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Liposome-Based Delivery Systems for Sustained and Controlled Scent Release: Enhancing Stability and Retention in Cosmetic and Perfumery Applications

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1. Introduction

The volatility and instability of fragrance molecules present significant challenges in the formulation of cosmetic and perfumery products[1]. Conventional solutions often rely on ethanol and high concentrations of surfactants, co-surfactants, and solvents, which can compromise both the sensory quality and safety of the final product[2]. To address these limitations, novel delivery systems are being explored to enhance fragrance stability and prolong olfactory performance[3].

Liposome-based delivery systems have emerged as promising technology for enhancing stability, sustained release, and controlled retention of volatile compounds in cosmetic and perfumery applications. Liposomes, which are spherical vesicles composed of phospholipid bilayers, provide a versatile platform for encapsulating and protecting delicate scent molecules from degradation due to environmental factors such as oxygen, light, and temperature fluctuations[4]. Their biocompatibility and ability to merge with biological membranes make them especially attractive for formulations where user safety and prolonged sensory experience are critical[5]. Recent research has focused on optimizing the physicochemical properties of liposomes, such as size, lamellarity, and lipid composition, to tailor the release kinetics of entrapped scents[6]. Such refinements aim not only to prolong the fragrance life but also to achieve a controlled and predictable release profile, thereby ensuring that the aromatic notes are maintained over an extended period[7]. In turn, this addresses a major challenge faced in the cosmetic and perfumery industries, where the volatility and rapid evaporation of natural and synthetic fragrances often limit product performance.

In the present study, we describe the synthesis and comprehensive characterization of an innovative liposome-based delivery system for the encapsulation of fragrances in cosmetic and perfumery applications. In contrast to prior studies that have broadly applied liposomal encapsulation to diverse active compounds, our formulation has been meticulously designed to accommodate the distinct physicochemical attributes of perfume molecules, with the primary objective of enhancing both formulation stability and controlled release. Furthermore, the liposome composition and preparation parameters were systematically optimized to maximize encapsulation efficiency while rigorously maintaining the structural integrity of the vesicles under conventional storage and usage conditions.

2. Materials and Methods

All reagents were obtained from commercial suppliers and used without further purification.

2.1 Selection of the Model Perfume Compound

The analysis of the samples was conducted using headspace gas chromatography coupled to a high-resolution quadrupole time-of-flight mass spectrometer (GC-Q-TOF), following a pre-concentration step performed with a thermal desorption unit with a cryogenic trap. The samples for analysis were collected from the headspace of the vial, allowing for the evaluation of the analyte's volatility. A 2.5 mL volume of the headspace vapor was injected into an adsorption cartridge, where it was adsorbed. Subsequently, the sample was thermally desorbed at 200°C and introduced into the GC-Q-TOF system. This instrument provides high resolution and accuracy for the identification and quantification of volatile compounds. The vapor-phase sample was injected in split mode, which reduces the amount of sample entering the chromatography column, ensuring that volatile compounds present at lower concentrations are detected with greater precision. The GC-Q-TOF system would separate sample components based on their retention times, while the mass spectrometer would identify each compound based on its exact mass. Linalyl acetate (LA) was selected as a model analyte among three potential candidates (tetrahydrolinalool, linalool, and linalyl acetate) by analyzing all options and choosing the compound that produced the most robust signal.

2.2 Development of liposomal LA

Liposomes were prepared following standard procedures in our lab using thin film hydration, a standard method normally used in the development of liposomes for different applications[8]. Initially, all lipids were dissolved in ethanol and combined in a round-bottom flask. A mixture of phosphatidylcholine, 1-hexadecanol, ceramides, sodium cocoyl glutamate, caprylic/Capric Triglyceride, mannitol and linalyl acetate, in a molar ratio 11/5/1/4/34/37/21 was dissolved in ethanol. The solvent evaporated using a rotary evaporator at 50 °C and decreasing the pressure for 45 min. The lipid film (6.13% w/v) was hydrated by adding ultrapure water and glycerin and heating up to 58ºC for 20 minutes. The resulting liposomal suspension was homogenized for 10 min at 16000 rpm using a micra homogenizer obtaining a white colloidal dispersion of liposomes.

2.3 Characterization of liposomes by dynamic light scattering and M3PALS

Liposomal average diameter and polydispersity index were evaluated by DLS measurements using a Zetasizer Ultra Red (Malvern Instruments, Worcestershire, UK). A 1:100 dilution of the product was prepared with Milli-Q water and moved into a PS cuvette for analysis.
Zeta potential was estimated by using a Zetasizer Ultra Red by electrophoretic light scattering. Samples were measured by diluting the product 5:1000 in Milli-Q water and using a capillary cuvette.

2.4 High performance liquid chromatography with ultraviolet/light detection (HPLC/UV-VIS)

LA was quantified by HPLC using an Agilent® Series Infinity 1200 Infinity equipped with a UV detector and a Kromaphase 100 C18, 5.0 µm (150 × 4.6 mm). LA was quantified at 210 nm, and a mixture MilliQ water and acetonitrile (20:80) was used as mobile phase, delivered at a flow rate of 1 ml min−1. A calibration curve (peak area of LA vs known concentration) was built

using working, standard solutions (420.7 − 2145.2 µg mL−1). The correlation coefficient (R2) obtained was 0.99995. LA retention time was approximately 5.1 min, the limits of detection and quantification achieved were 21.1 µg mL−1 and 63.8 µg mL−1.

2.5 Encapsulation efficacy

To calculate the encapsulation efficiency, empty liposomes were spiked with LA to reach the same analyte concentration that in the product liposomal formulation. A 1:4 mixture of the encapsulated product and the spiked blank in Milli-Q water were prepared and loaded into Vivaspin® 6 centrifugal concentrators (10000 MWCO). The non-entrapped free analyte, which was able to cross the concentrator membrane, was separated from the vesicles by centrifugation at 4500 FCR for 15 min. Each filtrate was filtered through 0.22 µm filters and analyzed by HPLC as described above. The encapsulation efficacy was calculated using the following formula:

$$\mathrm{EE\%} = 1 - \frac{\text{LA concentration of the product}}{\text{LA concentration of the spiked sample}}$$

2.6 Controlled release

Two samples of liposomal LA and free LA were prepared to perform a comparative controlled release study. Both samples were prepared in a similar manner, achieving a final LA concentration of 1.50% w/v. The required amounts of liposomal and free LA were weighed and diluted to 50 gr with Milli-Q water, and 0.5% w/v of xanthan gum was added to each sample to ensure homogeneity. Each sample was divided into several GC-MS vials and stored at 32ºC, to simulate physiological conditions on the skin surface. The headspace concentration of LA for each sample was measured daily by triplicate for a week, and the total concentration of LA in solution was measured by HPLC the first and last days of the experiments, following the above-mentioned procedure. To normalize the obtained areas, hexadecane-d34 was used as an internal standard due to its retention time being close to that of linalyl acetate.

3. Results

3.1. Selection of the Model Perfume Compound

Chromatograms obtained for each analyte are presented in Figure 1. Based on these chromatograms, subsequent tests were conducted using linalyl acetate (LA), as it exhibited the peak with the highest resolution among the three compounds studied.

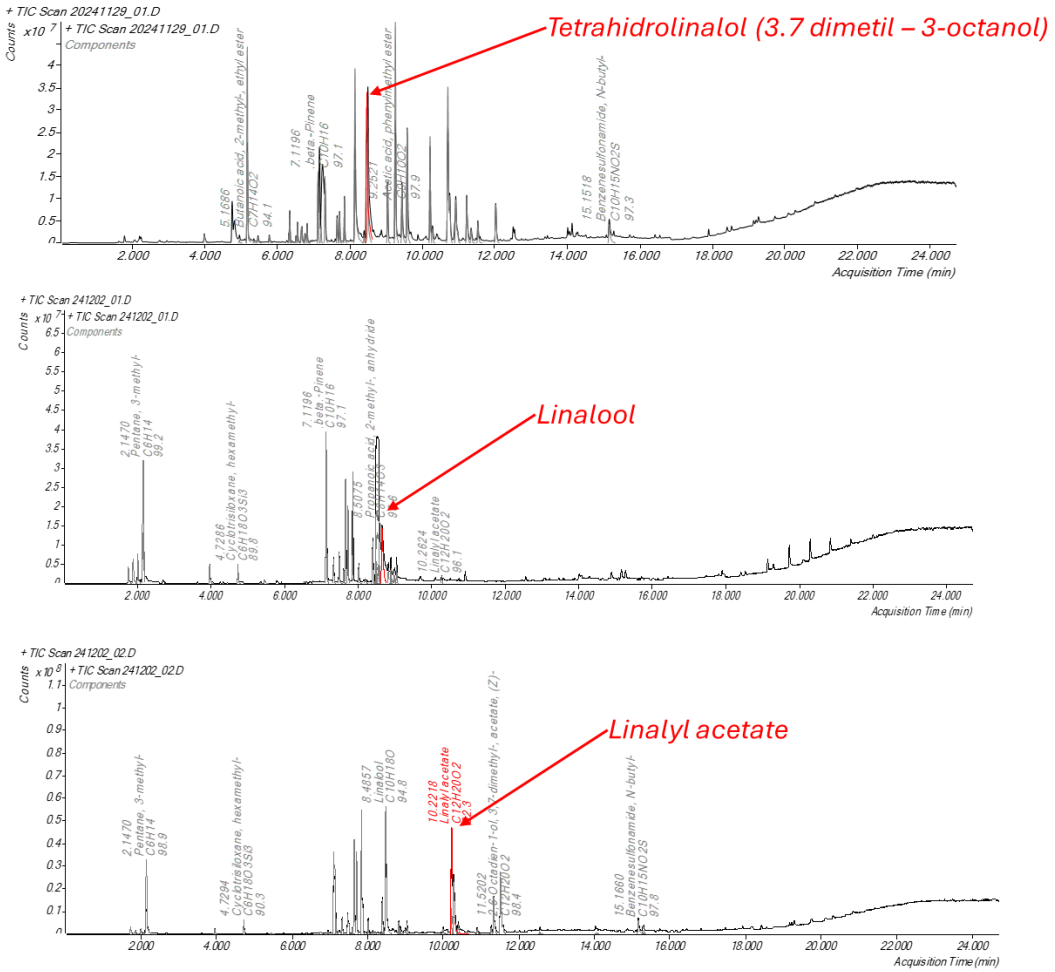

Figure 1. GC-MS chromatograms for Tetrahydrolinalol, Linalol and Linanlyl acetate

3.2. Development and characterization of liposomal LA

The mean diameter, PDI, zeta potential and encapsulation efficacy (EE%) of the liposomes are presented in Table 1.

Table 1. Characterization of unloaded liposomes and liposomes loaded with LA

Sample	Diameter (nm)	PDI	Zeta potential (mV)	EE (%)
Unloaded	156.8 ± 4.1	0.0971 ± 0.040	-2.128 ± 0.157	-
Loaded LA	164.7 ± 2.2	0.2230 ± 0.024	-16.94 ± 0.965	30 ± 2

Both loaded and unloaded liposomes exhibit similar diameters ranging between 150 and 300 nm, with PDI below 0.5, indicating a moderate particle size distribution appropriate for drug delivery. Lower PDI values suggest uniformity in the size of the liposomes. The zeta potential, which indicates the surface charge of the liposomes, plays a significant role in determining their stability and interaction with biological membranes. Unloaded liposomes exhibit surface charges close to zero, attributable to the neutral electric charge of the phosphatidylcholine (PC) used in their preparation. However, loaded liposomes present a negative charge, possibly due to the presence of linalyl acetate.

3.3. Controlled release

Total concentration of LA in solution at the beginning and at the end of the stability period, measured by HPLC, is shown in Table 2.

Table 2. Total LA concentration for both free and liposomal products, at the beginning and at the end of the stability period (1 week storage at 32ºC)

Sample	t0	1 week	Difference	Relative difference
Free LA	1.351%	1.334%	-0.017%	-1.258%
Loaded LA	1.324%	1.238%	-0.086%	-6.495%

Total concentration for both products was expected to be 1.5%. In both cases it can be observed that there is just a slight decrease in total concentration over the course of the stability period. This seems to indicate a good retention of the analyte in solution, independently of the encapsulation.

The results of the headspace GC-MS measurements are shown in Figure 1. Areas were normalized with respect to hexadecane-d34 and with respect to the total concentration of LA found by HPLC. t0 was arbitrarily set to an area of 0, assuming that when vials were prepared, there was no time for the diffusion processes to act, so all the LA would be in solution.

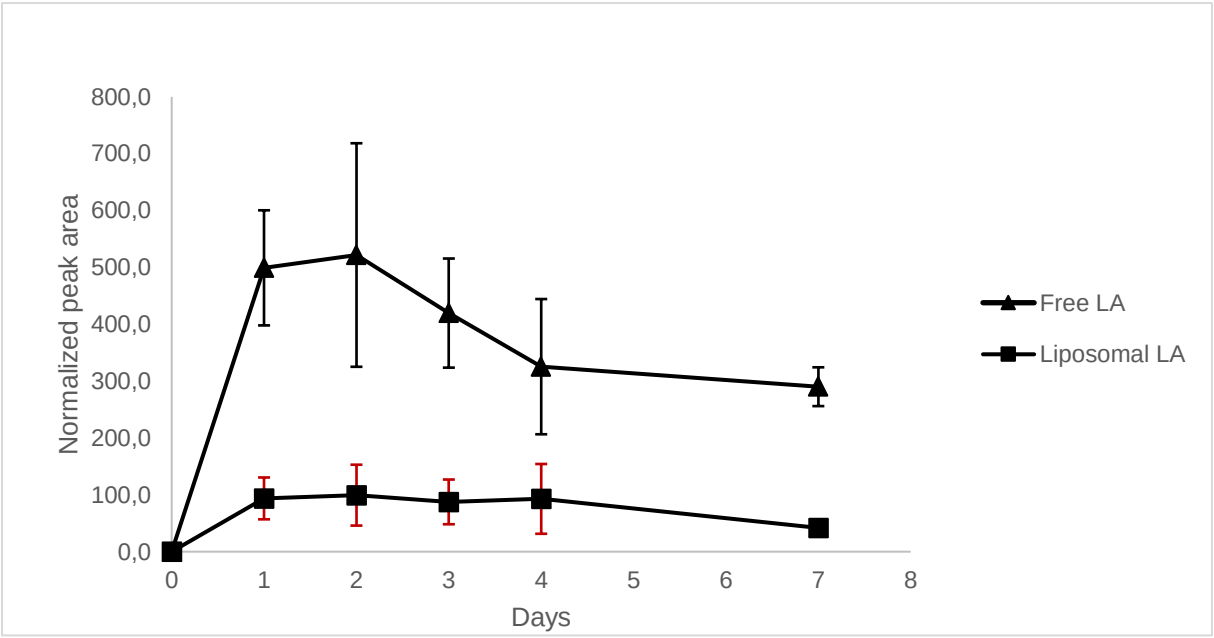

Figure 1. Normalized peak areas for liposomal and free LA, stored at 32ºC for 1 week. Areas were normalized with respect to an internal standard (hexadecane-d34) and with respect to the total amount of LA in solution measured by HPLC

Areas observed for liposomal LA remained constant during the duration of the experiment, whereas areas for free LA present a significant fluctuation. A significant increase in headspace concentration is found for free LA during the first two days of stability. After that time, the concentration begins to decrease until the end of the experiment. In the case of liposomal LA, this initial increase in vapor concentration is five times less intense, and the concentration remains

constant during the first 4 days of experiment, decreasing slightly after 7 days. It has been shown that linalyl acetate can be easily degraded in the presence of air, and that this process is accelerated by heat[9]. Therefore, a possible explanation of the results observed can be that liposomes are able to protect LA from external stressors that can cause its degradation, increasing the lifetime of the molecule.

4. Discussion

The results obtained in this study reinforce the potential of liposome-based delivery systems for the controlled release and enhanced stability of fragrance molecules in cosmetic and perfumery formulations.

Dynamic light scattering and zeta potential analyses confirmed the formation of stable liposomes with suitable physicochemical properties for dermal applications. The observed particle size (approximately 160 nm) and low polydispersity index (PDI < 0.3) are consistent with desirable characteristics for topical delivery systems and minimal aggregation during storage. The increase in negative surface charge upon loading LA (-16.94 mV) may be attributed to partial localization of the fragrance molecule at the liposomal interface, slightly altering the surface potential and contributing to colloidal stability. This charge shift is in line with previous studies reporting similar trends upon incorporation of non-ionic and hydrophobic compounds into liposomal bilayers[10]

Encapsulation efficiency was determined to be approximately 30%, which, while moderate, is acceptable given the volatility and low aqueous solubility of LA. Optimization of encapsulation efficiency for fragrance molecules often requires a balance between lipid composition, phase transition temperature, and the physicochemical nature of the scent compound. Further exploration into lipid derivatives, multilamellar systems, or hybrid vesicles may enhance entrapment yields in future studies.

Comparative release profiles highlight a significant advantage of the liposomal system. While both free and encapsulated LA showed minimal changes in total concentration over a one-week period at 32°C, the headspace GC-MS analysis revealed a markedly different volatilization behavior. Free LA exhibited a sharp increase in vapor phase concentration during the initial 48 hours, followed by a gradual decline, suggesting rapid diffusion and potential degradation. In contrast, liposomal LA showed a delayed and flattened release curve, indicating a more sustained and controlled evaporation rate. This aligns with the expected diffusion barrier role of liposomal bilayers, which slows the migration of volatile compounds toward the headspace, thereby preserving olfactory performance over time.

Notably, the stability of LA in liposomal formulations is likely enhanced by the physical protection offered by the bilayer and possibly by the antioxidative properties of some excipients used in the formulation. These findings are consistent with prior literature emphasizing the ability of liposomes to reduce oxidative degradation and extend the functional half-life of encapsulated actives in aqueous environments[11].

5. Conclusion

This study demonstrates that liposome-based delivery systems can be effectively employed for the encapsulation and controlled release of fragrance molecules, offering notable advantages in terms of stability, volatility control, and retention. The formulated liposomal system successfully encapsulated linalyl acetate and displayed favorable size distribution, colloidal

stability, and reproducible encapsulation efficiency. More importantly, the headspace GC-MS analysis confirmed that liposomal encapsulation significantly modulates the evaporation dynamics of LA, reducing its initial volatilization and maintaining a more constant release profile over time.

These findings are of relevance to the cosmetic and perfumery industries, where prolonged scent retention and fragrance stability are key formulation challenges. By offering a bio-compatible and efficient delivery system, liposomes open new opportunities for developing alcohol-free or low-solvent products with improved sensorial performance and environmental resilience.

Future studies should aim to validate these findings across a wider range of fragrance molecules, explore perceptual impacts through sensorial panel testing, and assess the performance of liposomal fragrances within final product matrices such as creams, lotions, or sprays. In addition, exploring large-scale manufacturing techniques and regulatory compliance pathways will be essential for the commercial translation of this technology.

6. Bibliography

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