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Liposomal Calcium: A Novel Nutraceutical Delivery Approach by West Bengal Chemical Industries Ltd., Kolkata, India

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ABSTRACT

Liposomes are advanced nutraceutical delivery systems renowned for their ability to enhance pharmacokinetics, improve bioavailability, and enable targeted therapeutic interventions. This study focuses on the synthesis, characterization, and application of calcium-responsive liposomes developed by West Bengal Chemical Industries Ltd., Kolkata, India. These innovative formulations of liposomal calcium leverage the critical role of calcium in cellular processes to achieve controlled and precise nutraceutical release. Comprehensive analyses, including encapsulation characteristics, scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), and differential scanning calorimetry (DSC), confirmed the liposomes' structural integrity, thermal stability, encapsulation efficiency (88%), and calcium assay (31.2% w/w). Accelerated stability studies revealed minimal leakage and robust performance under varying conditions, demonstrating their practicality for clinical use. Zeta potential (-30.67 mV), polydispersity index (0.2231), and particle size (142.5 nm) further validated their suitability for therapeutic applications. These findings underscore the potential of liposomal calcium in addressing conditions such as osteoporosis, cardiovascular diseases, and heavy metal detoxification, marking a significant step forward in targeted nutraceutical delivery systems.

Keywords: *Liposomes, Delivery Systems, Scanning Electron Microscopy, Encapsulation Characteristics, Cardiovascular Diseases, Osteoporosis*

1. Introduction

Liposomes have emerged as clinically approved supramolecular nutraceutical delivery systems, renowned for their ability to encapsulate therapeutic agents and enhance their pharmacokinetic and pharmacodynamic properties [1]. These nanocarriers provide a unique platform for targeted nutraceutical delivery, reducing systemic toxicity and increasing therapeutic efficacy [2]. Liposomal calcium plays a crucial role in improving calcium bioavailability and absorption, making it an effective supplement for bone health, muscle function, and cellular signaling. Encapsulation in liposomes enhances calcium stability, protects it from degradation, and facilitates targeted delivery to specific tissues. This advanced formulation minimizes gastrointestinal discomfort and maximizes calcium uptake, addressing deficiencies more efficiently than conventional calcium supplements [3].

West Bengal Chemical Industries Ltd., Kolkata, India, has revolutionized this domain with the development of advanced liposomal calcium formulations. These innovative formulations are designed to exploit calcium's critical role in cellular signaling, disease progression, and tissue repair. The calcium liposomes manufactured by West Bengal Chemical Industries Ltd. are engineered to ensure stability, biocompatibility, and high selectivity, making them ideal for applications in treating conditions such as osteoporosis, calcium deficiency disorders, and cardiovascular diseases.

The benefits of liposomal calcium include enhanced bioavailability, targeted delivery to affected tissues, and minimized side effects due to the controlled release mechanism [3,4].

Furthermore, these formulations integrate cutting-edge lipid chemistry to ensure efficient calcium encapsulation and release, as evidenced by fluorescence-based assays, dynamic light scattering (DLS), and scanning transmission electron microscopy (STEM) studies [4]. The inclusion of phosphatidylcholine (PC) in the liposomal composition mirrors the biological composition of cellular membranes, further enhancing their effectiveness and biocompatibility [5].

This article explores comprehensive analyses, including scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), and differential scanning calorimetry (DSC), to confirm the structural integrity, thermal stability, and encapsulation efficiency of liposomal calcium. By leveraging the expertise of West Bengal Chemical Industries Ltd., these liposomal systems offer a promising therapeutic solution for a range of diseases, redefining the scope of targeted and efficient nutraceutical delivery.

2. Comparative Analysis of Liposomal Calcium

Bioavailability, Stability, and Physicochemical Properties

Liposomes have proven to be a versatile platform in nutraceutical delivery systems, particularly for enhancing the effectiveness of therapeutic agents. Recent studies have emphasized the advantages of liposomal formulations in improving nutrient bioavailability, targeted nutraceutical release, and therapeutic efficacy. The following sections of the literature review will focus on the applications of liposomal calcium for improving calcium absorption in our body.

2.1. Analyses of Liposomal and Non-Liposomal Calcium

The efficacy profiles of liposomal versus non-liposomal multivitamin and mineral (MVM) formulations have been investigated by previous studies. The study demonstrated that liposomal encapsulation significantly enhances nutrient absorption and clearance rates, as reflected in lower mineral volume distribution, higher bioavailability, and improved nutrient efficacy parameters such as clearance rates and elimination half-life [6]. Notably, liposomal formulations exhibited greater clearance and absorption of minerals, such as calcium, compared to non-liposomal counterparts, indicating their potential to improve health outcomes. The findings highlight the importance of liposomal encapsulation in optimizing nutrient delivery and underscore the need for further research into the long-term health benefits of liposomal calcium supplements [6].

2.2. Comparison of Bioavailability and Absorption of Liposomal and Non-Liposomal Calcium

Bioavailability and absorption are key determinants of calcium supplementation efficacy. Studies have shown that liposomal calcium exhibits superior bioavailability compared to conventional calcium salts, such as calcium carbonate or calcium citrate [7]. The phospholipid bilayer of liposomes enhances calcium transport across the intestinal epithelium, bypassing traditional ionized calcium absorption pathways, which are often limited by gastric pH and the presence of competing ions. [6, 7] While specific quantitative data on the percentage increase in bioavailability of liposomal calcium over conventional forms is limited, some studies have indicated improved absorption and retention rates with liposomal formulations. For instance, clinical trials have reported higher plasma calcium levels in individuals consuming liposomal calcium, suggesting enhanced absorption efficiency and reduced gastrointestinal side effects compared to non-liposomal counterparts. The number of clinical trials specifically investigating liposomal calcium is limited. One study, titled "Effects of Liposomal Encapsulation on Calcium Powder Absorption and Metabolism," aimed to evaluate the absorption and metabolism of liposomal calcium powder. However, detailed results from this trial are not readily available. Studies have shown that as gastric pH increases, the disintegration and dissolution of calcium carbonate decrease significantly from 96% at pH 1 to 23% at pH 6.1.[7] The pharmacokinetics of liposomal calcium involve its absorption, distribution, metabolism, and excretion. Liposomal encapsulation enhances the transport of calcium across the intestinal epithelium by bypassing traditional ionized calcium absorption pathways. This mechanism is less affected by gastric pH and the presence of competing ions, potentially leading to improved bioavailability. Once absorbed, liposomal calcium is distributed through the bloodstream to various tissues. The liposomal delivery system may facilitate more efficient cellular uptake due to the nanoscale size and phospholipid composition of the vesicles. Calcium itself is not metabolized but is utilized in various physiological processes, including bone mineralization, muscle contraction, and nerve transmission. The liposomal carrier may be metabolized by hepatic enzymes or other pathways, depending on its composition. Excess calcium is excreted primarily through the kidneys. The impact of liposomal delivery on the excretion rates of calcium has not been extensively studied and warrants further research.

2.3. Physicochemical Characterization and Thermal Stability of Liposomal Calcium

Previous studies have extensively characterized liposomal calcium using techniques such as dynamic light scattering (DLS), zeta potential measurements, and transmission electron microscopy (TEM).[8] These analyses have confirmed the nanoscale size distribution of liposomal calcium formulations, typically ranging from 50 to 200 nm, which contributes to their enhanced cellular uptake [8]. Encapsulation efficiency and loading capacity studies have further validated the stability of calcium within the liposomal core. Additionally, the differential scanning calorimetry (DSC) study provided insights into the structural integrity of liposomal calcium under physiological conditions, demonstrating its potential as a stable and effective supplement [8].

2.4. Encapsulation Efficiency and Stability of Liposomal Calcium

Encapsulation efficiency is a crucial factor in determining the effectiveness of liposomal calcium delivery, as it directly influences bioavailability and stability. Studies report encapsulation efficiencies ranging between 70% and 90%, depending on factors such as lipid composition, preparation techniques, and the calcium salt used [8,9]. Among different formulation methods, thin-film hydration followed by sonication or extrusion has been found to yield higher encapsulation rates compared to traditional emulsion-based techniques.[9] This is attributed to the uniform size distribution and better integration of calcium within the liposomal bilayer. Furthermore, cholesterol incorporation into liposomal membranes has been shown to play a significant role in enhancing encapsulation efficiency and structural stability. Cholesterol stabilizes the lipid bilayer, reducing permeability and minimizing calcium leakage over time [9]. This is particularly beneficial for applications requiring extended shelf life and sustained calcium release. Additionally, optimizing the lipid-to-calcium ratio and adjusting preparation conditions such as hydration temperature, sonication time, and extrusion pressure can further improve encapsulation efficiency and prevent premature calcium release. Overall, achieving high encapsulation efficiency is essential for developing a stable and effective liposomal calcium formulation, ensuring enhanced bioavailability, controlled release, and prolonged stability in various pharmaceutical and nutraceutical applications. Further research into novel lipid compositions and optimization strategies could help enhance encapsulation efficiency, making liposomal calcium an even more reliable and versatile supplement.

2.5. Physical and Chemical Stability of Liposomal Calcium in Solution

The stability of liposomal calcium in aqueous solutions is influenced by factors such as pH, ionic strength, and storage conditions. [7, 9] Research indicates that liposomal calcium exhibits prolonged stability in neutral or slightly acidic environments, with minimal aggregation or precipitation over extended periods. The presence of stabilizing agents like cholesterol and polyethylene glycol (PEG) further enhances liposomal integrity, preventing vesicle fusion and degradation. [7, 8, 9] Long-term storage studies have demonstrated that liposomal calcium retains its encapsulation efficiency and size distribution for several months under refrigeration, making it a viable formulation for clinical and commercial applications [8].

2.6. FTIR Analysis for Liposomes, Calcium, and Liposomal Calcium

Beyond encapsulation efficiency and stability, a deeper understanding of liposomal calcium formulations requires an exploration of the molecular interactions between calcium ions and the lipid bilayer. The structural organization and binding mechanisms within liposomal systems play a crucial role in their functional properties, influencing stability, bioavailability, and release kinetics. Spectroscopic and analytical techniques provide valuable insights into these interactions, helping to optimize liposomal formulations for enhanced performance. Various analytical techniques, including Fourier-transform infrared (FTIR) spectroscopy, have been employed to characterize the physicochemical interactions within liposomal calcium systems. These methods enable researchers to assess molecular bonding, lipid phase transitions, and encapsulation success, offering a comprehensive view of liposomal integrity. Fourier-transform infrared (FTIR) spectroscopy is widely used to characterize the interactions between calcium and lipid bilayers in liposomal formulations.[9] FTIR spectra of free calcium salts typically exhibit strong absorption peaks corresponding to carbonate or phosphate functional groups.[10] In contrast, liposomes show characteristic peaks associated with phospholipid headgroups and hydrocarbon chains. Upon encapsulation, liposomal calcium demonstrates spectral shifts or peak broadening, indicating calcium-lipid interactions [10]. These shifts, particularly in the phosphate stretching and carbonyl regions, confirm successful encapsulation and potential binding between calcium ions and lipid molecules, contributing to the stability of the liposomal system [10].

2.7. Factors Affecting Liposome Integrity

Liposome integrity is crucial for effective nutraceutical delivery, and factors like salt concentration, pH, temperature, and excipients significantly influence the stability and performance of liposomal formulations. Salt concentration affects electrostatic interactions within the lipid bilayer; high concentrations can cause leakage, while low concentrations may stabilize the liposomes [20]. pH variations impact the charge and polarity of the membrane, potentially destabilizing the liposomes, which in turn affects nutraceutical release and encapsulation efficiency. [17, 18] Temperature also plays a critical role- higher temperatures can increase permeability, leading to premature nutraceutical leakage, while lower temperatures may cause aggregation or fusion [18-19]. Excipients such as stabilizers and surfactants can enhance stability and modify nutraceutical release, but their interactions with liposomal components need to be carefully monitored to avoid negatively affecting liposome integrity.[19] Understanding how these factors affect liposome integrity is essential for optimizing formulations, ensuring sustained release, improved bioavailability, and regulatory compliance for clinical use [18]. Together, these studies illustrate the versatility and effectiveness of liposomal formulations across various applications, from improving nutrient bioavailability to enabling precise nutraceutical release and enhancing chelation therapy.

Materials and Methods

3.1 Liposome Characterization

Liposome characterization was carried out according to the "Guidance for Industry on Liposome Nutraceutical Products" published by the USFDA to assess the quality, stability, and performance of liposomal calcium [9]. The Important physicochemical parameters such as morphology, surface properties, and zeta potential were examined to evaluate the stability and functionality of the formulation. Morphology was assessed using Scanning Electron Microscopy (SEM), which confirmed uniformity in shape and structure, crucial for reproducibility in therapeutic applications. Surface properties were analyzed with imaging and spectroscopic techniques, providing insights into the physicochemical composition.[9] These properties impact liposome interactions with biological fluids, stability, and bioavailability. The zeta potential, indicating the net charge of liposomes, was measured to ensure colloidal stability. A zeta potential value > +30 mV and < -30 mV indicates strong repulsive forces, preventing aggregation and ensuring long-term dispersion and stability. These measurements adhere to ICH guidelines and optimize liposome formulation for better nutraceutical delivery and therapeutic efficacy. This characterization ensures that the liposomal calcium formulation meets stability, integrity, and functionality standards, facilitating optimization for targeted delivery and controlled release.

3.2 Encapsulation Efficiency and Nutraceutical Loading

Encapsulation efficiency and nutraceutical loading capacity are key parameters in evaluating liposomal calcium formulations. Encapsulation efficiency was calculated to determine the percentage of calcium enclosed within the liposomes, with acceptance criteria set at not less than 85%. High efficiency is crucial for therapeutic efficacy, bioavailability, and controlled release, as low efficiency can lead to free calcium, increasing the risk of precipitation and instability. The calcium content in liposomes was maintained at not less than 31.2% w/w of elemental calcium, ensuring consistent dosing and formulation performance. Maintaining precise calcium concentration is essential for achieving the desired therapeutic benefits. Nutraceutical loading capacity was calculated to evaluate the proportion of calcium in the total formulation weight. Higher nutraceutical loading enhances the amount of calcium delivered per dose, reduces dosing requirements, and improves stability by preventing premature leakage or degradation. These studies are vital for developing an effective liposomal calcium formulation that ensures sustained release, improved absorption, and enhanced therapeutic benefits, positioning it as a viable alternative to conventional supplements.

3.3 Particle Size Distribution

The particle size distribution of liposomal formulations was measured according to ICH guidelines to ensure uniformity, as particle size significantly affects stability, bioavailability, and therapeutic efficacy. Uniform size distribution optimizes nutraceutical delivery, pharmacokinetics and minimizes side effects. Dynamic Light Scattering (DLS) was used to measure the hydrodynamic diameter, providing a size distribution profile. Encapsulation efficiency exceeded 85% for sieved samples within the 595–45 microns and above 80% for retention samples within the 250–89 microns. By following ICH guidelines and using these methods, we ensure the liposomal formulations maintain optimal particle size distribution, enhancing their performance and reliability in therapeutic use.

3.4 Stability and Leakage Rate

The stability and leakage rate of calcium from liposomes were assessed under accelerated stability conditions (40˚C ± 2˚C, 75% ± 5% relative humidity) to simulate long-term storage and predict shelf life, per ICH Q1A(R2) guidelines [17]. The study focused on two key parameters: elemental calcium content and encapsulation efficiency. These studies are crucial to ensure that calcium remains encapsulated without significant leakage, as leakage can reduce bioavailability and therapeutic efficacy. These accelerated stability studies provide valuable insights into the product's long-term performance under harsh conditions, helping manufacturers optimize formulations, establish proper storage conditions, and meet regulatory standards for safety and effectiveness [19]. Elemental calcium content, targeted at no less than 30% (w/w), ensures the product retains its active ingredient throughout its shelf life, guaranteeing efficacy for the end user [17]. Encapsulation efficiency, with a target of no less than 85%, is crucial for assessing the release profile and bioavailability of calcium, ensuring the product delivers the desired therapeutic effect [18].

3.5 FTIR analysis

Fourier Transform Infrared Spectroscopy (FTIR) analysis is crucial for ensuring API encapsulation, detecting chemical interactions, and confirming the chemical stability of the formulation. It also helps assess encapsulation efficiency, which influences nutraceutical release, stability, and bioavailability. The data support the suitability of the formulation for pharmaceutical applications, ensuring structural consistency and chemical compatibility between the lipid layer and the API, while enhancing controlled release and stability.[13, 14]The FTIR spectra were recorded using an FTIR spectrometer (Agilent, USA)within the range of 4000–400 cm^{-1} using Attenuated Total Reflectance (ATR) mode. ATR mode is a sampling technique in FTIR spectroscopy that enables direct analysis of solid, liquid, or gel samples without extensive preparation. In ATR mode, an infrared beam is directed onto a high-refractive-index crystal, where it undergoes internal reflection, creating an evanescent wave that penetrates a few micro-meters into the sample, allowing for efficient spectral acquisition with minimal interference from sample thickness or surface irregularities [21].

3.6 Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry (DSC) was used to assess the thermal stability of liposomal calcium, an important parameter for ensuring formulation integrity under temperature stress. DSC analysis helps evaluate phase transitions, crystallinity, and thermal degradation, confirming that encapsulated calcium remains stable without decomposition or undesirable phase changes at elevated temperatures [15]. Thermal stability is crucial for pharmaceutical and nutraceutical applications as it protects calcium from degradation, helps determine optimal storage conditions, and ensures a consistent nutraceutical release profile. It also aligns with ICH guidelines, ensuring quality and safety standards [16]. The present sample was sent to Sapalaorganics (Secunderabad, India) for DSC.

3.7. Nutraceutical Loading

The total amount of nutraceutical loaded in the liposomes was determined by digesting the liposomal formulation with a solvent (e.g., ethanol or acetic acid), followed by UV-Vis spectrophotometry at a predetermined wavelength.

The percentage of nutraceutical loading was calculated using the formula:

$$Drug\ loading\ (\%) = \frac{Mass\ of\ Drug\ in\ liposomal\ formulation}{Mass\ of\ liposomal\ complex\ recovered} \times 100$$

Results and Discussion

4.1. Encapsulation Efficiency and Calcium Content

The encapsulation efficiency was calculated to be 88%, exceeding the minimum acceptable threshold of 70%. The calcium content of liposomes was measured at 31.2% w/w, which met the criteria of being NLT 30.0% w/w. These results demonstrate successful encapsulation and nutraceutical loading [17].

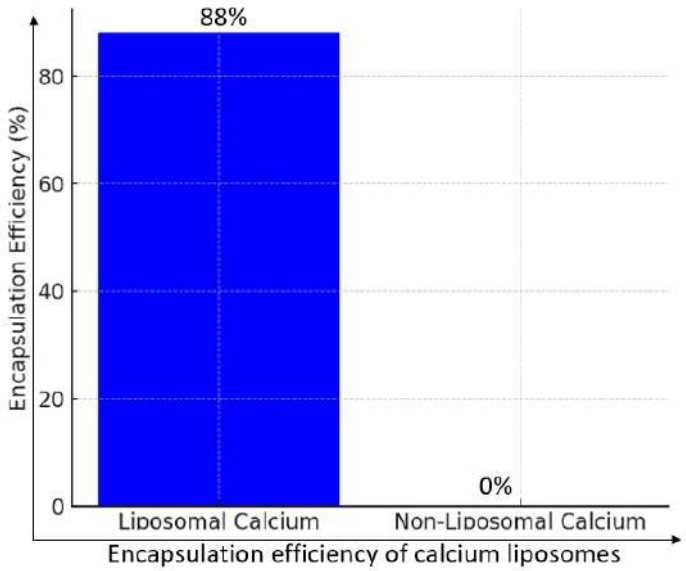

Figure 1: Encapsulation Efficiency of Liposomal versus Non-Liposomal Calcium

4.2. Liposome Morphology and Surface Properties

- **Scanning Electron Microscopy (SEM)** imaging revealed uniform liposome structures with consistent surface texture and encapsulation. This uniformity confirmed the successful formulation of liposomes as per the quality evaluation guidelines [16].

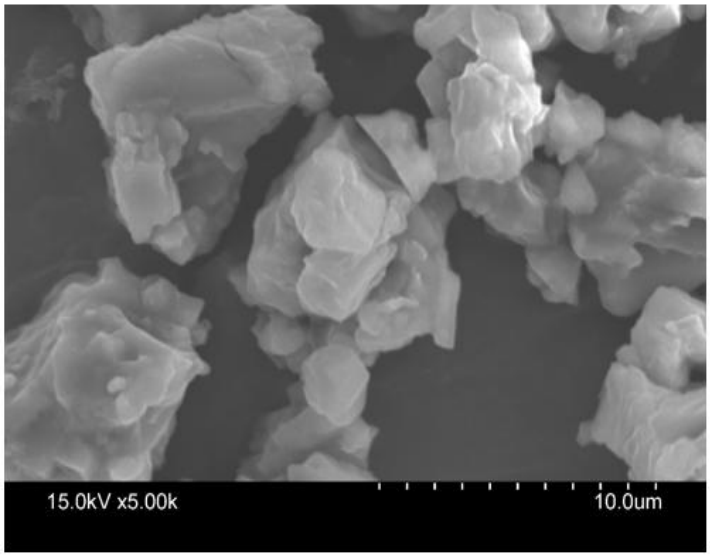

Figure 2: SEM Study of Liposomal Calcium

4.3. Zeta Potential and Stability

Zeta potential measurements confirmed the colloidal stability of the liposomes, remaining within the acceptable range defined by ICH guidelines.

These findings indicate that the liposomes are well-suited for dispersion and have a low risk of aggregation.[16]

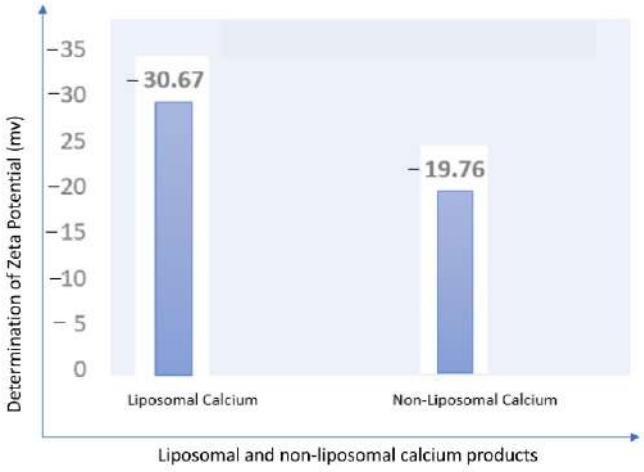

Figure 3: Zeta Potential measurements

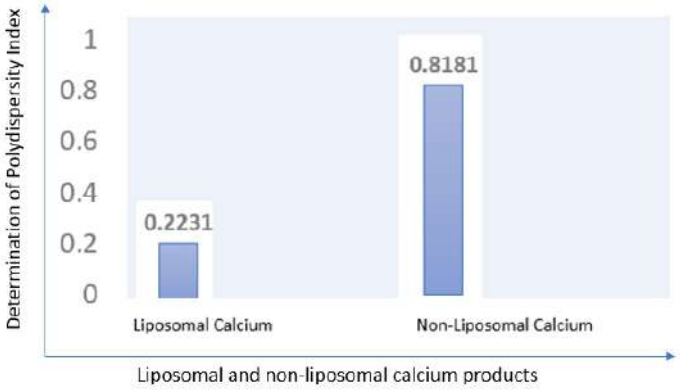

Figure 4: Polydispersity measurements

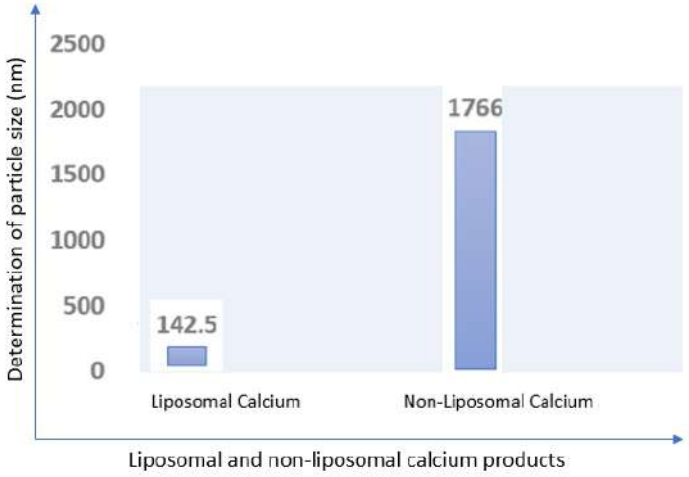

Figure 5: Particle Size

4.4 Particle Size Distribution

The particle size distribution analysis confirmed uniformity, with the average size of liposomes falling within the acceptable range for effective nutraceutical delivery. This consistency is crucial, as it ensures reliable bioavailability, meaning that the body can absorb and utilize the nutraceutical efficiently. The encapsulation efficiency and calcium content analysis further support this, showing that within a certain range of particle sizes, the encapsulation efficiency remained stable. This is particularly important in pharmaceutical formulations, where maintaining a high encapsulation efficiency directly impacts the nutraceutical's therapeutic efficacy.

The study also demonstrated that reducing particle size had little influence on encapsulation efficiency when microspheres were in the 595-500 microns range, calcium content was consistently observed to be more than 30% w/w across all mesh sizes, indicating uniform label claims throughout the batch.

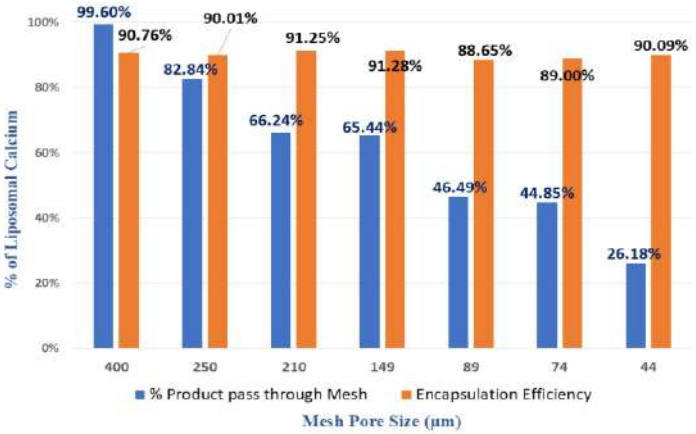

Figure 6: Chart comparing the % of liposomal calcium products that can pass through the mesh of varied porosity with their respective encapsulation efficiency percentages.

From the retention sample analysis, encapsulation efficiency was highest within the 250-105 microns pore size range, ranging from 84.11% to 82.70%, indicating that microspheres of these sizes were well-suited for encapsulating the calcium-based compound. A slight reduction in encapsulation efficiency was observed as the particle size decreased beyond 74 microns (78.94%) and 44 microns (77.62%), but it remained above 77%, which is still within an acceptable range for controlled nutraceutical release formulations. The calcium content was consistently above 30%, irrespective of mesh size, suggesting that the formulation ensured a uniform distribution of the active ingredient throughout the different particle sizes.

The past sample analysis showed similar trends, with encapsulation efficiencies remaining above 88% for all particle sizes. Interestingly, encapsulation efficiency peaked at around 177-149 microns, with values of 91.25% and 91.28%, respectively, indicating that these particle sizes might offer optimal nutraceutical retention and controlled release properties. The slight variations in calcium content between the retention and passed samples suggest minor losses during sieving, but overall, uniformity was maintained.

These findings have significant implications for nutraceutical formulation and controlled-release technologies. First, they confirm that encapsulation efficiency remains consistently high across a broad range of particle sizes, allowing for flexibility in formulation design. The high encapsulation efficiency (>80%) within the 250- 105 microns range suggests that these particle sizes are optimal for ensuring sustained nutraceutical release and effective absorption, particularly for microsphere-based nutraceutical delivery systems.

Additionally, the particle size range of 595- 45 microns demonstrated minimal impact on encapsulation efficiency, reinforcing the robustness of the formulation process. This suggests that the formulation can withstand slight variations in particle size without compromising its effectiveness. Moreover, the uniform calcium content (>30%) across all mesh sizes indicates that nutraceutical distribution remains stable throughout the batch, ensuring dose consistency and regulatory compliance.

In pharmaceutical applications, consistent particle size and high encapsulation efficiency are crucial for ensuring predictable nutraceutical release, minimizing side effects, and improving

patient adherence to medication regimens. These results also suggest that formulations using similar encapsulation methods could be effectively scaled up for industrial production without significant loss of efficiency, making them highly suitable for commercial nutraceutical development [18], the particle size study highlights the effectiveness of this encapsulation method in ensuring high encapsulation efficiency, uniform nutraceutical distribution, and reliable bioavailability. This strengthens the potential of such formulations for controlled-release nutraceutical delivery, offering improved therapeutic outcomes for patients while maintaining manufacturing efficiency and regulatory compliance.

4.5 Stability and Leakage

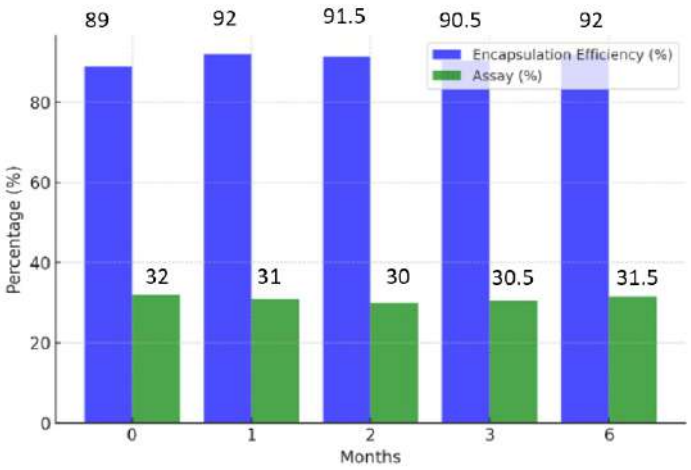

Figure 7: Stability Study of Liposomal Formulation

Stability studies conducted under accelerated conditions confirmed the robustness of the liposomal formulation, with encapsulation efficiency maintained at 86% and calcium content retained at 30.5% w/w over six months. These results indicate that the formulation is chemically and physically stable, ensuring that the active ingredient remains effectively encapsulated without significant degradation or loss. In pharmaceutical applications, such stability is crucial for maintaining nutraceutical potency and therapeutic efficacy over extended storage periods, particularly under stress conditions that mimic real-world handling and distribution environments.

The minimal leakage rates of calcium further demonstrate the structural integrity of the liposomal formulation. Leakage of encapsulated compounds is a common challenge in liposomal nutraceutical delivery systems, as degradation of the lipid bilayer can lead to premature nutraceutical release, reducing efficacy and increasing potential side effects. However, in this study, it can be inferred that the liposomal structure effectively prevents the premature escape of calcium, ensuring that the formulation remains intact until it reaches its intended biological target. This controlled retention is particularly valuable for sustained and targeted nutraceutical delivery, as it allows for a gradual and predictable release of the active ingredient, improving bioavailability and therapeutic outcomes [16].

The findings from this stability and leakage study have important implications for pharmaceutical formulation development, long-term storage, and regulatory approval. The ability to retain 86% encapsulation efficiency and 30.5% calcium content over six months suggests that this formulation has excellent long-term stability. This is essential for pharmaceutical manufacturing and distribution, ensuring that the product remains effective throughout its shelf life. Stability under accelerated conditions further indicates that even under temperature fluctuations and storage stresses, the formulation can maintain its potency, making it suitable for large-scale production and commercial applications. The low leakage rates of calcium confirm that the liposomal structure remains intact, preventing premature release of the active ingredient. This is particularly critical for controlled-release nutraceutical formulations, where maintaining a consistent nutraceutical release profile ensures prolonged therapeutic action, reducing the need for frequent dosing and improving patient compliance. Pharmaceutical formulations must meet stringent regulatory standards for stability and efficacy. The demonstrated retention of encapsulation efficiency and calcium content ensures that this liposomal formulation aligns with ICH (International Council for Harmonisation) guidelines for nutraceutical stability, increasing its potential for regulatory approval. Stability testing data like this provides evidence of formulation reliability, which is crucial for gaining approval from regulatory agencies such as the FDA (Food and Nutraceutical Administration) and EMA (European Medicines Agency). Since liposomal formulations are widely used in targeted nutraceutical delivery, nutraceutical, and vaccine delivery, the robust stability and minimal leakage observed in this study suggest that similar encapsulation techniques could be adapted for other bioactive compounds. This could enhance nutraceutical formulations for calcium-based therapeutics, as well as other minerals, vitamins, or pharmaceuticals requiring controlled release.

4.6 Spectroscopic Analysis Findings

The FTIR spectroscopic characterization provides crucial insights into the structural integrity and successful encapsulation of calcium carbonate within the liposomal formulation. The spectrum of non-liposomal calcium showed characteristic peaks at 1795 cm^{-1}, corresponding to the C=O bonds from carbonates, while a peak at 872 cm^{-1} was attributed to Ca-O bonds. Additionally, the peaks at 713 cm^{-1} and 874 cm^{-1} confirmed the presence of calcite, which is the crystalline form of calcium carbonate. The spectrum of liposomes displayed prominent O-H stretching vibrations between 3400 cm^{-1} and 2600 cm^{-1}, signifying the presence of hydroxyl groups. The band at 2300 cm^{-1} indicated carbon dioxide stretching, while peaks at 1900 cm^{-1} (aromatic C-H bending) and 1750 cm^{-1} (C=O stretching) were characteristic of a liposome. Additionally, a peak at 1460 cm^{-1} represented C-H bending from alkane groups. These spectral features confirmed the identity of liposomes as a lipid component of the liposomal system. The FTIR spectrum of the liposomal sample demonstrated the presence of a peak at 2900 cm^{-1}, attributed to O-H stretching from the liposome, suggesting successful encapsulation of calcium carbonate within the liposomal formulation. Furthermore, the characteristic peaks of calcite (714 cm^{-1} and 874 cm^{-1}) were observed but with reduced intensity, indicating that the liposome encapsulation effectively masked the calcium carbonate core. This reduction in peak intensity suggests the formation of a stable liposomal structure surrounding the active ingredient, reducing direct exposure to the external environment.

Table 1: FTIR Spectral Peaks

Sample	Wavenumber (cm\u207B\u00B9)	Functional Group/Observation
Non-Liposomal Calcium	1795	C=O bonds from carbonates [21]
	872	Ca-O bonds [20]
	713 & 874	Characteristic peaks for calcite
Liposome	3400 - 2600	O-H stretching [21]
	2300	O=C=O (carbon dioxide) stretching
	1900	C-H (aromatic) bending
	1750	C=O stretching
	1460	C-H (alkane) bending [22]
Liposomal calcium	2900	O-H stretching of the liposome in liposomal calcium
	714 & 874	Characteristic peaks of calcite (less intense)

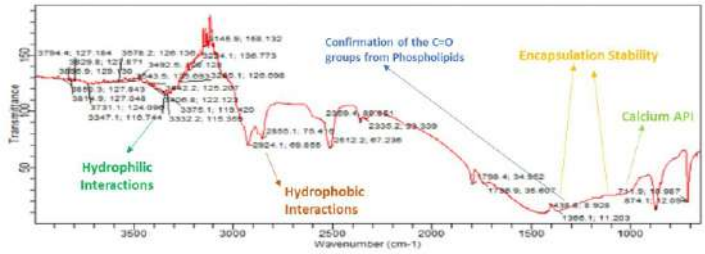

Figure 8: FTIR Spectrum of Liposomal Calcium

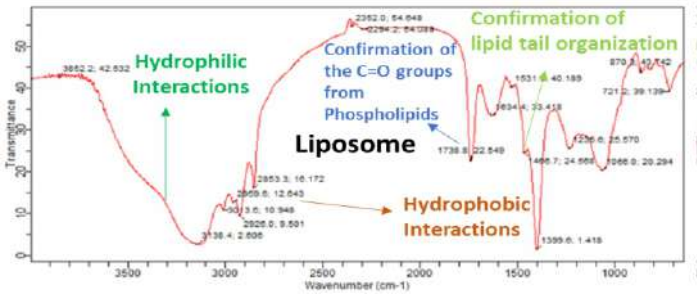

Figure 9: FTIR Spectrum of Liposome

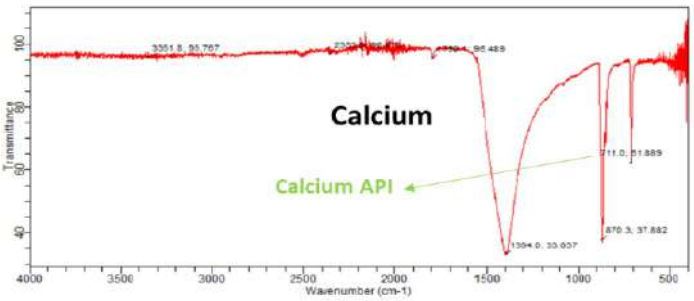

Figure 10: FTIR Study of Non-liposomal Calcium

Overall, the study demonstrates the potential of liposomal encapsulation in improving the physicochemical properties of calcium carbonate, making it more suitable for various biomedical and commercial applications.

4.7 Thermal Stability

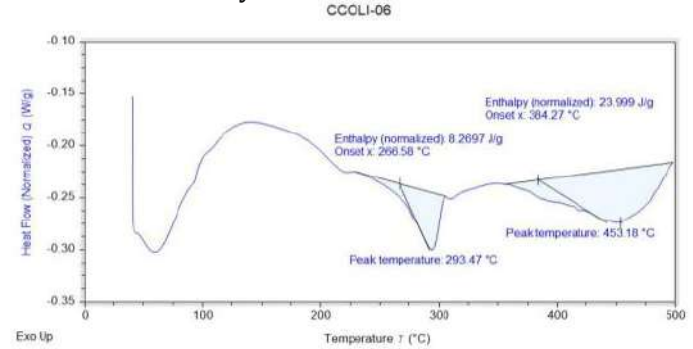

Figure 11: DSC Analysis of Liposomal Calcium

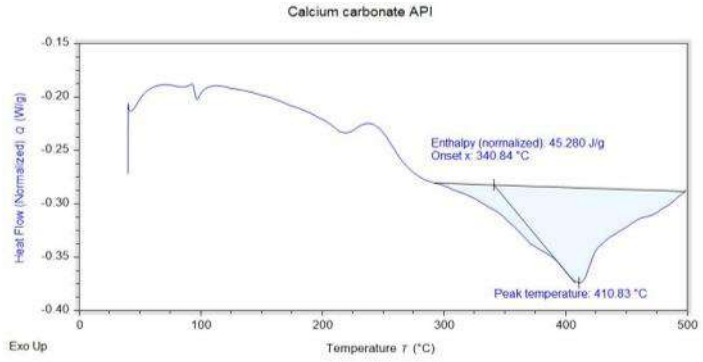

Figure 12: DSC Analysis of Non-liposomal Calcium

The Differential Scanning Calorimetry (DSC) analysis confirmed the thermal stability of the liposomal formulation containing calcium carbonate. The DSC analysis demonstrated that the liposomal calcium formulation exhibits superior thermal stability compared to non-liposomal calcium carbonate. The liposomal formulation showed a higher onset temperature (384.27°C) and peak temperature (453.18°C), indicating greater resistance to heat-induced degradation. In contrast, non-liposomal calcium began decomposing at a lower onset temperature of 340.84°C, with a peak at 410.83°C. Additionally, the enthalpy change (ΔH) was lower in the liposomal sample, suggesting improved thermal protection. Encapsulation within the liposomal matrix effectively preserved the calcium content, with only a minimal variation from 32.25% w/w to 33.61% w/w after exposure to 150°C for 4 hours. This demonstrates the robustness of liposomal coating in preventing leakage or degradation under high temperatures. The enhanced stability makes liposomal calcium suitable for industries requiring heat-resistant formulations, such as pharmaceuticals, nutraceuticals, and functional foods, ensuring extended shelf life and improved bioavailability. At room temperature, the calcium content was measured at 32.25% w/w, indicating a well-maintained encapsulation within the liposomal matrix. Following exposure to an elevated temperature of 150˚C for 4 hours, the calcium content exhibited minimal variation, remaining stable at 33.61% w/w. This negligible change in calcium content suggests that the formulation effectively resists thermal degradation and does not undergo significant compositional breakdown under high-temperature conditions.

The stability of the calcium content even after thermal treatment highlights the robustness of the liposomal encapsulation system, ensuring that calcium carbonate remains protected from thermal-induced alterations. This finding suggests that the structural integrity of the liposomal encapsulation is maintained, preventing the leakage or decomposition of the encapsulated material.

The demonstrated thermal stability of the liposomal formulation has significant implications for its practical applications, particularly in industries requiring temperature-resistant formulations, such as pharmaceuticals, nutraceuticals, and functional foods. Many bioactive compounds degrade under high temperatures, leading to reduced efficacy. However, the ability of the liposomal system to retain its calcium content even at 150˚C suggests enhanced formulation durability, making it suitable for heat-intensive processing (e.g., sterilization, drying, or baking) [20]. Additionally, this stability is particularly valuable for oral supplement formulations, as it ensures that the encapsulated calcium remains intact during storage and transportation, even under fluctuating temperature conditions. In pharmaceutical applications, maintaining stability at high temperatures is essential for extended shelf life and improved bioavailability, as it prevents premature degradation of the active ingredient.[19, 20] Overall, these results reinforce the efficacy of liposomal encapsulation as a protective mechanism, enhancing both the stability and usability of calcium carbonate formulations across diverse industrial and biomedical applications. Further studies on long-term storage stability and *in vivo* bioavailability would be beneficial to establish its effectiveness in real-world conditions.

4.8. Nutraceutical loading

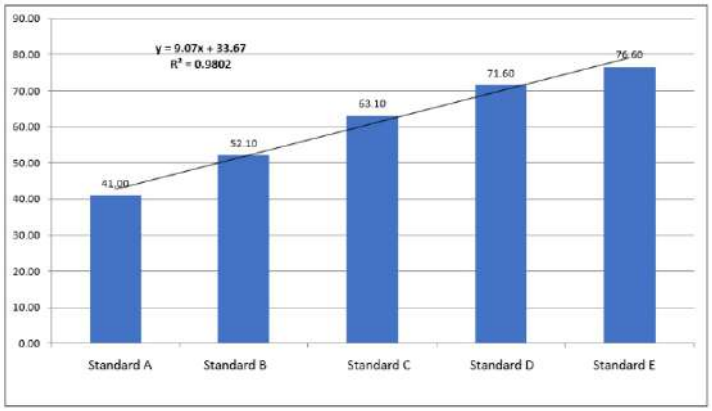

Figure 13: Nutraceutical Loading Capacity of Liposomal Formulation

The nutraceutical loading capacity of the liposomal formulation was determined to be 0.76 mg of nutraceutical per mg of liposomal product, indicating a high encapsulation efficiency. The nutraceutical-to-lipid ratio, which is a crucial parameter in liposomal formulations, was calculated to assess the loading efficiency of the nutraceutical within the liposomal matrix. The percent nutraceutical load was evaluated across different standards (A to E), showing a consistent increasing trend to determine the correlation coefficient. Standard A showed a nutraceutical load of 41.00%, Standard B was 52.10%, Standard C was 63.10%, Standard D was 71.60%, and Standard E reached 76.60%. The data follows a linear regression model with the equation $y = 9.07x + 33.67$ and a high correlation coefficient ($R^2 = 0.9802$), suggesting a strong linear relationship between nutraceutical concentration and encapsulation efficiency. This implies that increasing nutraceutical concentration leads to a proportionate increase in the nutraceutical load, highlighting the effectiveness of the liposomal system in accommodating higher nutraceutical doses without significant losses.

The high nutraceutical loading capacity observed in this study is a key indicator of the efficiency and stability of the liposomal formulation.

The ability to encapsulate 0.76 mg of nutraceutical per mg of liposome suggests that this system can effectively enhance nutraceutical bioavailability by ensuring a higher proportion of the active compound is delivered to the target site. The increasing trend in the percent nutraceutical load across standards signifies that the liposomal system exhibits a scalable nutraceutical-loading capability, making it suitable for applications requiring higher nutraceutical doses without compromising stability [21]. This is particularly beneficial in pharmaceutical and nutraceutical applications, where improved nutraceutical encapsulation efficiency enhances therapeutic efficacy and reduces toxicity by controlling the release of active ingredients, the linear correlation observed ($R^2 = 0.9802$) reinforces the predictability and consistency of the formulation process. This allows for precise optimization of liposomal nutraceutical delivery systems for clinical and commercial applications, ensuring dose accuracy and reproducibility [22]. These results demonstrate the potential of liposomal technology as a reliable and efficient nutraceutical delivery system. Further studies, including *in vitro* release kinetics and stability assessments, would be beneficial to validate its performance under real-world conditions [23].

5. Conclusion

This study highlights the innovative potential of calcium-responsive liposomes as advanced nutraceutical delivery systems. The formulations developed by West Bengal Chemical Industries Ltd. demonstrated excellent structural integrity, high encapsulation efficiency, and remarkable stability across varying conditions, confirming their suitability for clinical applications. The integration of calcium's biological role into the liposomal design allows for controlled, targeted release, minimizing systemic toxicity and enhancing therapeutic efficacy. Analytical techniques such as SEM, FTIR, and DSC validated the physicochemical properties of the liposomes, while stability studies confirmed their robustness under accelerated conditions. These liposomal systems hold immense promise for treating calcium-related disorders, improving nutrient delivery, and even supporting detoxification therapies. Future research should focus on expanding their clinical applications, optimizing their formulations, and conducting *in vivo* studies to fully realize their potential in enhancing patient outcomes.

6. References

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