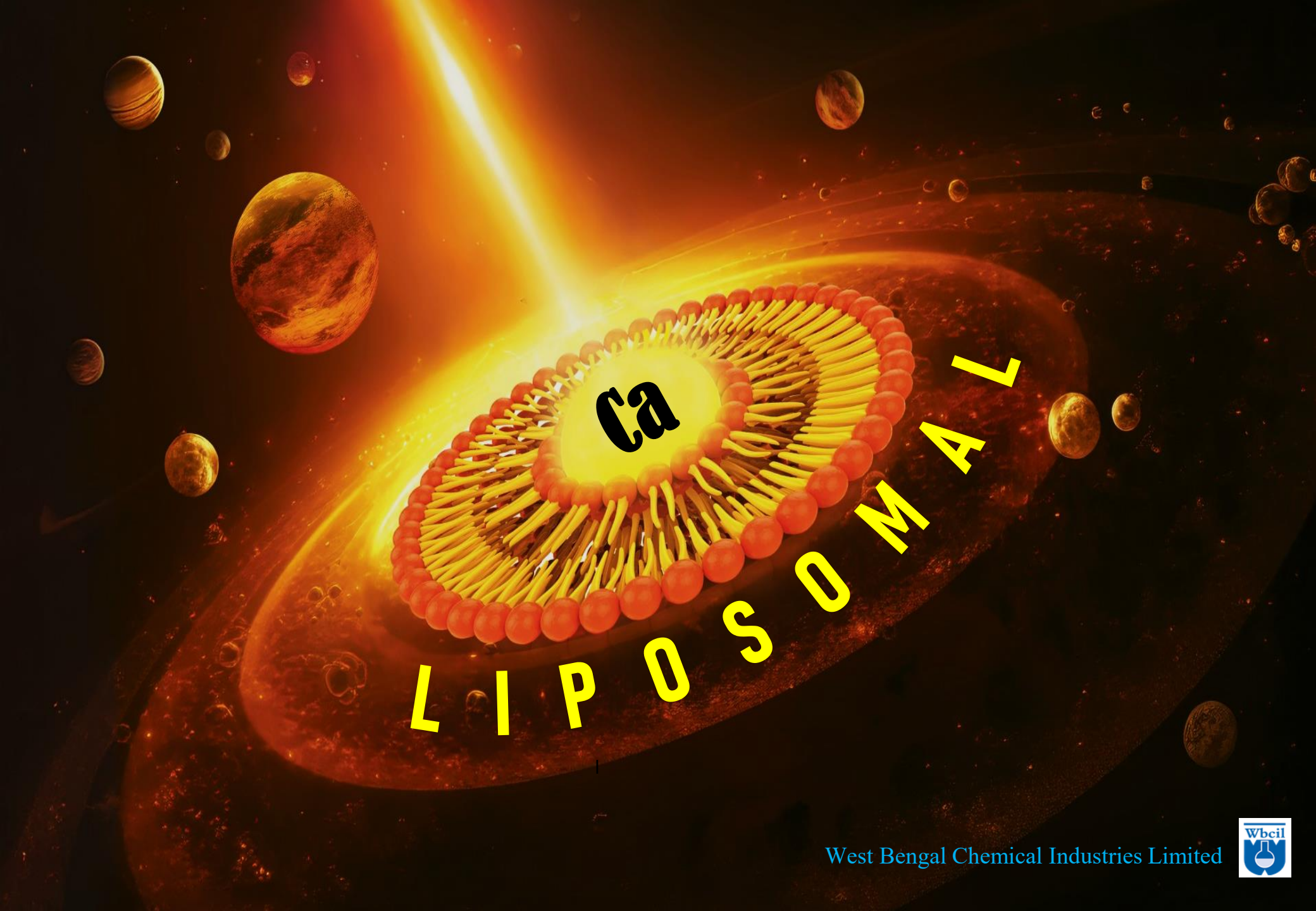
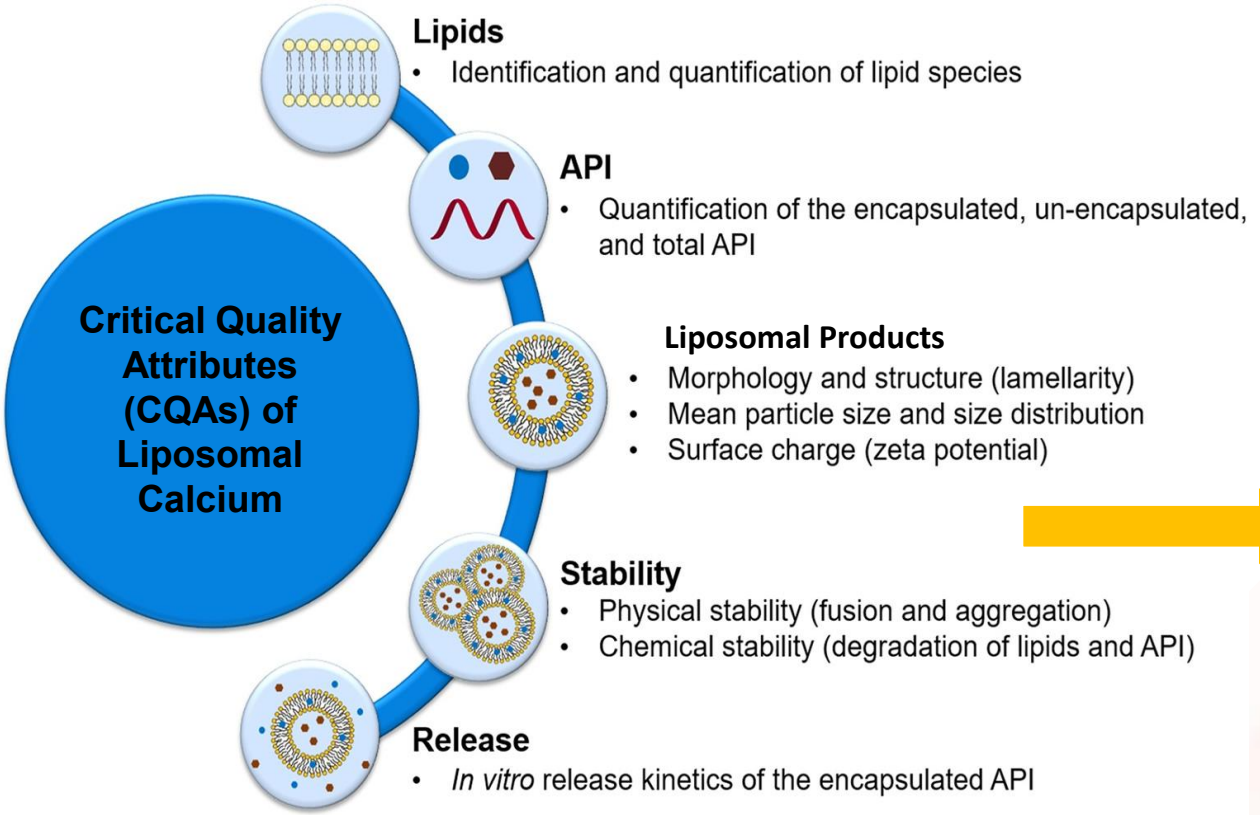


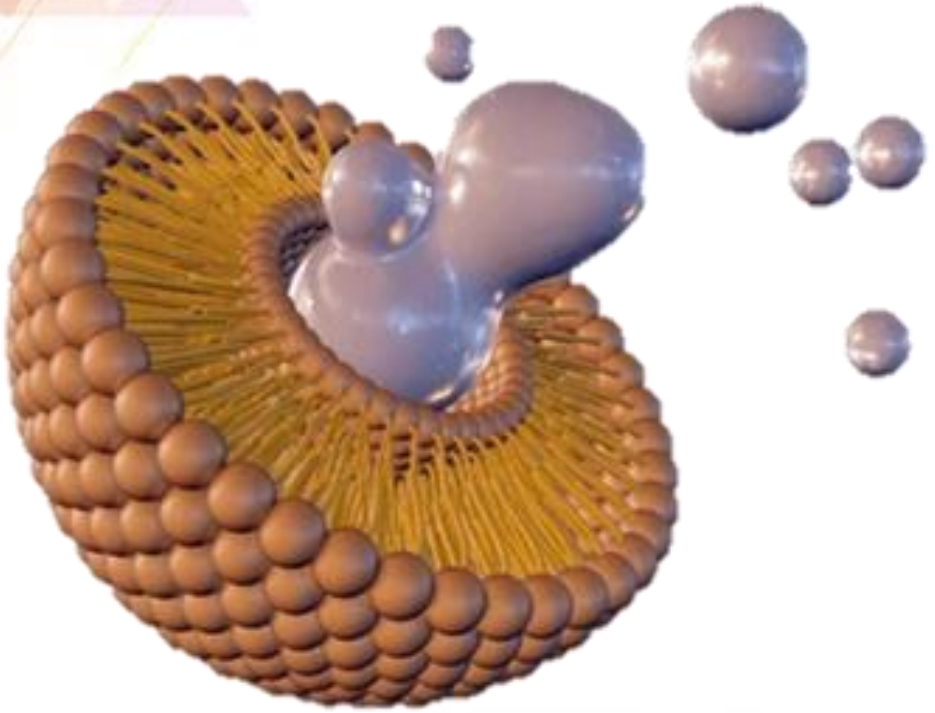
West Bengal Chemical Industries Limited

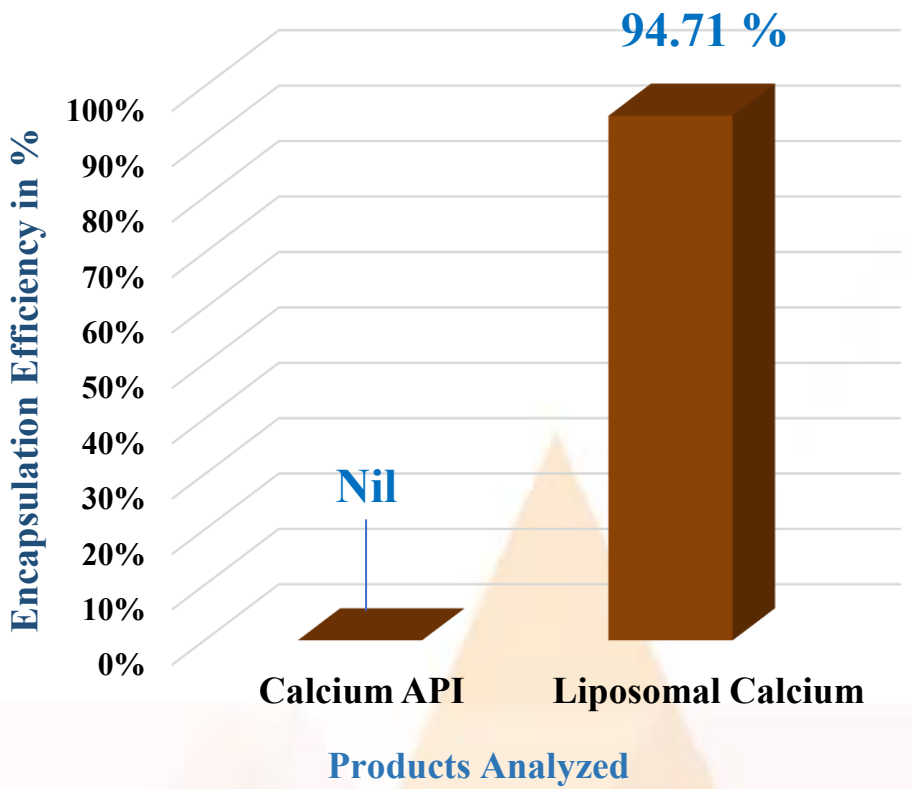
Summary of Characterizations Performed on Liposomal Calcium



1. ***Encapsulation efficiency of Liposomal Calcium***
2. ***Analysis of particle size and uniformity of Liposomal Calcium using DLS***
3. ***Behavior of Liposomal Calcium particles in liquid medium using DLS Zeta-sizer***
4. ***FTIR analysis of Liposomal Calcium***
5. ***Elemental Analysis of Liposomal Calcium***
6. ***Morphology analysis of Calcium Liposomes using SEM***
7. ***Analysis of Calcium leakage from Liposomes***
8. ***Stability analysis of Liposomes at 105^{o} C temperatures***
9. ***Endothermic study of Liposomal Calcium using DSC analysis***
10. ***Mineral Loading Capacity***
11. ***Product Uniformity – Mesh Size vs EE%***

1. Encapsulation Efficiency of 29.02% Liposomal Calcium





- **Acceptance criteria: 30% to 34% of Elemental Calcium**
- **Acceptance criteria: NLT 70% Encapsulation efficiency**

Encapsulation Efficiency measured by validated titrimetric analytical data

- Liposomal encapsulation ensures **94.71% efficiency**, significantly surpassing the **minimum requirement of 70%**.
- Efficient encapsulation minimizes **mineral loss**, improving **bioavailability** and **therapeutic efficacy**.
- Offers **protection against oxidation and gastrointestinal irritation**, common with conventional calcium forms.

2. Dynamic Light Scattering Analysis of Liposomal Calcium

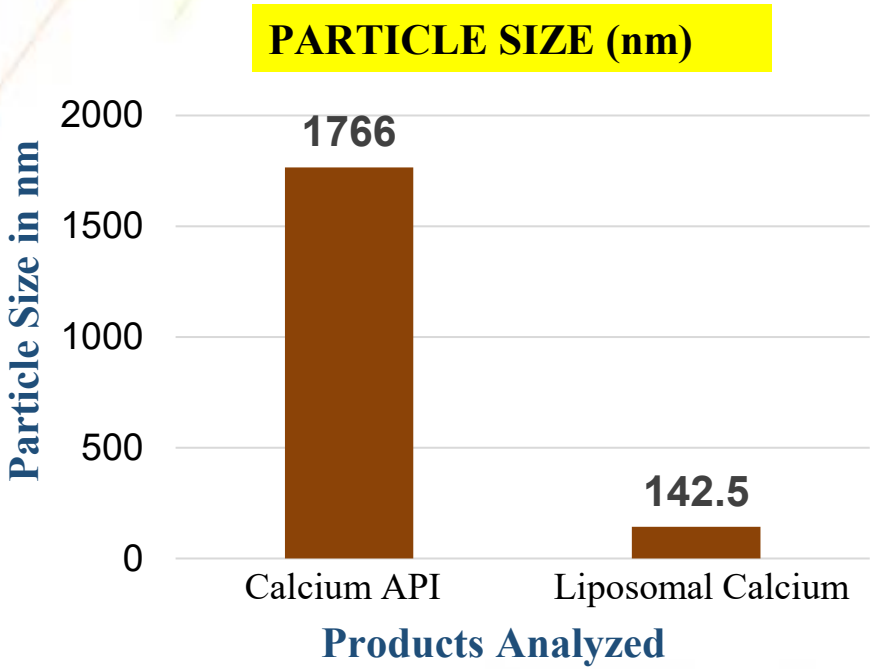


Figure 1 – Chart showing the particle size of Calcium API with Liposomal Calcium

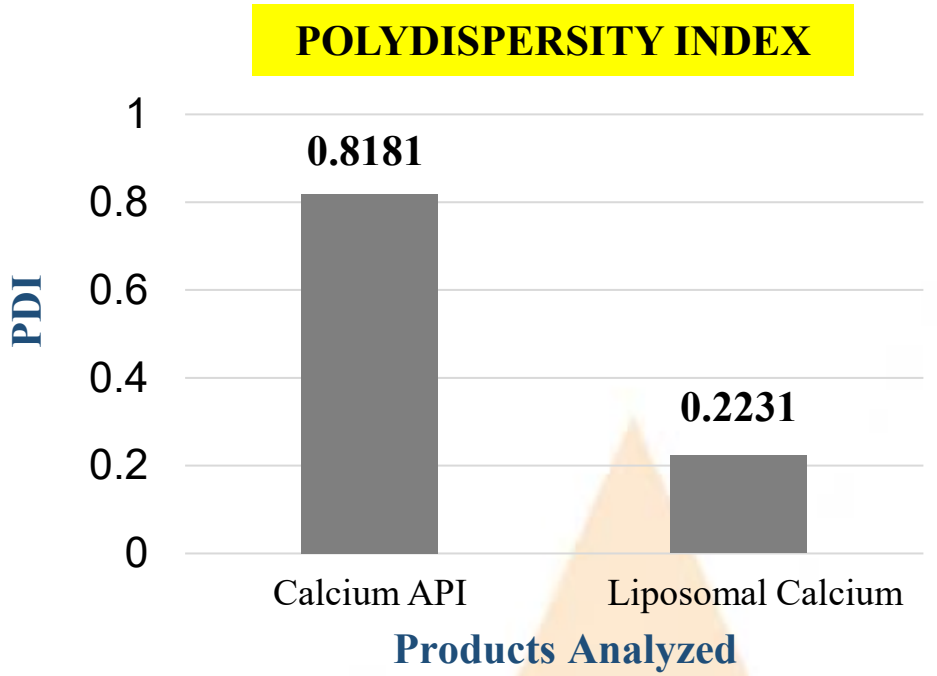


Figure 2 – Polydispersity Index (PDI) of Liposomal Calcium in solution

❖ **Acceptance criteria:**

- **Particle Size : < 220 nm**
- **Polydispersity Index : <1**

- Nanosized, uniform particles offer greater colloidal stability and improved shelf life.
- Smaller particles (particle size: 142.5 and PDI 0.2231) support **increased mucosal permeability** and cellular uptake.
- DLS characterization confirms high formulation control and **batch-to-batch reproducibility**.

3a. Behavior of Liposomal Calcium

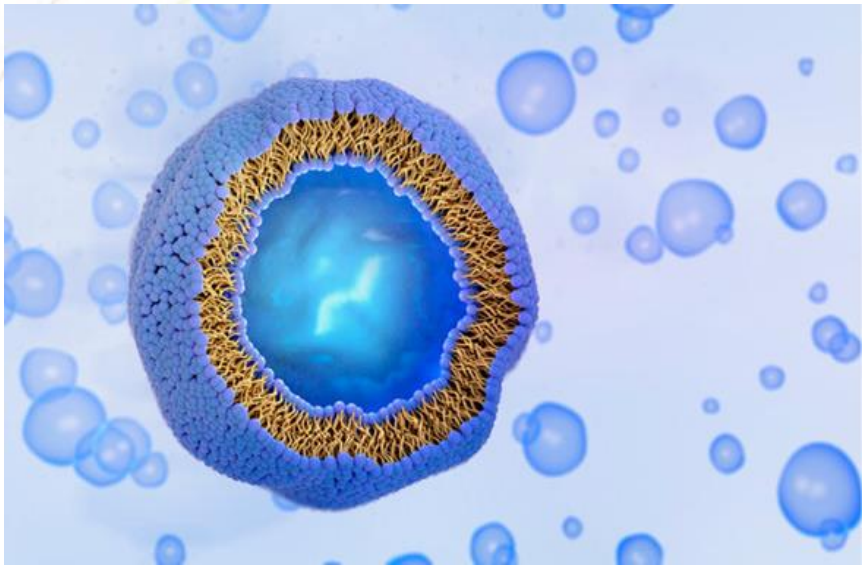


Figure 1 – A figure showing the balance of attractive and repelling forces which determine how particles behave in a medium.

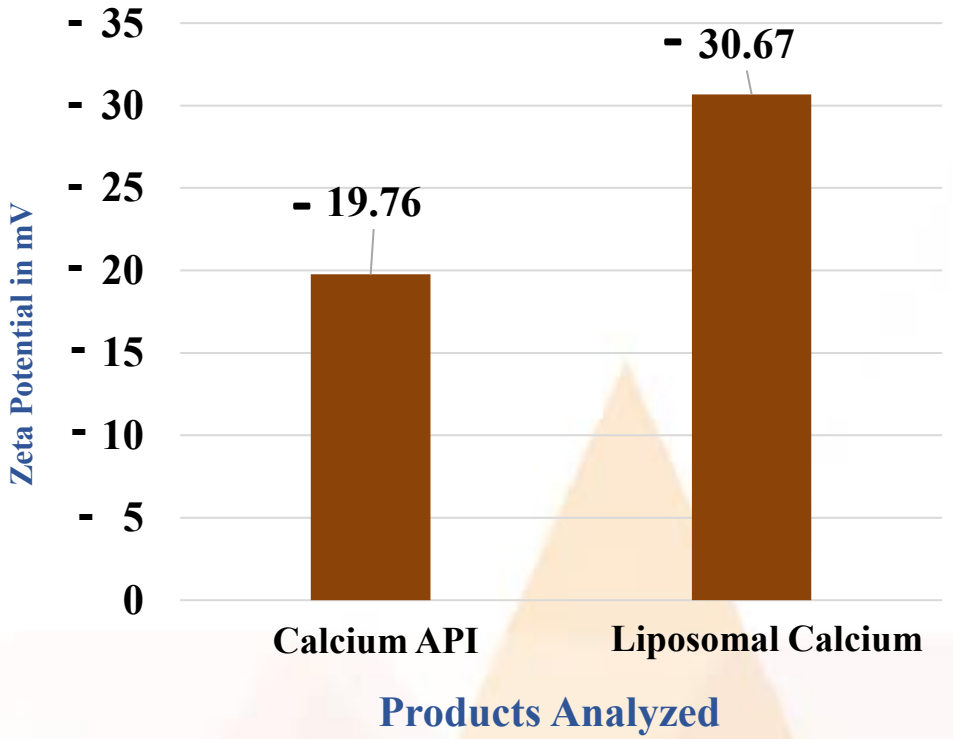


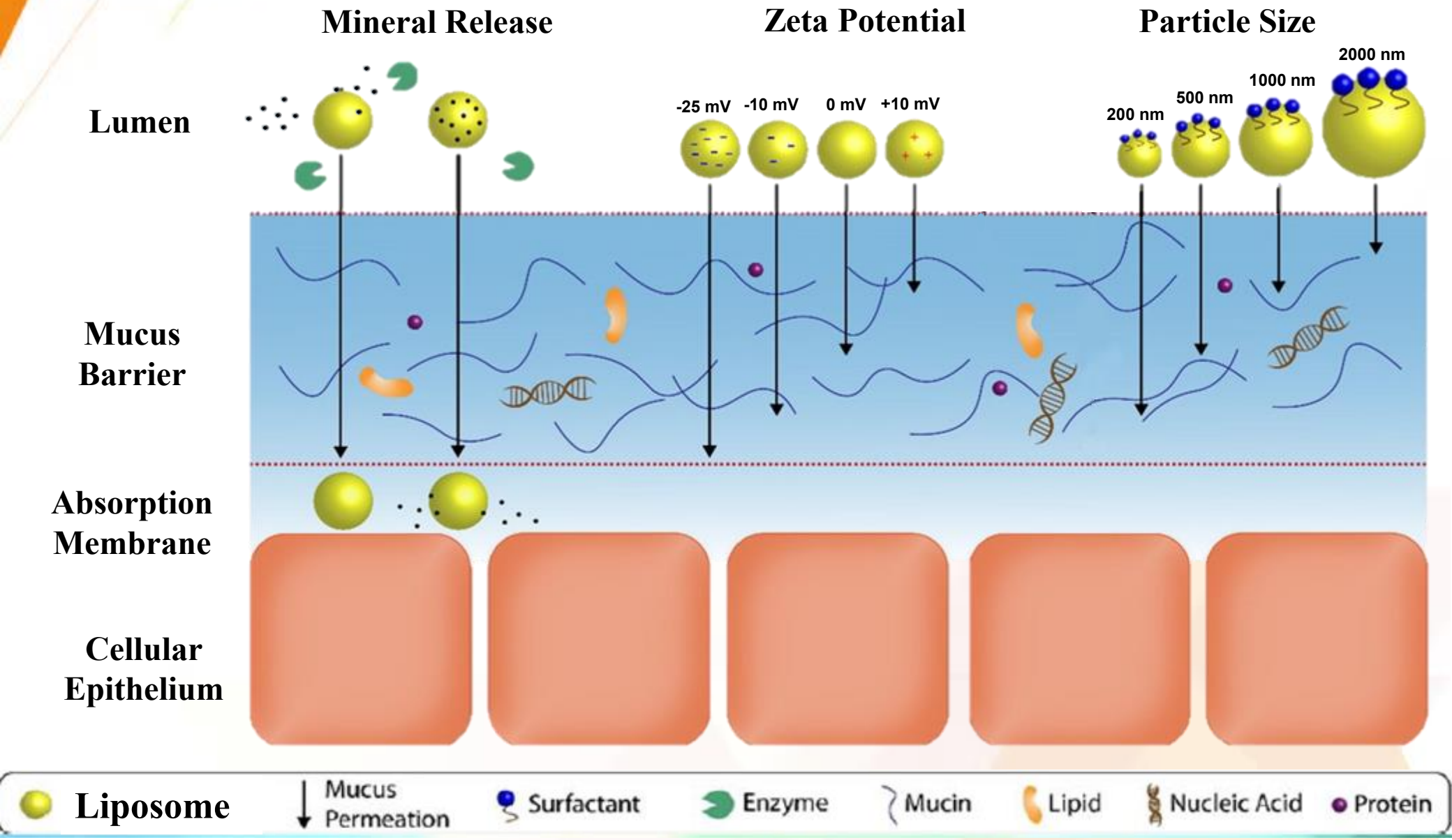
Figure 2 – Chart comparing the Zeta potential of Calcium API and Liposomal Calcium showing Calcium in Liposomal form is stable and unlikely to agglomerate in solution.

❖ **Acceptance criteria:**

➢ **Zeta Potential : < -30 mV**

- Liposomal Calcium shows **high zeta potential (-30.67 mV)** → excellent colloidal stability.
- Prevents particle aggregation → ensures **uniform suspension**.
- Enhances **product shelf life** and **bioavailability** in liquid form.

3b. Absorption of Liposomal Calcium Represented Schematically on a Cellular Cross-Section



4a. FTIR Spectra of Calcium, Liposome & Liposomal Calcium

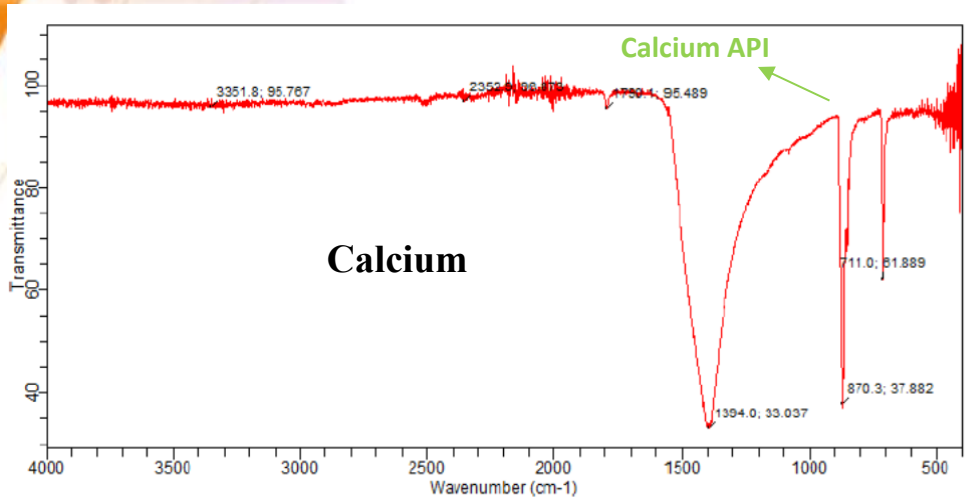


Figure 1: IR Transmission spectrum showing bands at different wavelengths of Calcium Carbonate API

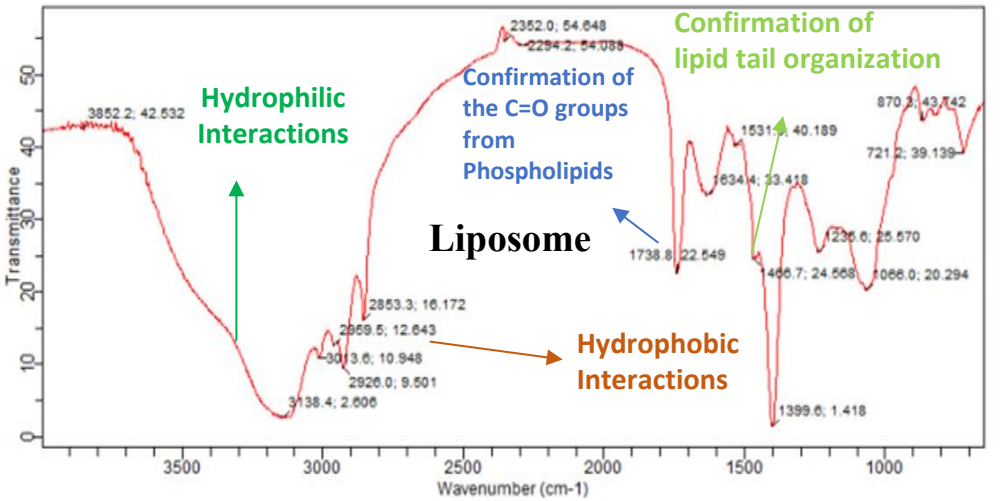


Figure 2: Hydrophobic and Hydrophilic interactions within Empty Liposome

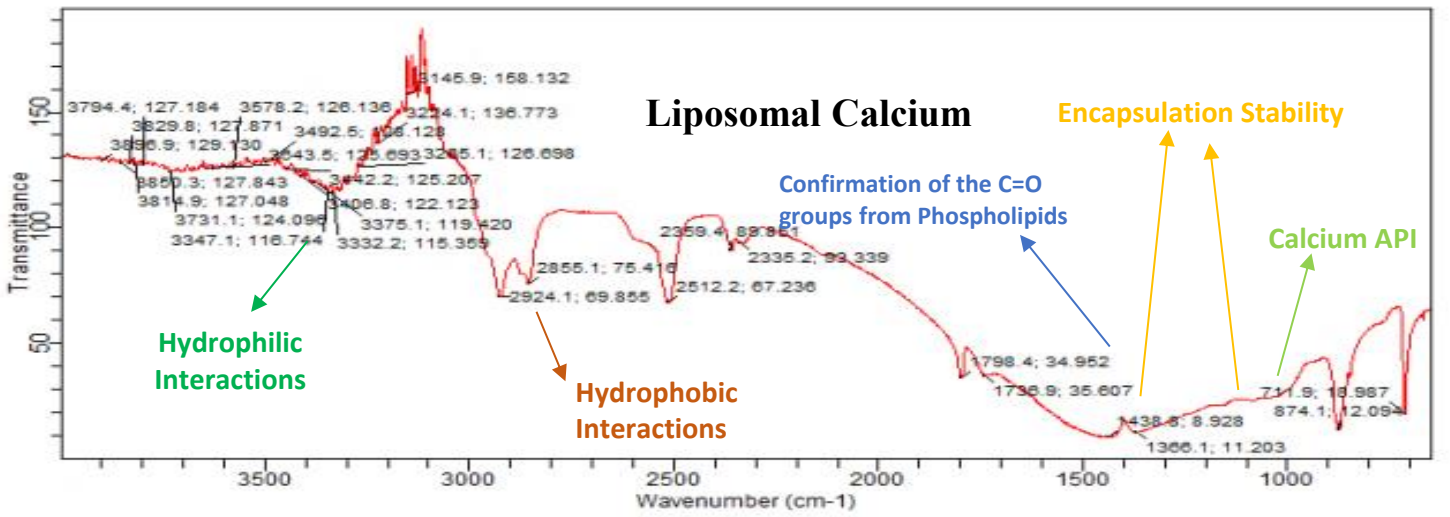


Figure 3: IR Transmission spectrum of Liposomal Calcium is shown

4b. Summary of FTIR Analysis of Liposomal Calcium

1. Confirmation of the C=O and OH groups - Broad **-OH peaks (~3400 cm^{-1})** and **C=O shifts (1399.6 cm^{-1})** indicate controlled release.

2. Hydrophobic Interactions - Distinct CH_2 peaks at **2924 cm^{-1} and 2855 cm^{-1}** confirm ordered lipid tail packing with Calcium carbonate.

3. Hydrophilic Interactions - Broad -OH peaks (~3400 cm^{-1}) and **CO_3^{2-} peaks (711.9 cm^{-1})** confirm strong hydrophilic interactions.

4. API - Shifts in **C=O stretching (~1366.1 cm^{-1})**, consistent **CH_2 bands (~2924, 2855 cm^{-1})** and **CO_3^{2-} peaks (~711.9 cm^{-1})** confirm the integration of Calcium carbonate within the phospholipid bilayer.

5. Encapsulation Stability - Peaks at **1366.1 cm^{-1} (C=O stretching)** and **711.9 cm^{-1} (CO_3^{2-} bending)** confirm successful interaction between API and lipid bilayer.

5. Elemental Analysis of Liposomal Calcium

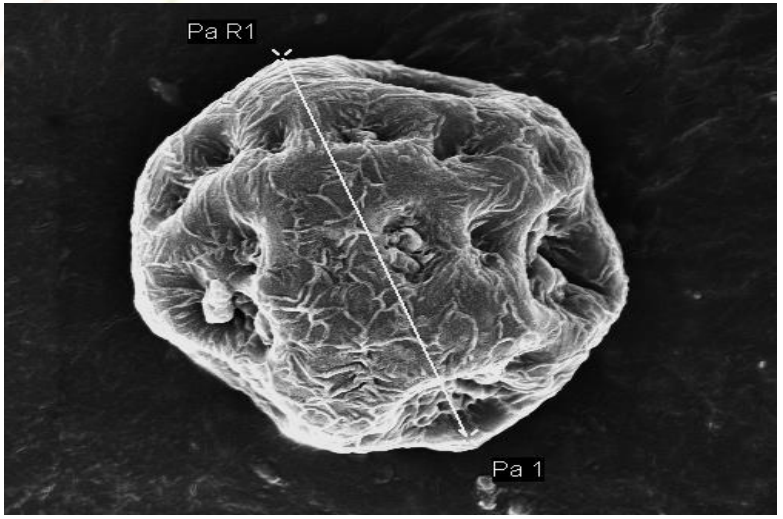
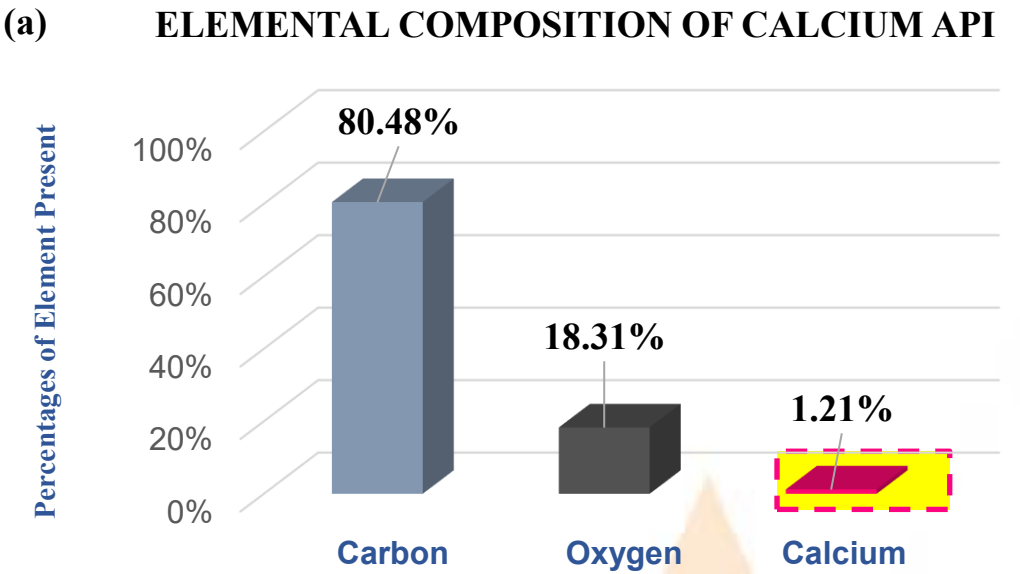


Figure 1 – SEM imaging showing the area scanned using Energy Dispersive X-Ray Spectroscopy (EDAX)

- **No surface calcium detected** in the liposomal calcium spectrum, confirming that calcium is fully enclosed and not exposed on the surface.
- **EDAX scan** shows that only the liposomal shell elements are detected, proving that the calcium core is completely encapsulated within the liposome.



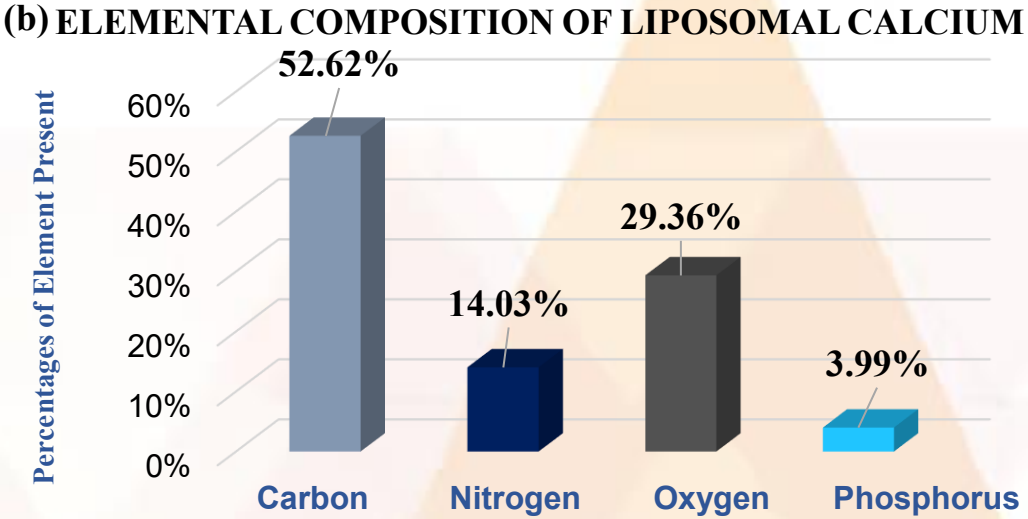


Figure 2 – A graphical representation of the percentages of elements composing (a) Calcium API and (b) Liposomal Calcium

6. Morphology of Liposomal Calcium as Viewed Under a Scanning Electron Microscope

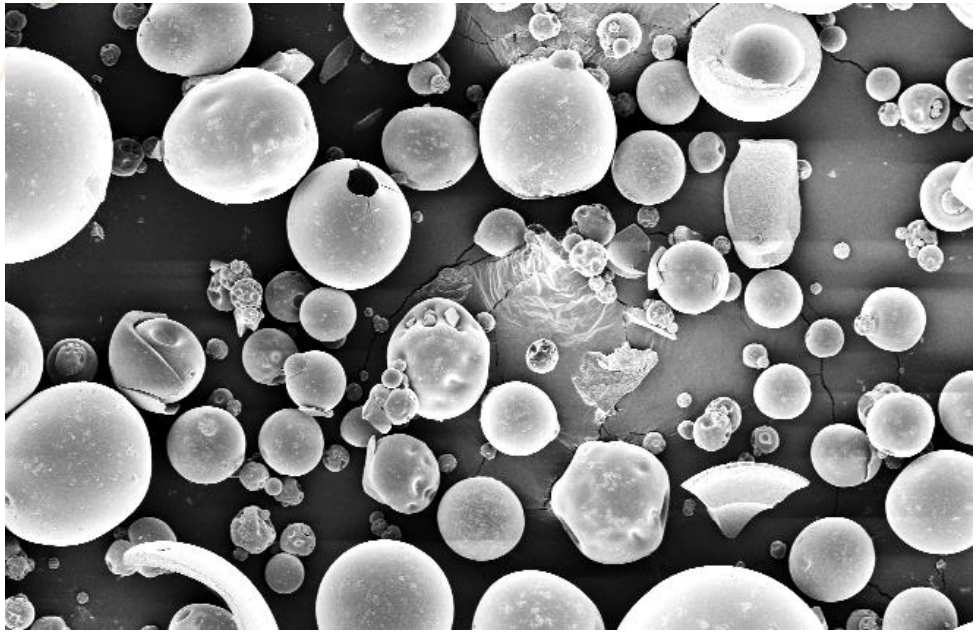


Figure 1 – SEM image of few Calcium Liposomes scattered within the field of view under observation

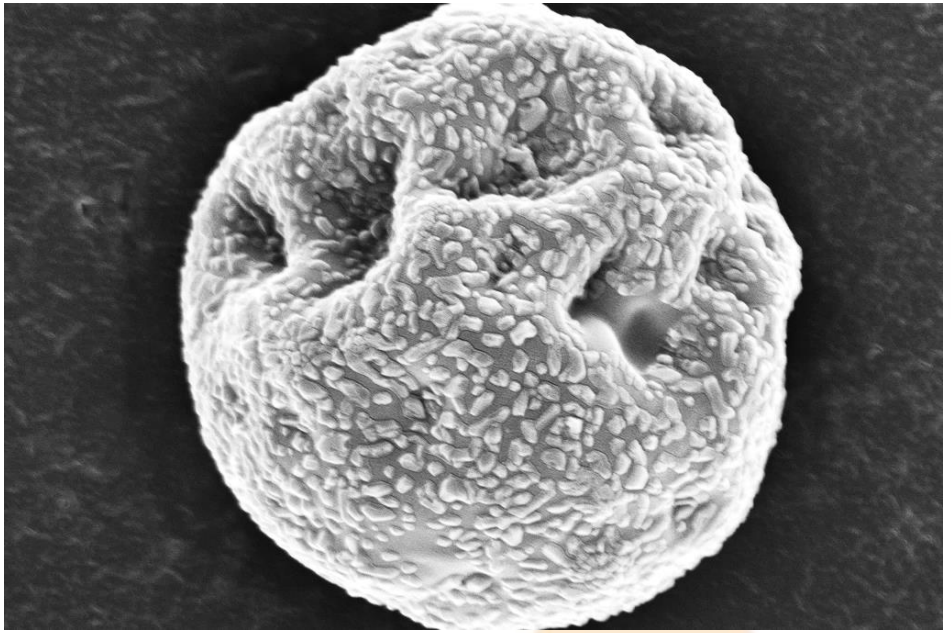


Figure 2 – Zoomed-in view of a Calcium Liposome

- Spherical morphology observed in liposomal calcium particles.
- Uniform size distribution seen across the field (Figure 1).
- Particles appear round surfaced.
- Spherical and uniform morphology enhances **stability, encapsulation efficiency, and cellular uptake**, making it ideal for liposomal drug delivery.

7. Leakage of Calcium from Liposomes

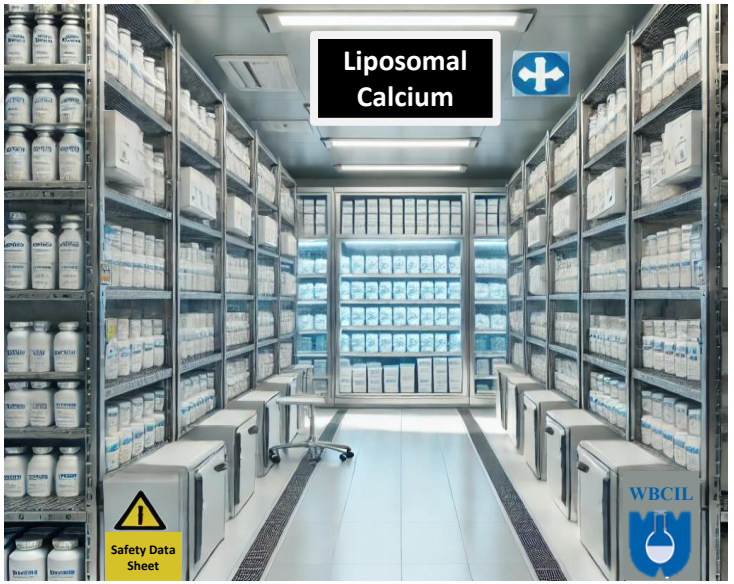


Figure 1 – An image representing the storage of formulations stored on shelves

- **Encapsulation efficiency remains high (~94)** throughout 3 years of storage, indicating stable liposome structure.
- **Assay values for free calcium remain low (~29%)**, showing minimal leakage over time.
- The formulation shows **excellent retention of calcium**, confirming its suitability for long-term shelf storage.

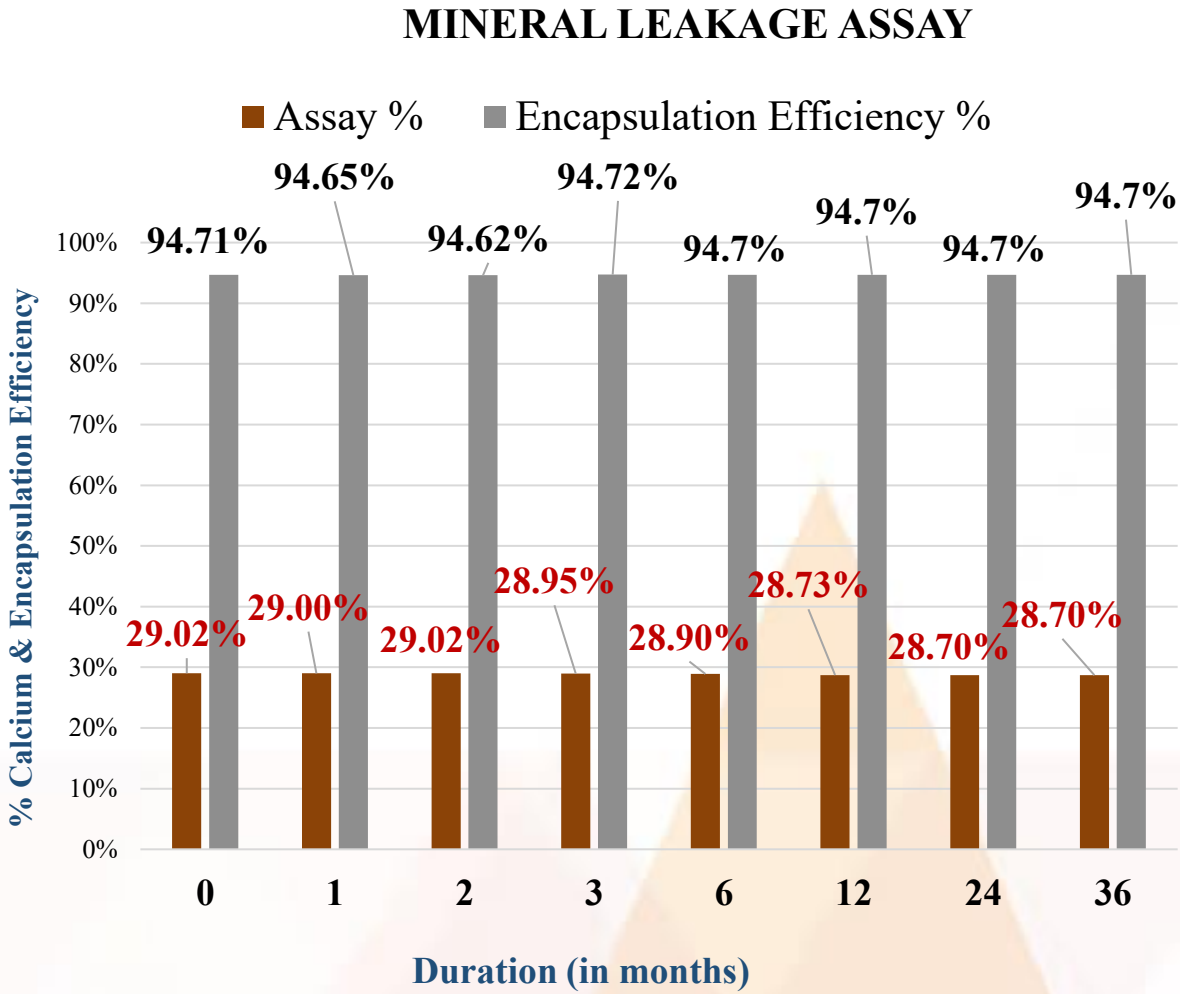


Figure 2 – Chart comparing the stability of Liposomal Calcium stored over a period of 3 years at 40°C ± 2 °C and a relative humidity of 75% ± 5%.

8. Stability of Liposomal Calcium at Elevated Temperatures

Figure 1 – An image representing the transport of formulations at elevated temperatures.

- **Encapsulation efficiency remains high (~94.7%)** even after exposure to 105°C for 4 hours.
- **Assay values (29.02% at RT vs. 28.74% at 105°C)** show minimal variation, indicating **negligible calcium leakage**.
- Demonstrates **thermal robustness**, making the formulation suitable for transport and storage in hot climates.

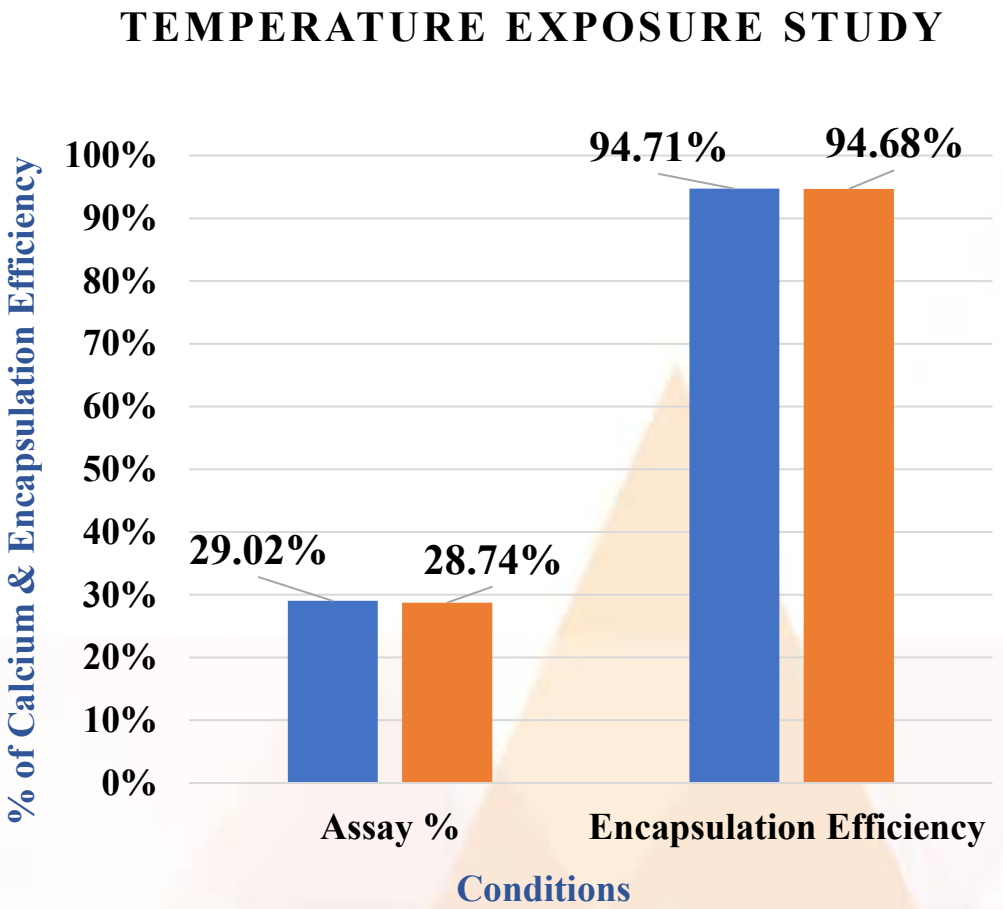


Figure 2 – Chart comparing the stability of Liposomal Calcium both at room temperature (RT) and at 105°C (exposure for 4 hours).

9a. Differential Scanning Calorimetry Analysis

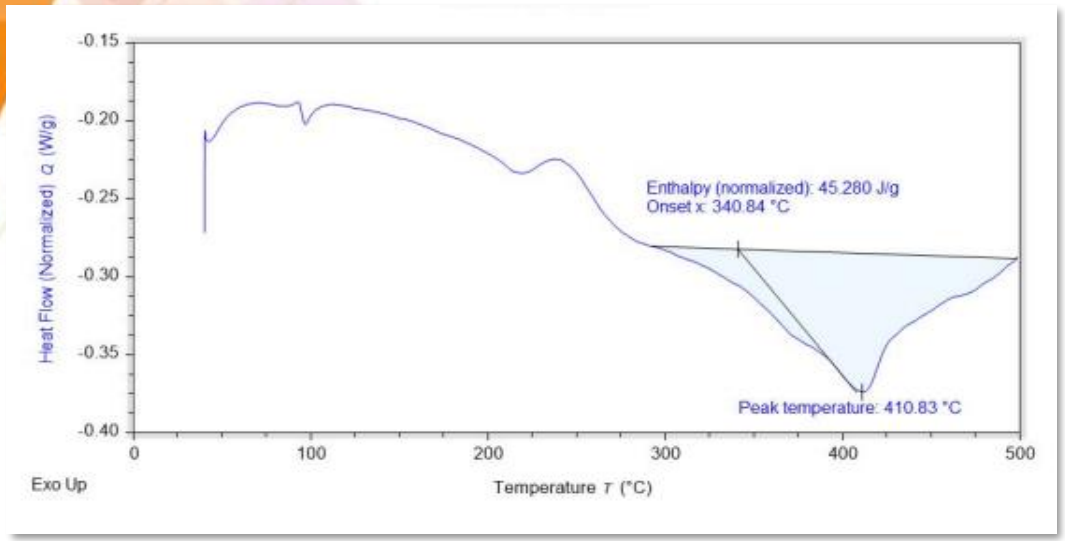


Figure 1: DSC Thermogram of Calcium API

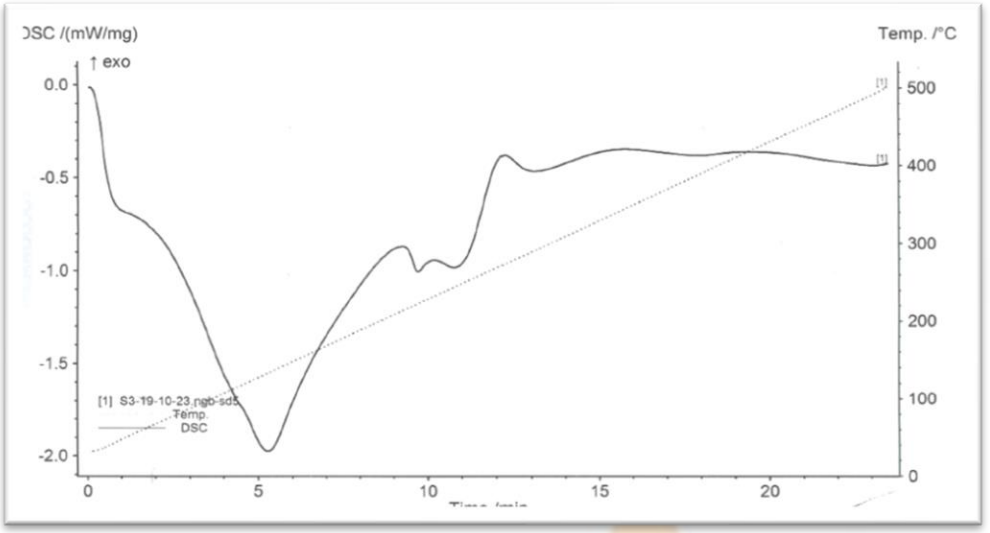


Figure 2: DSC Thermogram of Empty Liposome

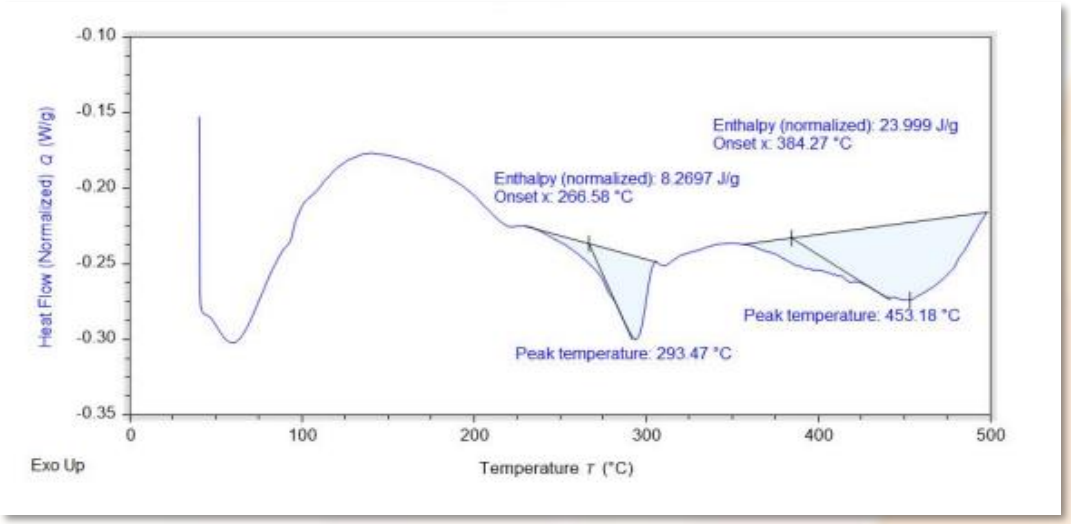
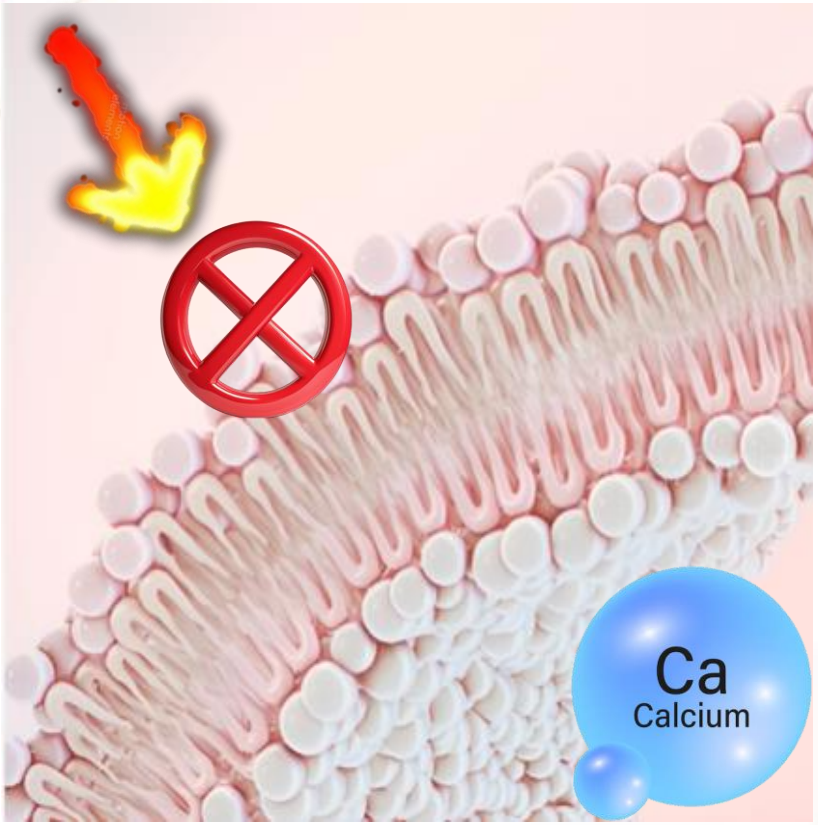


Figure 3: DSC Thermogram of Liposomal Calcium

9b. Endothermic study of Liposomal Calcium Using Differential Scanning Calorimetry Analysis

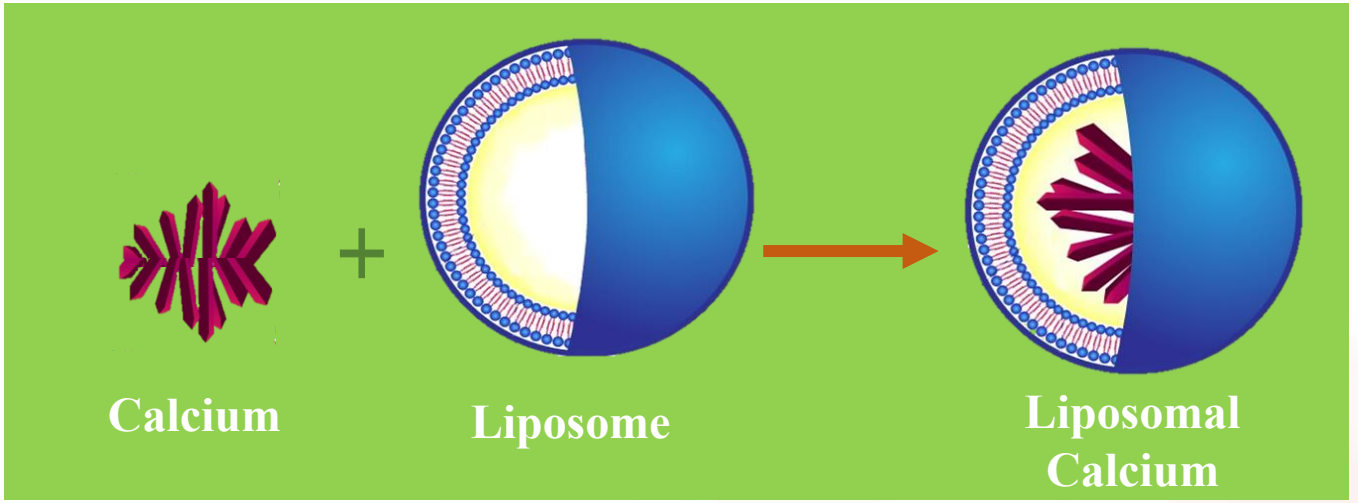


An illustration showing how the phospholipid bilayer is preventing the heat to accessing Calcium API from getting disintegrated.

Sample	Thermal Events (°C)	Inference
Calcium Carbonate API	410.83	Distinct thermal transitions indicate phase changes or melting points associated with Calcium carbonate*.
Liposome	136.85, 212.78, 278.42	Exhibits multiple transitions related to phospholipid structural changes and thermal stability*.
Liposomal Calcium	293.47, 453.18	Enhanced thermal stability indicates strong molecular interaction between the lipid bilayer and calcium carbonate*.

10. Mineral Loading Capacity

Calcium Loading **0.76 mg per mg of Liposomal Product**



Formulation of Calcium in Liposomes

- Calcium loading capacity in Liposomes refers to the amount of Calcium encapsulated within the Liposome relative to the total weight of the Liposomal formulation.
- A higher Calcium loading capacity in Liposomes ensures more efficient mineral delivery, reduces the amount of Liposome required, and improves therapeutic outcomes.

$$\textit{Calcium loading capacity} = \frac{\textit{Mass of calcium in Liposomal Calcium}}{\textit{Total mass of Calcium and Liposome}}$$

11. Product Uniformity – Mesh Size vs EE%

Figure 1 - A representative image of Liposomal Calcium powder.

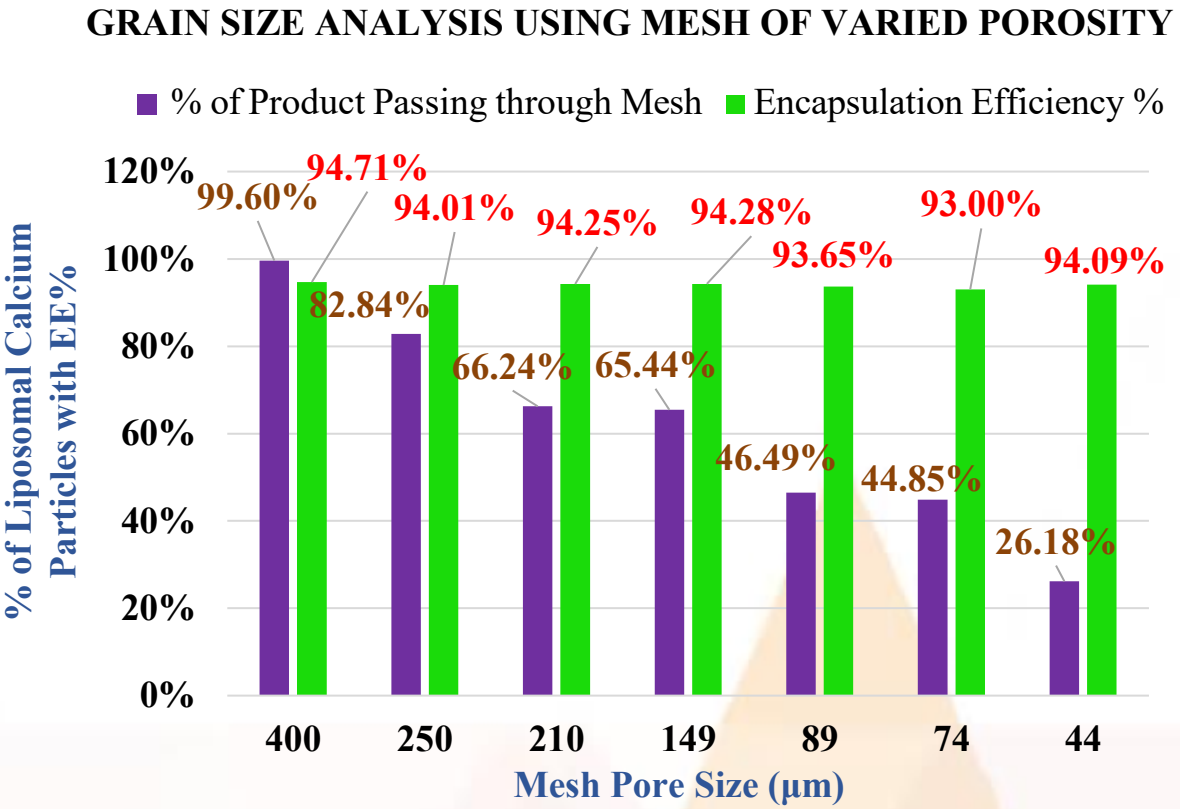


Figure 2 – Chart comparing the % of Liposomal Calcium that can pass through mesh of varied porosity with their respective encapsulation efficiency percentages.

- **Encapsulation efficiency remained stable (93-94%)** across all mesh sizes, regardless of particle retention.
- This indicates that **calcium is effectively entrapped** within liposomes of all sizes, confirming **formulation integrity and uniform encapsulation**.

Thank You!!!