving co-delivery of Retinol and Vit C *in vivo* remain limited, highlighting a gap for future research.

The innovative aspect of this study lies in the dual loading of both a lipophilic and hydrophilic compound into stable nanoscale carriers using high-pressure microfluidization. The parallel assessment of physicochemical stability, release, skin performance, and antioxidant potential provides a comprehensive understanding of the system.

CONCLUSION

In this study, liposomal nanocarriers were successfully developed to co-encapsulate Retinol and Vit C for topical application. The dual-loading strategy offers a promising platform for the synchronized delivery of hydrophilic and lipophilic actives, addressing longstanding formulation challenges. Future investigations, particularly *in vivo* evaluations, are warranted to confirm the clinical relevance and long-term safety of these nano systems. A Turkish Patent application was made to register the co-encapsulation and easier production method for circulation in a microfluidic chamber under 1000-1500 bar pressure (Uzuner & Demirci, 2024).

ACKNOWLEDGEMENTS

We would like to thank Acıbadem University (ACU) Faculty of Pharmacy and all other departments for allowing us the necessary equipment and supplies, and our colleagues for their contributions. We also thank the ACU Scientific Projects Coordination Unit (ABAPKO) for supporting this project under the FAST SUPPORT program (2022/02-17).

AUTHOR CONTRIBUTION STATEMENT

Hypothesis development YYU, literature research BD/YYU/NSÖ, experimentations and data analysis YYU / BD, preparation and correction of the manuscript were carried out by YYU / NSÖ.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

Arangüren, A., Torres, C. E., Muñoz-Camargo, C., Osma, J. F., & Cruz, J. C. (2020). Synthesis of nanoscale liposomes via low-cost microfluidic systems. *Micromachines*, *11*(12), 1050. https://doi.org/10.3390/mi11121050

Arce, F. J., Asano, N., See, G. L., Itakura, S., Todo, H., & Sugibayashi, K. (2020). Usefulness of artificial membrane, Strat-M®, in the assessment of drug permeation from complex vehicles in finite dose conditions. *Pharmaceutics*, *12*(2), 173. https://doi.org/10.3390/pharmaceutics12020173

Ballacchino, G., Weaver, E., Mathew, E., Dorati, R., Genta, I., Conti, B., & Lamprou, D. A. (2021). Manufacturing of 3D-printed microfluidic devices for the synthesis of drug-loaded liposomal formulations. *Int J Mol Sci*, *22*, 8064. https://doi.org/10.3390/ijms22158064

Baspinar, Y., & Borchert, H. H. (2012). Penetration and release studies of positively and negatively charged nanoemulsions—Is there a benefit of the positive charge? *International Journal of Pharmaceutics, 430*(1–2), 247–252. https://doi.org/10.1016/j.ijpharm.2012.03.040

Burke, K. E. (2007). Interaction of vitamins C and E as better cosmeceuticals. *Dermatologic Therapy*, *20*, 314-321. https://doi.org/10.1111/j.1529-8019.2007.00145.x

Cárcamo, J. M., Pedraza, A., Bórquez-Ojeda, O., & Golde, D. W. (2002). Vitamin C suppresses TNF alpha-induced NF kappa B activation by inhibiting I kappa B alpha phosphorylation. *Biochemistry*, *41*(43), 12995–13002. https://doi.org/10.1021/bi0263210

Caritá, A. C., Fonseca-Santos, B., Shultz, J. D., Michniak-Kohn, B., Chorilli, M., & Leonardi, G. R. (2020). Vitamin C: One compound, several uses. Advances for delivery, efficiency and stability. *Nanomedicine: Nanotechnology, Biology, And Medicine*, *24*, 102117. https://doi.org/10.1016/j.nano.2019.102117

Carugo, D., Bottaro, E., Owen, J., Stride, E., & Nastruzzi, C. (2016). Liposome production by microfluidics: Potential and limiting factors. *Scientific Reports*, *6*, 25876. https://doi.org/10.1038/srep25876

Commission Regulation (EU) 2024/996 of 3 April 2024 amending Regulation (EC) No 1223/2009 as regards the use of Vitamin A, Alpha-Arbutin, Arbutin and certain substances with potential endocrine-disrupting properties in cosmetic products. OJ L, 2024/996. Retrieved from https://eur-lex.europa.eu/eli/reg/2024/996/oj/eng

Cosmetic Ingredient Review Expert Panel. (1987). Final report on the safety assessment of retinyl palmitate and retinol. *J Am Coll Toxicol*, *6*(3), 279-320 (re-affirmed 2005).

Darr, D., Dunston, S., Faust, H., & Pinnell, S. R. (1996). Effectiveness of antioxidants (vitamin C and E) with and without sunscreens as topical photoprotectants. *Acta Derm Venereol*, *76*(4), 264-268. https://doi.org/10.2340/0001555576264268

De Leo, V., Maurelli, A. M., Giotta, L., & Catucci, L. (2022). Liposomes containing nanoparticles: Preparation and applications. *Colloids and Surfaces B: Biointerfaces*, *218*, 112737. https://doi.org/10.1016/j.colsurfb.2022.112737

De Mel, J. U., Gupta, S., Harmon, S., Stingaciu, L., Roth, E. W., Siebenbuerger, M.,…Schneider, G. J. (2021). Acetaminophen interactions with phospholipid vesicles induced changes in morphology and lipid dynamics. *Langmuir*, *37*(31), 9560–9570. https://doi.org/10.1021/acs.langmuir.1c01458

Dragicevic-Curic, N., Scheglmann, D., Albrecht, V., & Fahr, A. (2008). Temoporfin-loaded invasomes: Development, characterization and in vitro skin penetration studies. *Journal of Controlled Release, 127*(1), 59–69. https://doi.org/10.1016/j.jconrel.2007.12.013

Dymek, M., & Sikora, E. (2022). Liposomes as biocompatible and smart delivery systems – The current state. *Advances in Colloid and Interface Science*, *309*, 102757. https://doi.org/10.1016/j.cis.2022.102757

Ekmekcioglu, A., Gok, O., Oz-Arslan, D., Erdal, M. S., Yagan Uzuner, Y., & Muftuoglu, M. (2024). Mitochondria-Targeted Liposomes for Drug Delivery to Tumor Mitochondria. *Pharmaceutics*, *16*(7), 950. https://doi.org/10.3390/pharmaceutics16070950

Elsayed, M. M. A., Abdallah, O. Y., Naggar, V. F., & Khalafallah, N. M. (2007). Lipid vesicles for skin delivery of drugs: Reviewing three decades of research. *International Journal of Pharmaceutics, 332*(1–2), 1–16. https://doi.org/10.1016/j.ijpharm.2006.12.005

Farris, P. K. (2005). Topical vitamin C: A useful agent for treating photoaging and other dermatologic conditions. *Dermatol Surg*, *31*(7 Pt 2), 814-817. https://doi.org/10.1111/j.1524-4725.2005.31725

Haq, A., Goodyear, B., Ameen, D., Joshi, V., & Michniak-Kohn, B. (2018). Strat-M® synthetic membrane: Permeability comparison to human cadaver skin. *Int J Pharm*, *547*, 432-437. https://doi.org/10.1016/j.ijpharm.2018.06.012

Herndon, J. H., Jr, Jiang, L. I., Kononov, T., & Fox, T. (2016). An Open Label Clinical Trial to Evaluate the Efficacy and Tolerance of a Retinol and Vitamin C Facial Regimen in Women With Mild-to-Moderate Hyperpigmentation and Photodamaged Facial Skin. *Journal of Drugs in Dermatology*, *15*(4), 476–482.

Johnson, W., Jr. (2017). Safety assessment of retinol and retinyl palmitate as used in cosmetics. *International Journal of Toxicology, 36*(5_suppl2), 53S–58S. https://doi.org/10.1177/1091581817716657

Kaźmierczak-Barańska, J., Boguszewska, K., Adamus-Grabicka, A., & Karwowski, B. T. (2020). Two faces of vitamin C-antioxidative and pro-oxidative agent. *Nutrients*, *12*(5), 1501. https://doi.org/10.3390/nu12051501

Lin, F. H., Lin, J. Y., Gupta, R. D., Tournas, J. A., Burch, J. A., Selim, M. A.,…Pinnell, S. R. (2005). Ferulic acid stabilizes a solution of vitamins C and E and doubles its photoprotection of skin. *The Journal of Investigative Dermatology*, *125*(4), 826–832. https://doi.org/10.1111/j.0022-202X.2005.23768.x

Lombardo, D., & Kiselev, M. A. (2022). Methods of liposomes preparation: Formation and control factors of versatile nanocarriers for biomedical and nanomedicine application. *Pharmaceutics*, *14*(3), 543. https://doi.org/10.3390/pharmaceutics14030543

Murray, J. C., Burch, J. A., Streilein, R. D., Iannacchione, M. A., Hall, R. P., & Pinnell, S. R. (2008). A topical antioxidant solution containing vitamins C and E stabilized by ferulic acid provides protection for human skin against damage caused by ultraviolet irradiation. *Journal of the American Academy of Dermatology*, *59*(3), 418–425. https://doi.org/10.1016/j.jaad.2008.05.004

Nsairat, H., Khater, D., Sayed, U., Odeh, F., Al Bawab, A., & Alshaer, W. (2022). Liposomes: Structure, composition, types, and clinical applications. *Heliyon*, *8*(5), e09394. https://doi.org/10.1016/j.heliyon.2022.e09394

Pando, D., Matos, M., Gutiérrez, G., & Pazos, C. (2015). Formulation of resveratrol entrapped niosomes for topical use. *Colloids Surf B Biointerfaces*, *128*, 398-404. https://doi.org/10.1016/j.colsurfb.2015.02.037

Pena-Rodríguez, E., Moreno, M. C., Blanco-Fernandez, B., González, J., & Fernández-Campos, F. (2020). Epidermal delivery of retinyl palmitate loaded transfersomes: Penetration and biodistribution studies. *Pharmaceutics*, *12*(2), 112. https://doi.org/10.3390/pharmaceutics12020112

Pradhan, S. J., Kuchekar, A., Gawade, A., & Kore, S. (2024). Retinoids—A unique ingredient for skin rejuvenation employing novel drug delivery systems. *J Appl Pharm Sci*, *14*(07), 029-044. https://doi.org/10.7324/JAPS.2024.168608

Pudney, P. D., Mélot, M., Caspers, P. J., Van Der Pol, A., & Puppels, G. J. (2007). An in vivo confocal Raman study of the delivery of trans retinol to the skin. *Applied Spectroscopy*, *61*(8), 804–811. https://doi.org/10.1366/000370207781540042

Pullar, J. M., Carr, A. C., & Vissers, M. C. M. (2017). The roles of vitamin C in skin health. *Nutrients*, *9*(8), 866. https://doi.org/10.3390/nu9080866

Rodriguez-Loya, J., Lerma, M., & Gardea-Torresdey, J. L. (2024). Dynamic light scattering and its application to control nanoparticle aggregation in colloidal systems: A review. *Micromachines*, *15*, 24. https://doi.org/10.3390/mi15010024

SCCS (Scientific Committee on Consumer Safety). (2016). *Opinion on Vitamin A (Retinol, Retinyl Acetate, Retinyl Palmitate). SCCS/1576/16, final version 6 October 2016, corrigendum 23 December 2016. European Commission, DG Health and Food Safety*. Retrieved from https://health.ec.europa.eu/publications/revision-scientific-opinion-sccs157616-vitamin-retinol-retinyl-acetate-retinyl-palmitate_en

SCCS. (2022). *Scientific Opinion on Vitamin A (Retinol, Retinyl Acetate, Retinyl Palmitate) — Revision. SCCS/1639/21, final version 2/3 December 2022*. Retrieved from https://health.ec.europa.eu/system/files/2023-08/sccs_o_261.pdf

Sevinç, H. (2020). *Development of liposomal carrier systems of hydrolyzed collagen, resveratrol, retinol and investigation of their penetrations* [Master's thesis]. İstanbul: Yeditepe University, Institute of Health Sciences, Department of Pharmaceutical Technology.

Sorg, O., Antille, C., Kaya, G., & Saurat, J. H. (2006). Retinoids in cosmeceuticals. *Dermatol Ther*, *19*(5), 289-296. https://doi.org/10.1111/j.1529-8019.2006.00086.x

Telang P. S. (2013). Vitamin C in dermatology. *Indian Dermatology Online Journal*, *4*(2), 143–146. https://doi.org/10.4103/2229-5178.110593

Touitou, E., Dayan, N., Bergelson, L., Godin, B., & Eliaz, M. (2000). Ethosomes — novel vesicular carriers for enhanced delivery: Characterization and skin penetration properties. *Journal of Controlled Release*, *65*(3), 403–418. https://doi.org/10.1016/S0168-3659(99)00222-9

Tsubone, T. M., de Oliveira Junior, P. N., Scanavachi, G., dos Santos, V. F., da Cunha, A. R., Ramos, A. P.,…Itri, R. (2026). Acidic pH modulates headgroup orientation and packing in bis(monoacylglycero)phosphate bilayers. *ACS Physical Chemistry Au*, *6*(3), 483–492. https://doi.org/10.1021/acsphyschemau.5c00150

Uzuner, Y. Y., & Demirci, B. (2024). *Anti-ageing liposomal and ethosomal facial care formulas (Yaşlanma Karşıtı Lipozomal ve Etozomal Yüz Bakım Formülleri). Assignee: Acıbadem Mehmet Ali Aydınlar University*. Turkish Patent TR 2022/021774 A1, filed 30 December 2022, (PCT/TR2023/051080, WO 2024/144526 A1).

Uzuner, Y. Y., Sevinç, H., & Kanlidere, Z. (2026). Ethosomal nanocarriers for hydrophilic peptide encapsulation: Formulation optimization, stability, and in vitro release performance. *Molecules*, *31*(4), 744. https://doi.org/10.3390/molecules31040744

Vašková, J., Stupák, M., Vidová Ugurbaş, M., Židzik, J., & Micková, H. (2025). Therapeutic uses of retinol and retinoid-related antioxidants. *Molecules*, *30*(10), 2191. https://doi.org/10.3390/molecules30102191

Verma, D. D., Verma, S., Blume, G., & Fahr, A. (2003). Particle size of liposomes influences dermal delivery of substances into skin. *Int J Pharm*, *258*, 141-151. https://doi.org/10.1016/S0378-5173(03)00183-2

Wang, P., & Keller, A. (2009). Natural and engineered nano and colloidal transport: Role of zeta potential in prediction of particle deposition. *Langmuir: The ACS Journal of Surfaces and Colloids*, *25*, 6856-6862. https://doi.org/10.1021/la900134f

Wang, Y., Zhang, Q., Wei, Y., Cai, X., Li, Z., Wu, Q.,… Xiang, Q. (2024). Retinol semisolid preparations in cosmetics: transcutaneous permeation mechanism and behaviour. *Scientific Reports*, *14*(1), 22793. https://doi.org/10.1038/s41598-024-73240-y

Wohlrab, J., & Kreft, D. (2014). Niacinamide—Mechanisms of action and its topical use in dermatology. *Skin Pharmacology and Physiology, 27*(6), 311–315. https://doi.org/10.1159/000359974

Yu, B., Lee, R. J., & Lee, L. J. (2009). Microfluidic methods for production of liposomes. *Methods in Enzymology*, *465*, 129–141. https://doi.org/10.1016/S0076-6879(09)65007-2

Yourick, J. J., Jung, C. T., & Bronaugh, R. L. (2008). In vitro and in vivo percutaneous absorption of retinol from cosmetic formulations: Significance of the skin reservoir and prediction of systemic absorption. *Toxicol Appl Pharmacol*, *231*, 117-121. https://doi.org/10.1016/j.taap.2008.04.006

Zasada, M., & Budzisz, E. (2019). Retinoids: Active molecules influencing skin structure formation in cosmetic and dermatological treatments. *Postep Dermatol Alergol*, *36*, 392-397. https://doi.org/10.5114/ada.2019.87443

Zhong, J., Zhao, N., Song, Q., Du, Z., & Shu, P. (2024). Topical retinoids: Novel derivatives, nano lipid-based carriers, and combinations to improve chemical instability and skin irritation. *J Cosmet Dermatol*, *23*, 3102-3115. https://doi.org/10.1111/jocd.16415