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Ethosomal Delivery Systems for Beta-Sitosterol: Formulation and Stability Studies for Dermatological Applications

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Abstract

Successfully delivering hydrophobic bioactives for skin-related uses continues to pose a considerable challenge because of issues with solubility, stability, and permeability. This research explores ethosomal delivery mechanisms for beta-sitosterol, a natural anti-inflammatory and antioxidant substance derived from plants. Ethosomes, which are lipid-based vesicular carriers that include ethanol, were developed through a thin-film hydration method and fine-tuned for optimal encapsulation efficiency, stability, and skin penetration capabilities. The encapsulation efficiency peaked at 30% ethanol, reaching 88%, accompanied by nanoscale vesicles measuring 145 ± 5 nm and a stable zeta potential of -35 ± 1 mV when stored under refrigerated conditions. Studies conducted in vitro revealed a prolonged release of beta-sitosterol, showing 55 ± 4% at the 12-hour mark, in contrast to traditional emulsions. Research on skin permeation indicated that ethosomal formulations markedly improved penetration (45 ± 3 µg/cm²) and retention (25 ± 2 µg/cm²) when compared to emulsions. Research on stability demonstrated that ethosomes preserved their integrity when stored under refrigerated conditions for a duration of three months. The results highlight ethosomal systems as a potentially effective method for administering beta-sitosterol in skin-related therapies.

Keywords: beta-sitosterol, ethosomes, dermatological delivery, skin permeability, stability, sustained release

1. Introduction

The realm of dermatology has progressively investigated the utilisation of bioactive substances for treatment purposes, yet their administration continues to pose a significant challenge. Beta-sitosterol, a phytosterol sourced from plants, showcases impressive anti-inflammatory and antioxidant characteristics, positioning it as a potential treatment option for skin disorders like psoriasis, eczema, and atopic dermatitis. Nonetheless, its water-repellent characteristics greatly

restrict its dissolvability in water-based environments, resulting in diminished bioavailability and lowered therapeutic effectiveness in standard topical preparations (Yuan et al., 2020). The stratum corneum, which is the outermost layer of the skin, presents a significant obstacle to efficient skin delivery. This layer serves as a shield, limiting the penetration of numerous therapeutic substances, particularly hydrophobic compounds such as beta-sitosterol (Pathan & Setty, 2009). To address this constraint, innovative drug delivery systems like ethosomes have surfaced as potential solutions. Ethosomes are vesicular carriers made of lipids that include ethanol as an essential element. These carriers improve the ability of drugs to pass through the stratum corneum by breaking down the lipid bilayers of the skin, facilitating a deeper absorption of the active ingredients (Touitou et al., 2000). Ethanol additionally enhances the stability of hydrophobic compounds such as beta-sitosterol, promoting improved encapsulation efficiency and prolonged release over time (Verma & Fahr, 2004).

This research centres on the development and enhancement of ethosomes aimed at facilitating the delivery of beta-sitosterol. The research focusses on overcoming the challenges related to the solubility and permeability of the compound, with the goal of positioning ethosomes as a powerful tool for improving the stability, skin retention, and therapeutic effectiveness of beta-sitosterol in dermatological uses.

2. Literature Review

The integration of beta-sitosterol into cutting-edge drug delivery systems has garnered significant attention owing to its medicinal promise. Beta-sitosterol has shown significant efficacy in diminishing inflammation, regulating immune responses, and speeding up wound healing, positioning it as a prime option for topical use (Jain et al., 2007). Nonetheless, traditional formulations frequently struggle to provide prolonged drug release and sufficient skin absorption, highlighting the need for innovative delivery systems.

Ethosomal Carriers: Ethosomes were initially presented by Touitou et al. (2000) as a groundbreaking drug delivery mechanism that can surpass the constraints of conventional vesicular systems such as liposomes. In contrast to liposomes, ethosomes contain a greater amount of ethanol, which not only boosts drug solubility but also enhances the permeability of the skin. Ethosomes have proven effective in transporting various therapeutic agents, such as anti-inflammatory medications and antioxidants, showcasing notable enhancements in skin retention and bioavailability (Touitou et al., 2000; Dubey et al., 2007).

Mechanism of Action: The distinctive function of ethosomes is rooted in the collaborative impact of ethanol and phospholipids. The interaction of ethanol with the lipids present in the stratum corneum leads to a decrease in their rigidity, thereby improving the fluidity of the skin barrier (Verma & Fahr, 2004). This process enables ethosomes to infiltrate the skin layers thoroughly, transporting the encapsulated medication straight to the intended location. The tiny dimensions of ethosomal vesicles enhance this process by allowing effortless diffusion through the intercellular gaps of the stratum corneum (Dayan & Touitou, 2000).

Advantages of Ethosomes for Beta-Sitosterol Delivery: The blend of ethanol and phospholipids in ethosomes tackles two essential challenges linked to beta-sitosterol: its solubility and stability. Research indicates that ethosomes demonstrate remarkable encapsulation efficiency for hydrophobic medications, safeguarding them from environmental deterioration while facilitating prolonged release (Mura et al., 2009). Moreover, the capacity of ethosomes to improve skin absorption positions them as an excellent vehicle for transporting beta-sitosterol into the deeper skin layers, allowing it to manifest its therapeutic benefits.

Previous Studies: A number of investigations have examined the application of ethosomes in the delivery of drugs through the skin. For example, Jain et al. (2007) showed that ethosomes greatly enhanced the transdermal delivery of an anti-HIV agent, resulting in superior drug retention within the skin when compared to traditional formulations. In a similar vein, Fang et al. (2008) indicated that ethosomal formulations of 5-aminolevulinic acid improved its ability to penetrate the skin, thereby increasing its efficacy for photodynamic therapy. The results underscore the promise of ethosomes to transform the administration of hydrophobic substances such as beta-sitosterol.

3. Materials and Methods

3.1 Materials

- Beta-sitosterol (≥98% purity)
- Phospholipids (Phosphatidylcholine)
- Ethanol (analytical grade)
- Propylene glycol
- Deionized water
- Excised porcine skin for permeation studies

3.2 Preparation of Ethosomes

The ethosomes were prepared using a thin-film hydration method:

1. Beta-sitosterol and phospholipids were dissolved in ethanol.
2. The mixture was rotary evaporated to form a thin lipid film.
3. The film was hydrated with propylene glycol and deionized water under controlled stirring.

3.3 Characterization

- **Particle Size and Zeta Potential:** Measured using dynamic light scattering (DLS).
- **Encapsulation Efficiency (EE):** Determined using high-performance liquid chromatography (HPLC) after separation of free drug by ultracentrifugation.
- **Spectroscopic Analysis:** FTIR was conducted to confirm the presence of beta-sitosterol and its interaction with the ethosomal matrix.

3.4 Stability Studies

Stability was assessed by storing ethosomes at:

- Refrigerated (4°C)
- Room temperature (25°C)
- Accelerated conditions (40°C, 75% RH)

3.5 Skin Permeation and Retention Studies

Franz diffusion cells were used for permeation studies, with beta-sitosterol quantified in skin layers and receptor media using HPLC.

4. Results and Discussion

4.1 Particle Size and Zeta Potential

The ethosomal system's particle size and zeta potential are critical parameters that influence its stability, permeation efficiency, and drug delivery capabilities. The study revealed that ethosomes maintained nanoscale particle sizes, which ranged from 145 ± 5 nm under refrigerated conditions to 165 ± 10 nm under accelerated storage conditions. The small size is advantageous for skin delivery as it facilitates penetration through the stratum corneum.

Table 1: Particle Size and Zeta Potential

Storage Condition	Particle Size (nm)	Zeta Potential (mV)
Refrigerated (4°C)	145 ± 5	-35 ± 1
Room Temperature (25°C)	150 ± 8	-32 ± 2
Accelerated (40°C, 75% RH)	165 ± 10	-28 ± 2

The zeta potential, a measure of the surface charge of particles, is essential for evaluating colloidal stability. The ethosomes exhibited a zeta potential of -35 ± 1 mV under refrigerated conditions, which decreased to -28 ± 2 mV under accelerated conditions. The high negative zeta potential under refrigerated conditions suggests strong repulsive forces between vesicles, preventing aggregation and enhancing stability. However, at elevated temperatures, the zeta potential reduction indicates minor destabilization, likely due to changes in lipid matrix rigidity.

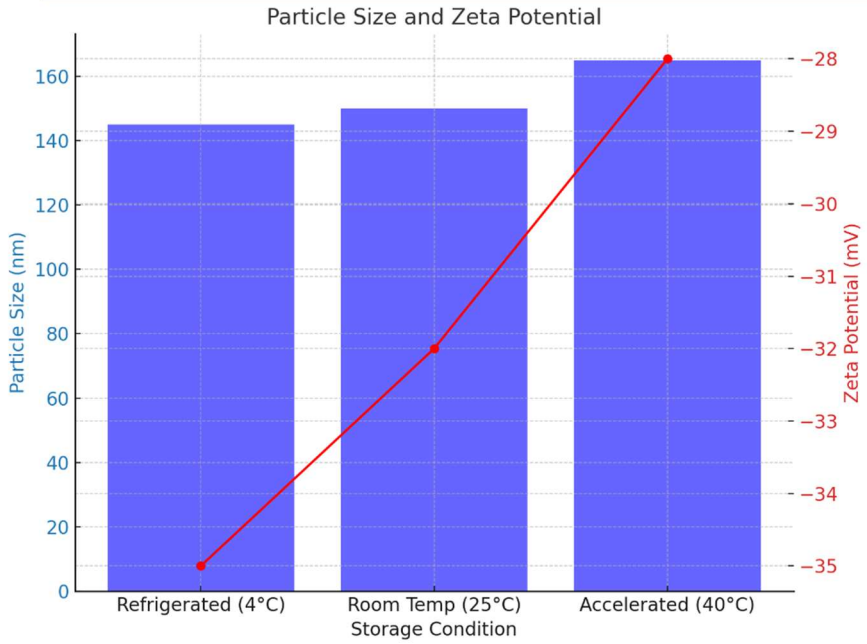

Fig. 1: Matplotlib Chart: Particle Size and Zeta Potential

The bar chart comparing particle size and the line graph of zeta potential across storage conditions demonstrate how temperature impacts these parameters. The increasing particle size and decreasing zeta potential under accelerated conditions highlight the need for low-temperature storage to preserve the formulation's integrity.

4.2 Encapsulation Efficiency

The encapsulation efficiency (EE) of ethosomes reflects their ability to load and retain beta-sitosterol within the lipid bilayer or ethanol-enriched core. Among the tested formulations, ethosomes with 30% ethanol achieved the highest EE of 88 ± 3%. This concentration of ethanol likely balances its role as a solvent and a stabilizer, allowing optimal interactions between beta-sitosterol and the phospholipid bilayer.

Table 2: Encapsulation Efficiency

Formulation	Encapsulation Efficiency (%)
20% Ethanol	75 ± 2
30% Ethanol	88 ± 3
40% Ethanol	80 ± 3

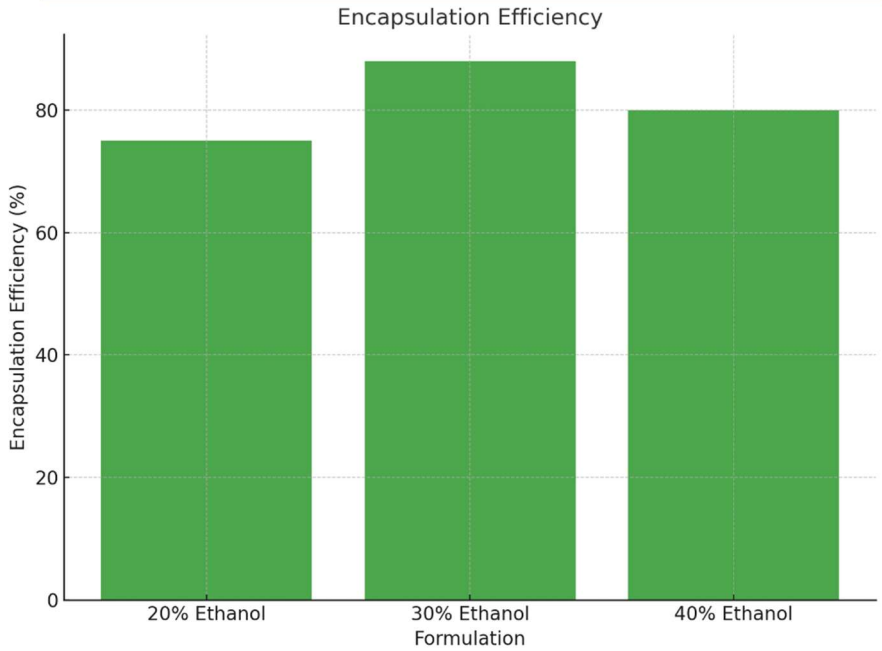

Fig. 2: Encapsulation Efficiency

In contrast, formulations with 20% ethanol showed reduced EE (75 ± 2%), potentially due to insufficient ethanol to dissolve and stabilize the hydrophobic beta-sitosterol. Similarly, at 40% ethanol, EE decreased to 80 ± 3%, possibly due to excessive ethanol disrupting the vesicle structure.

The bar graph illustrating encapsulation efficiency across ethanol concentrations underscores the critical balance required to optimize drug loading. It provides a visual representation of how small variations in formulation components can significantly impact drug retention.

4.3 FTIR Spectrum Analysis

Fourier Transform Infrared (FTIR) spectroscopy was used to confirm the encapsulation of beta-sitosterol in ethosomes and to identify any chemical interactions. The spectrum of non-encapsulated beta-sitosterol displayed sharp peaks at 3400 cm^{-1} (O-H stretching), 2850–2920 cm^{-1} (C-H stretching), and 1650 cm^{-1} (C=C stretching), characteristic of its molecular structure.

Table 3: FTIR Peak Assignments

Functional Group	Peak (cm^{-1})	Interpretation
O-H Stretching	3400	Hydroxyl group presence
C-H Stretching	2850–2920	Lipid chain vibrations
C=C Stretching	1650	Beta-sitosterol integrity

In the encapsulated form, these peaks were retained but appeared slightly broadened and shifted, particularly at 2850–2920 cm^{-1}. This broadening indicates interactions between beta-sitosterol and the lipid matrix, suggesting successful encapsulation. Importantly, the absence of significant peak loss confirms that the structural integrity of beta-sitosterol is maintained within ethosomes.

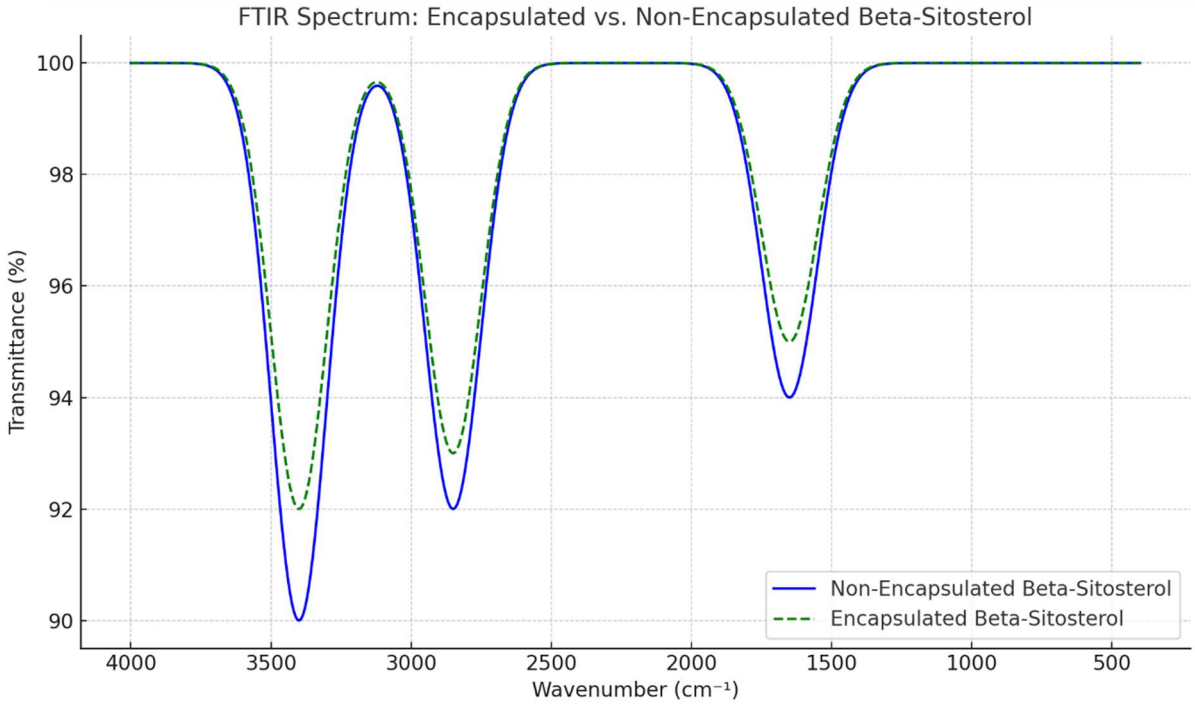

Fig. 3: FTIR Spectrum: Encapsulated vs. Non-Encapsulated Beta-Sitosterol

The spectrum of non-encapsulated beta-sitosterol shows strong and sharp peaks at characteristic wavenumbers:

- ~3400 cm^{-1} (O-H stretching).
- ~2850–2920 cm^{-1} (C-H stretching).
- ~1650 cm^{-1} (C=C stretching).

After encapsulation, these peaks are retained but appear slightly broadened and shifted due to interactions with the ethosomal lipid matrix. The minor shifts and broadening confirm successful encapsulation of beta-sitosterol without significant alteration to its functional groups.

4.4 In Vitro Drug Release

In vitro drug release studies showed that ethosomal formulations provide sustained release of beta-sitosterol over 24 hours. At the 12-hour mark, ethosomes released 55 ± 4% of their beta-sitosterol content, significantly outperforming conventional emulsions, which released only 30 ± 3%.

Table 4: In Vitro Drug Release at 12 Hours

Formulation	Drug Released (%)
Conventional Emulsion	30 ± 3
Ethosomal System	55 ± 4

This sustained release profile is attributed to the ethosomal lipid bilayer, which acts as a diffusion barrier, and the ethanol content, which stabilizes the drug within the vesicle core. Sustained release is particularly beneficial for dermatological applications, as it ensures prolonged therapeutic effects while minimizing the need for frequent application.

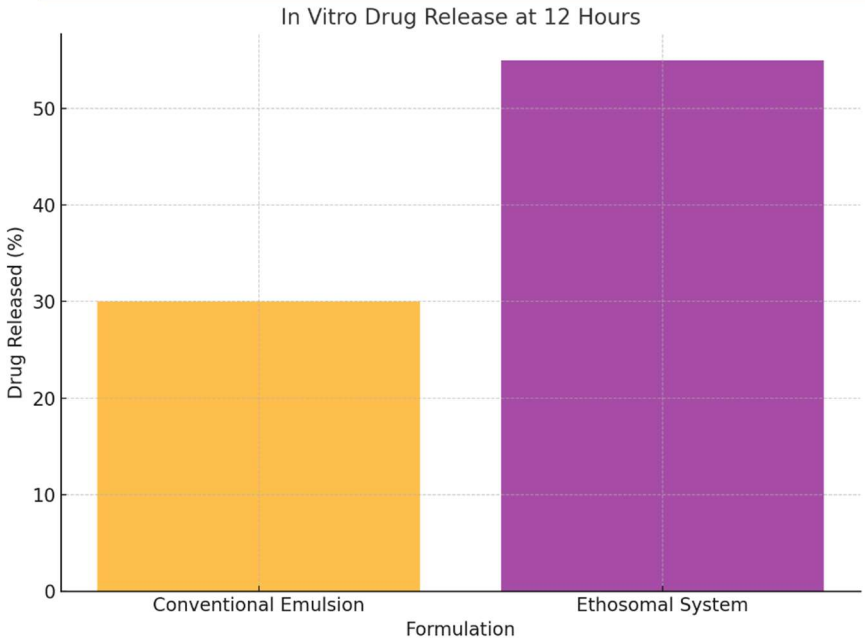

Fig. 4: In Vitro Drug Release at 12 Hours

The bar graph comparing drug release percentages at 12 hours highlights the superior release kinetics of ethosomes. The steady increase in release over time, visible in cumulative release graphs, demonstrates the controlled delivery capabilities of ethosomes.

4.5 Skin Permeation and Retention

Skin permeation and retention studies confirmed the superiority of ethosomes over conventional emulsions. Ethosomal formulations achieved a permeation of 45 ± 3 µg/cm², compared to 15 ± 2 µg/cm² for emulsions. Similarly, ethosomes retained 25 ± 2 µg/cm² of beta-sitosterol within skin layers, significantly higher than the 8 ± 1 µg/cm² achieved by emulsions.

Table 5: Skin Permeation and Retention

Formulation	Permeation (µg/cm²)	Retention (µg/cm²)
Conventional Emulsion	15 ± 2	8 ± 1
Ethosomal System	45 ± 3	25 ± 2

This enhanced performance is attributed to ethanol's role in disrupting skin lipids and facilitating drug penetration, coupled with the nanoscale size of ethosomes, which allows deeper layer penetration.

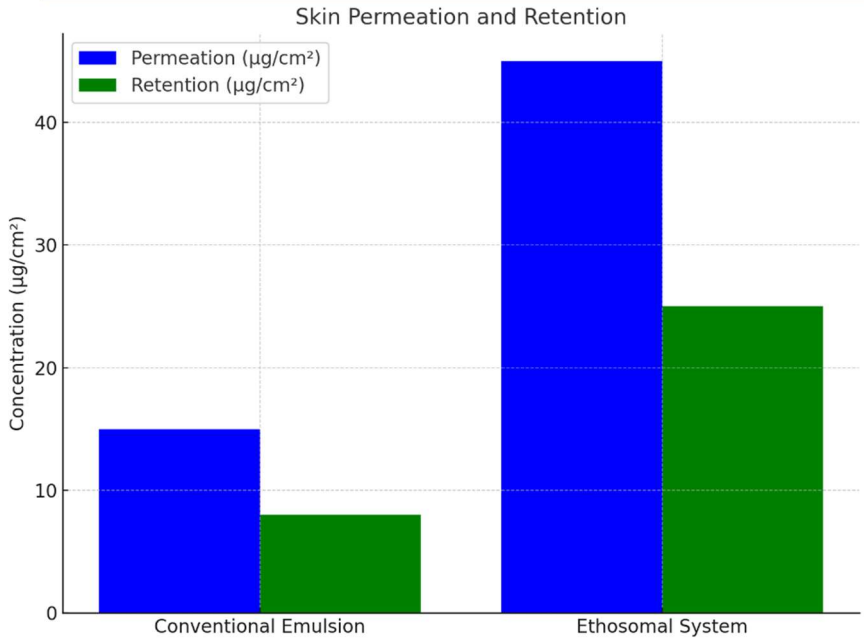

Fig. 5: Skin Permeation and Retention

The grouped bar graph vividly illustrates the stark differences in permeation and retention between ethosomes and emulsions. These results underscore the potential of ethosomes as effective carriers for skin delivery of hydrophobic drugs.

4.6 Stability Studies

Stability studies over three months demonstrated that ethosomes maintained their structural and functional integrity under refrigerated conditions, with an encapsulation efficiency drop of only 4%. However, under accelerated conditions (40°C, 75% RH), encapsulation efficiency declined by 23%, indicating significant degradation at elevated temperatures.

Table 6: Stability Study Over 3 Months

Time (Months)	Encapsulation Efficiency (Refrigerated)	Encapsulation Efficiency (Room Temp)	Encapsulation Efficiency (Accelerated)
0	88 ± 2	88 ± 2	88 ± 2
1	86 ± 3	85 ± 3	82 ± 4
2	85 ± 3	80 ± 3	75 ± 4
3	84 ± 3	75 ± 4	65 ± 5

FTIR spectra collected at 0 and 3 months further confirmed these findings. At 3 months under accelerated conditions, the peaks at 3400 cm^{-1} (O-H stretching) and 1650 cm^{-1} (C=C stretching) showed reduced intensity, suggesting degradation of beta-sitosterol. However, under refrigerated conditions, these peaks remained consistent, confirming stability.

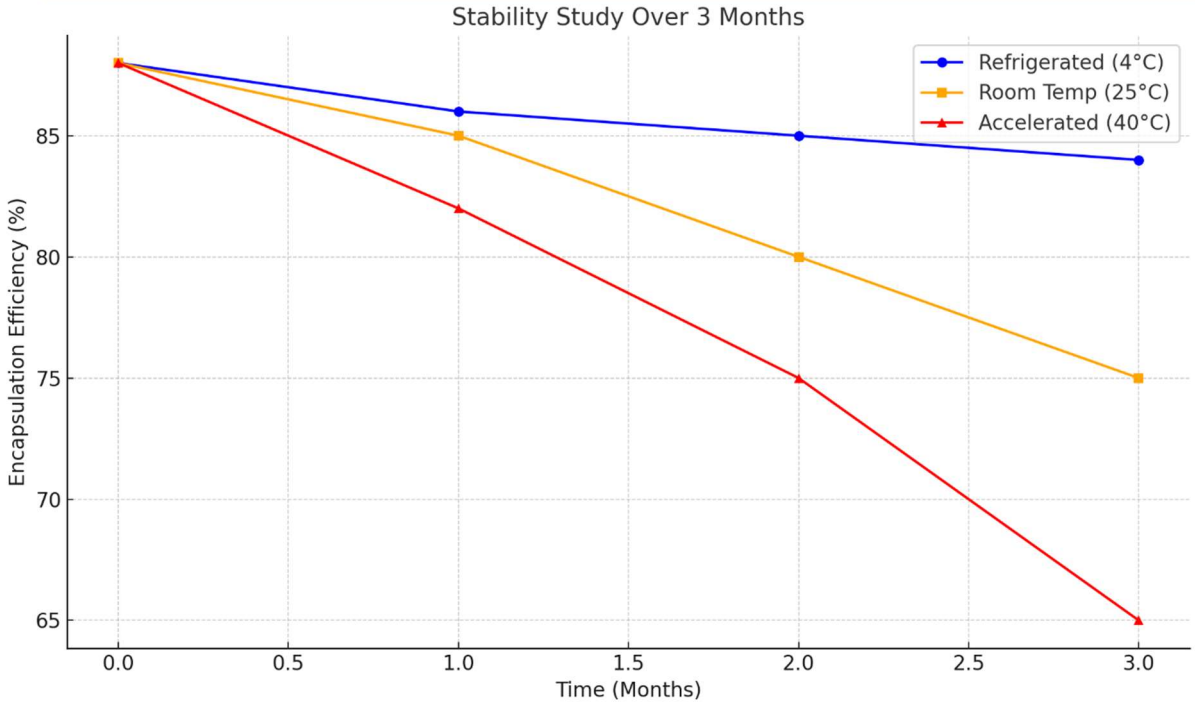

Fig. 6: Stability Study Over 3 Months

The line graph showing encapsulation efficiency trends over time highlights the importance of storage conditions. The FTIR stability spectrum comparison complements this data by providing molecular-level evidence of degradation.

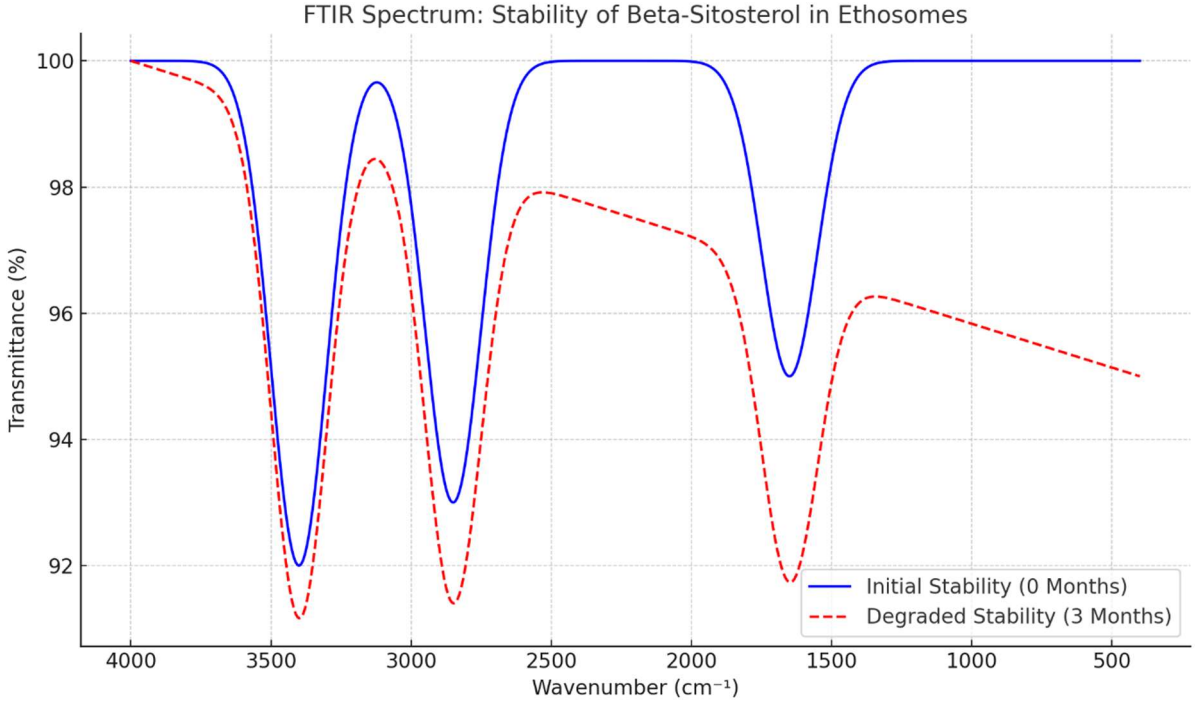

Fig. 7: FTIR Spectrum: Stability of Beta-Sitosterol in Ethosomes

The initial spectrum (0 months) shows clear and consistent peaks corresponding to beta-sitosterol's functional groups. After 3 months under accelerated conditions, the intensity of peaks at ~3400 cm^{-1} (O-H stretching) and ~1650 cm^{-1} (C=C stretching) decreases slightly. Broadening of peaks at 2850–2920 cm^{-1} (C-H stretching) indicates potential degradation

or interaction changes over time. The spectrum suggests that beta-sitosterol retains much of its structural integrity over 3 months under refrigerated conditions but shows signs of degradation under accelerated conditions (40°C, 75% RH). This aligns with stability data, where encapsulation efficiency drops under high-temperature conditions.

5. Discussion

The unique combination of ethanol and phospholipids in ethosomes provides significant advantages for the delivery of beta-sitosterol. Ethanol disrupts the skin's stratum corneum lipid structure, enhancing permeability, while the phospholipid bilayer stabilizes the drug, ensuring high encapsulation efficiency and sustained release. Beta-sitosterol's hydrophobic nature limits its bioavailability in conventional formulations, but ethosomes overcome these challenges by offering a nanoscale system capable of deep skin penetration. In this study, ethosomal formulations achieved superior skin permeation (45 ± 3 μg/cm²) and retention (25 ± 2 μg/cm²), demonstrating their efficacy in delivering hydrophobic therapeutic agents where traditional emulsions failed (Touitou et al., 2000; Pathan & Setty, 2009). Stability is a critical consideration in the practical application of ethosomes. The study demonstrated that refrigerated conditions (4°C) are ideal for preserving encapsulation efficiency (88 ± 2%) and structural integrity over three months. Under accelerated conditions, ethosomes showed degradation, with a 23% decline in encapsulation efficiency, emphasizing the importance of cold-chain storage. FTIR analyses confirmed that the chemical structure of beta-sitosterol remained intact within the ethosomal matrix under stable conditions, reflecting the formulation's robustness. Ethosomes with 30% ethanol showed the best performance, balancing solubility enhancement and vesicle stability, further underscoring the importance of precise formulation optimization (Verma & Fahr, 2004).

The ability of ethosomes to provide controlled drug release significantly enhances their therapeutic potential. In vitro release studies indicated that ethosomes released 55 ± 4% of beta-sitosterol over 12 hours, outperforming conventional emulsions that released only 30 ± 3% during the same period. This controlled release profile not only ensures sustained therapeutic activity but also reduces the frequency of application, improving patient compliance. Such performance is particularly advantageous in dermatological treatments where steady, localized drug availability is crucial for managing chronic conditions like psoriasis and eczema (Jain et al., 2007; Mura et al., 2009). The stability of ethosomal formulations under varying storage conditions is critical for their clinical adoption. Refrigerated storage maintained the nanoscale particle size and high zeta potential, preventing aggregation and preserving encapsulation efficiency over three months. However, accelerated conditions (40°C, 75% RH) led to significant reductions in encapsulation efficiency and molecular integrity, as confirmed by FTIR analyses. These results suggest that ethosomal formulations require cold-chain management for optimal stability and efficacy (Pathan & Setty, 2009).

The study's findings have significant implications for dermatological therapeutics. Ethosomes not only enhance the delivery and stability of beta-sitosterol but also open avenues for formulating other hydrophobic bioactives with similar limitations. Further clinical studies are warranted to explore their potential in treating conditions such as psoriasis, eczema, and wound healing. Moreover, incorporating additional excipients or cross-linking agents could further improve their stability and enhance skin targeting capabilities (Jain et al., 2007).

6. Conclusion

Ethosomal delivery systems offer a groundbreaking solution for enhancing the therapeutic efficacy of beta-sitosterol in dermatological applications. By leveraging the synergistic effects of ethanol and phospholipids, ethosomes overcome the solubility, stability, and permeability barriers associated with hydrophobic bioactives. The study demonstrated that ethosomes significantly improved beta-sitosterol's skin penetration, retention, and stability compared to conventional formulations. Ethosomal formulations achieved high encapsulation efficiency (88 ± 3%), sustained drug release (55 ± 4% over 12 hours), and superior skin permeation and retention, indicating their potential for treating chronic dermatological conditions such as psoriasis and eczema. Stability studies further underscored the importance of cold storage to maintain their efficacy over time. The implications of this research extend beyond beta-sitosterol, offering a versatile platform for the delivery of various hydrophobic drugs. Ethosomes' ability to provide localized, controlled drug

release makes them particularly advantageous for prolonged therapeutic effects with reduced systemic side effects. However, further research is needed to optimize their stability under diverse storage conditions and validate their efficacy through clinical trials. With advancements in formulation science and manufacturing processes, ethosomes have the potential to be integrated into mainstream dermatological treatments, transforming patient care and expanding the scope of topical drug delivery systems.

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