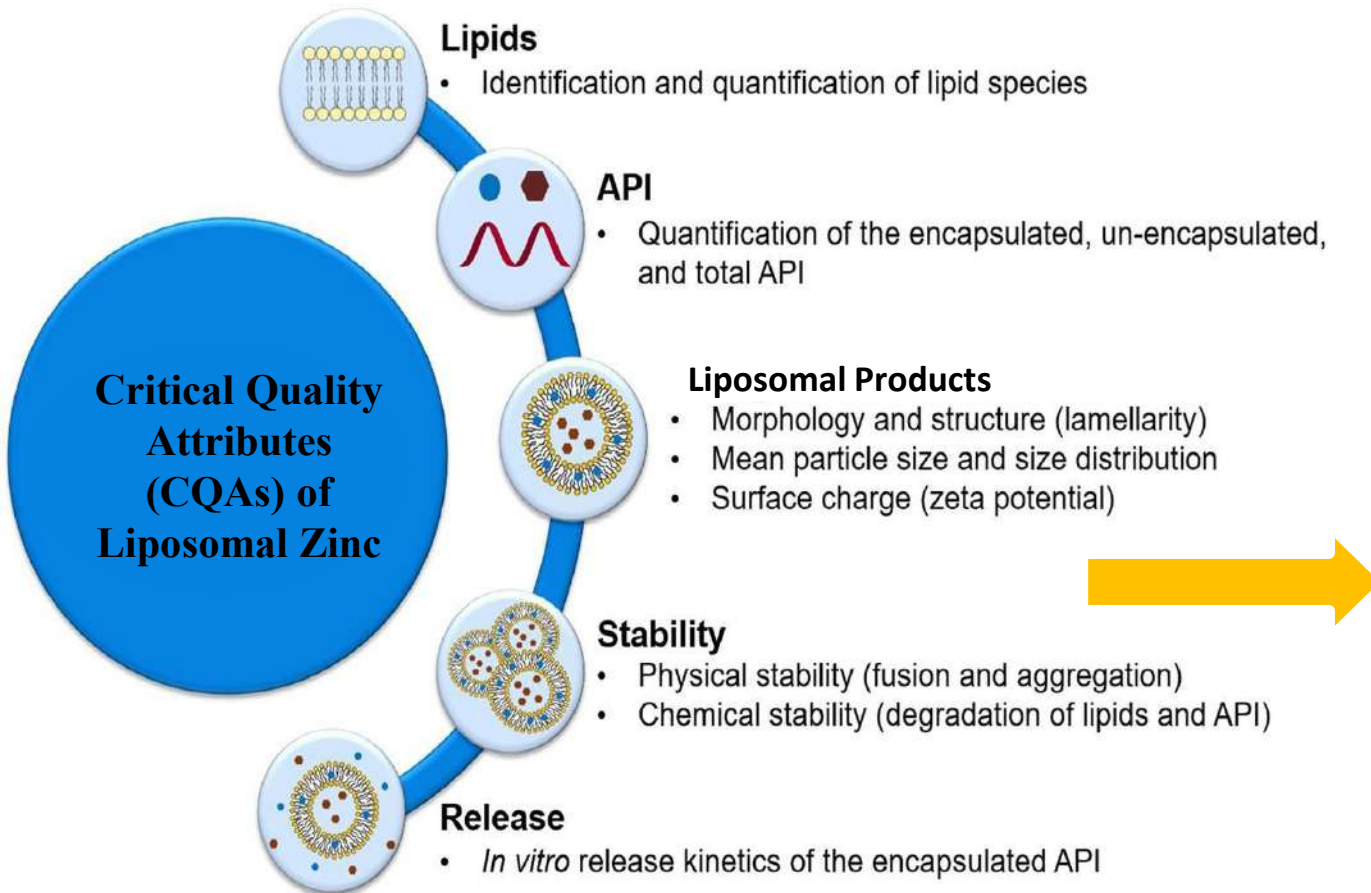




Zn

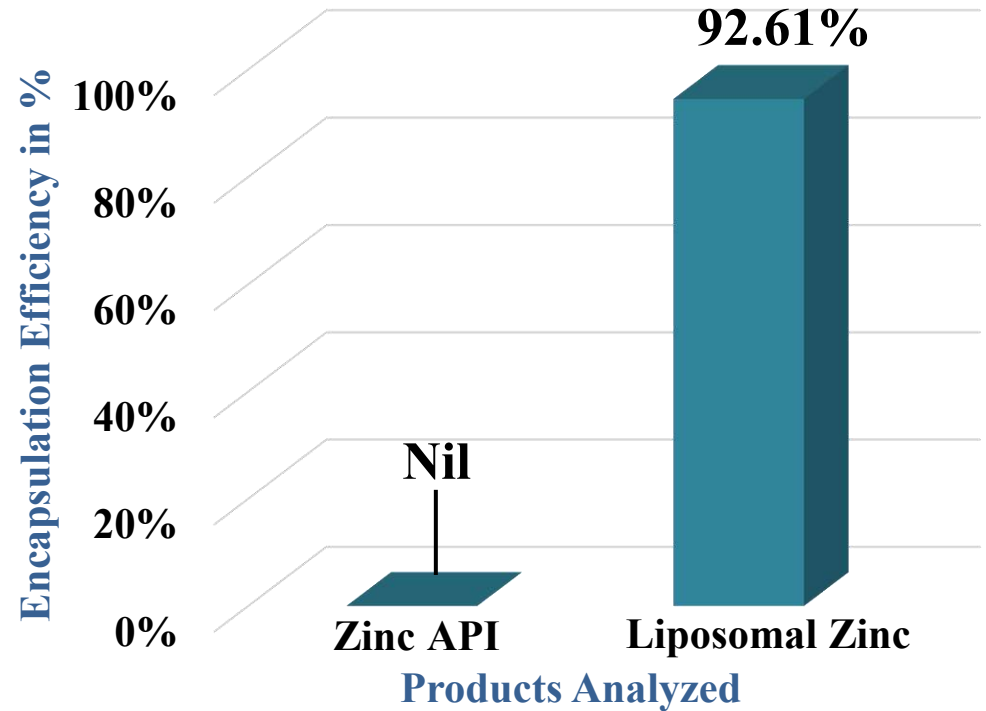
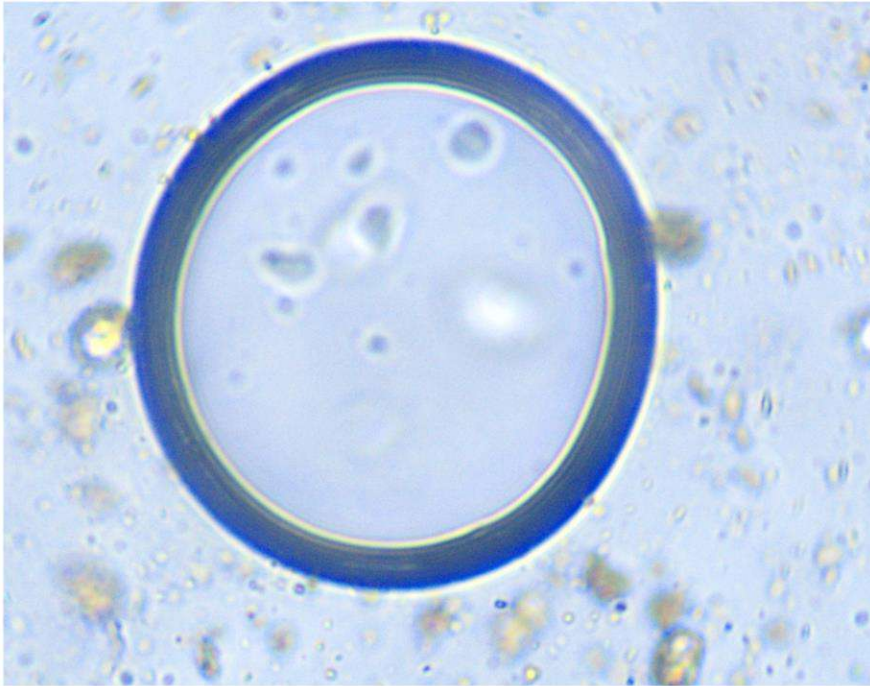
LIPOSOMAL

Summary of Characterizations Performed on Liposomal Zinc



1. *Encapsulation efficiency of Liposomal Zinc*
2. *Analysis of particle size and uniformity of Liposomal Zinc using DLS*
3. *Behavior of Liposomal Zinc particles in liquid medium using DLS Zeta-sizer*
4. *FTIR analysis of Liposomal Zinc*
5. *Elemental analysis of Liposomal Zinc*
6. *Morphology analysis of Zinc Liposomes using SEM*
7. *Analysis of Zinc leakage from Liposomes*
8. *Stability analysis of Liposomes at 105° C temperatures*
9. *Product Uniformity - Mesh Size vs EE%*

1. Encapsulation Efficiency of 20.23% Liposomal Zinc



Encapsulation Efficiency measured by validated titrimetric analytical data

❖ Acceptance criteria:

- Assay : **20 - 24%**
- Encapsulation efficiency : **NLT 70%**

- Liposomal encapsulation ensures **92.61% 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 zinc forms.



2. Dynamic Light Scattering Analysis of Liposomal Zinc

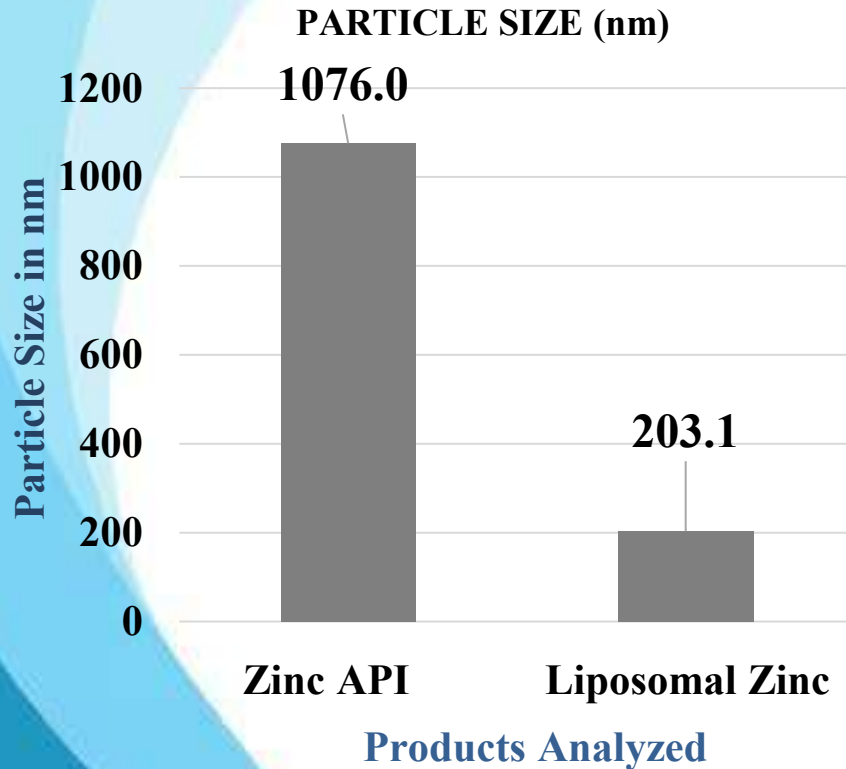


Figure 1 – Chart showing the particle size of Zinc API with Liposomal Zinc

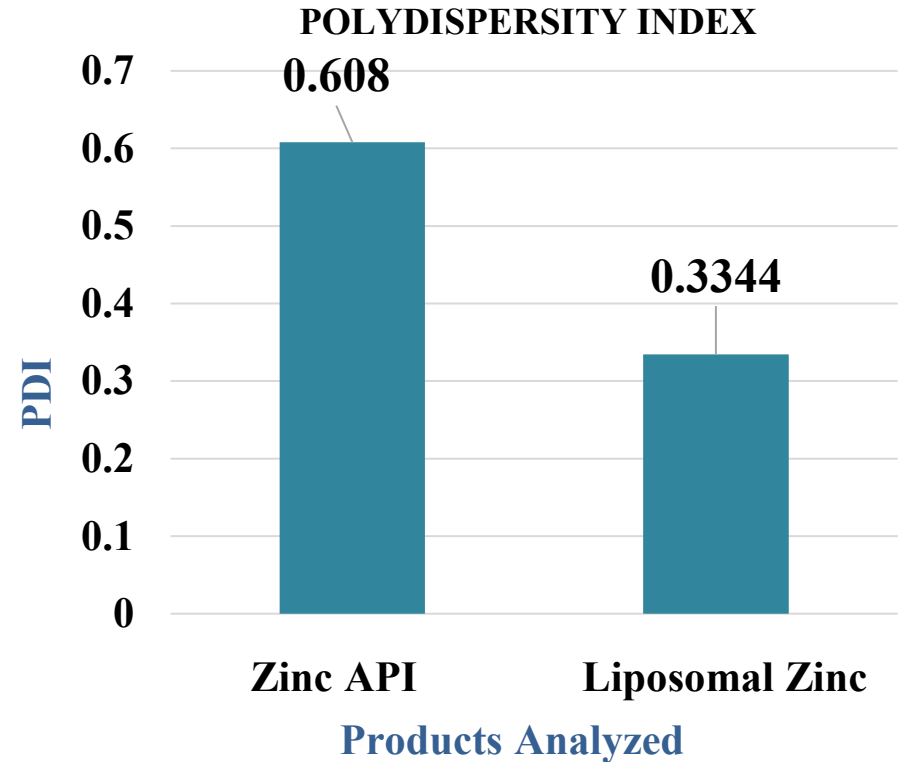


Figure 2 – Chart comparing the Polydispersity Index of Zinc API and Liposomal Zinc in solution

- Nanosized, uniform particles offer greater colloidal stability and improved shelf life.
- Smaller particles (particle size: 203.1 and PDI 0.3344 support **increased mucosal permeability** and cellular uptake.
- DLS characterization confirms high formulation control and **batch-to-batch reproducibility**.

❖ Acceptance criteria:

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

3a. Behavior of Liposomal Zinc

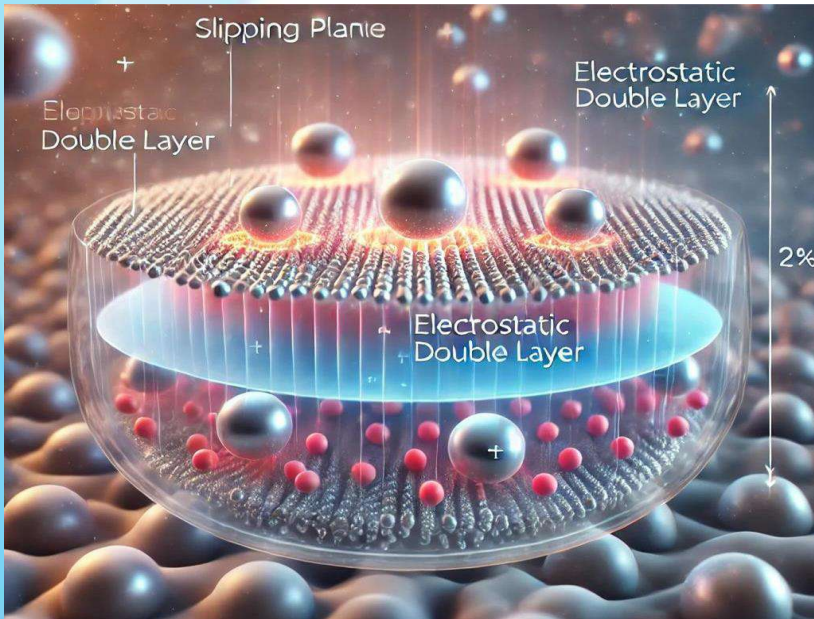


Figure 1 – A figure showing the balance of attractive and repelling forces which determine how particles behave in a medium.

- Liposomal Zinc shows **high zeta potential (-40.79 mV)** → excellent colloidal stability.
- Prevents particle aggregation → ensures **uniform suspension**.
- Enhances **product shelf life** and **bioavailability** in liquid form.

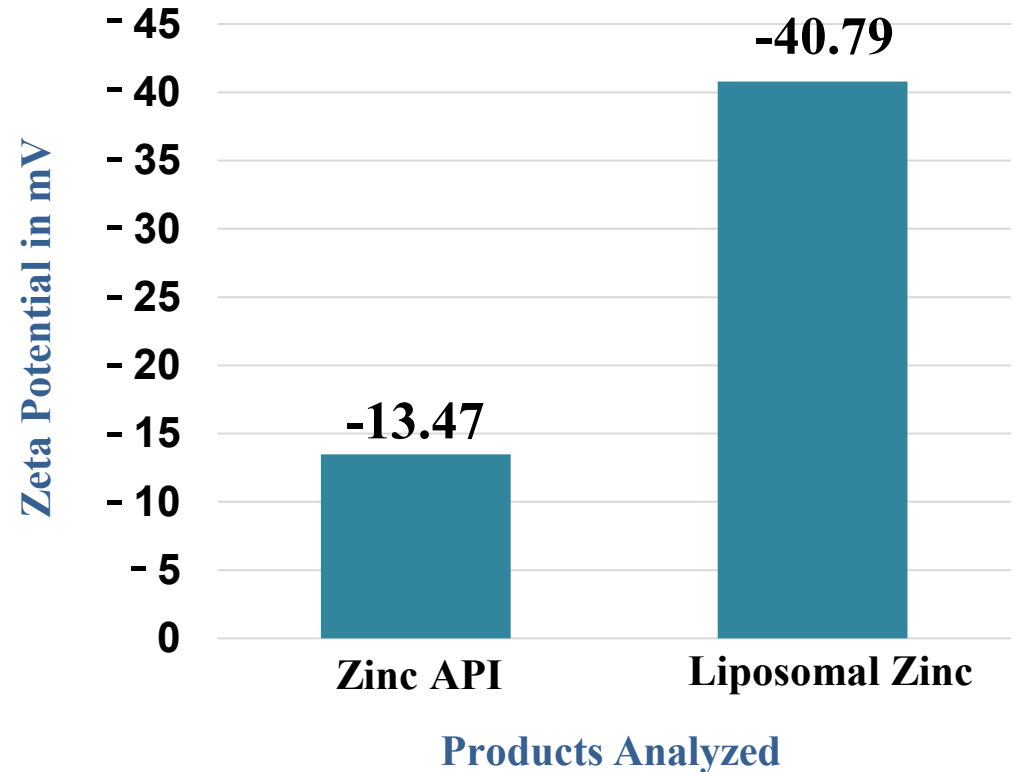


Figure 2 – Chart comparing the zeta potential of Zinc API and Liposomal Zinc showing Zinc in Liposomal form is stable and unlikely to agglomerate

❖ Acceptance criteria:

- **Zeta Potential : < -30 mV**

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

Mineral Release

Zeta Potential

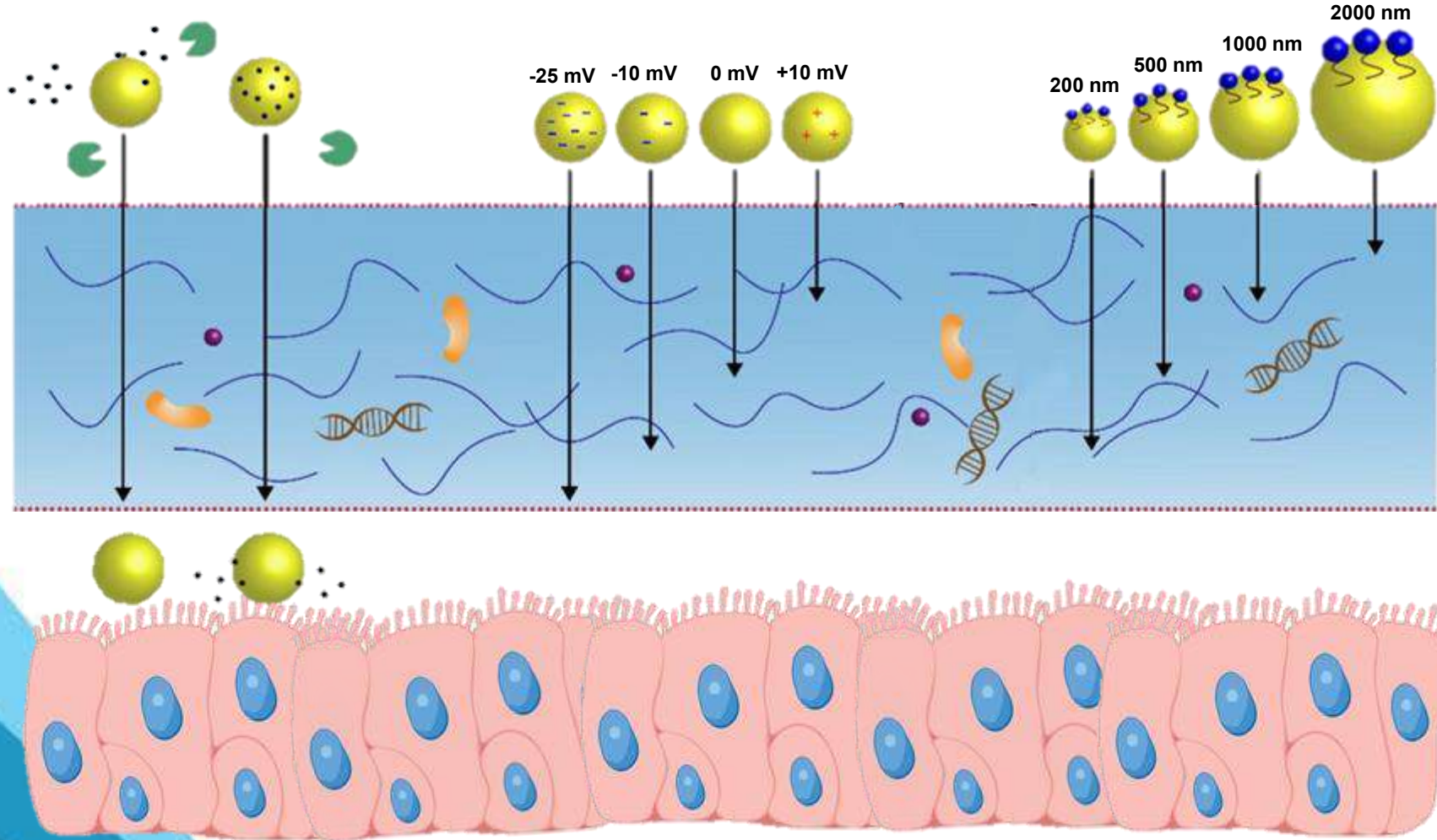
Particle Size

Lumen

Mucus Barrier

Absorption Membrane

Cellular Epithelium



Liposome

Mucus Permeation

Surfactant

Enzyme

Mucin

Lipid

Nucleic Acid

Protein

4a. FTIR Spectra of API, Liposome & Liposomal Zinc

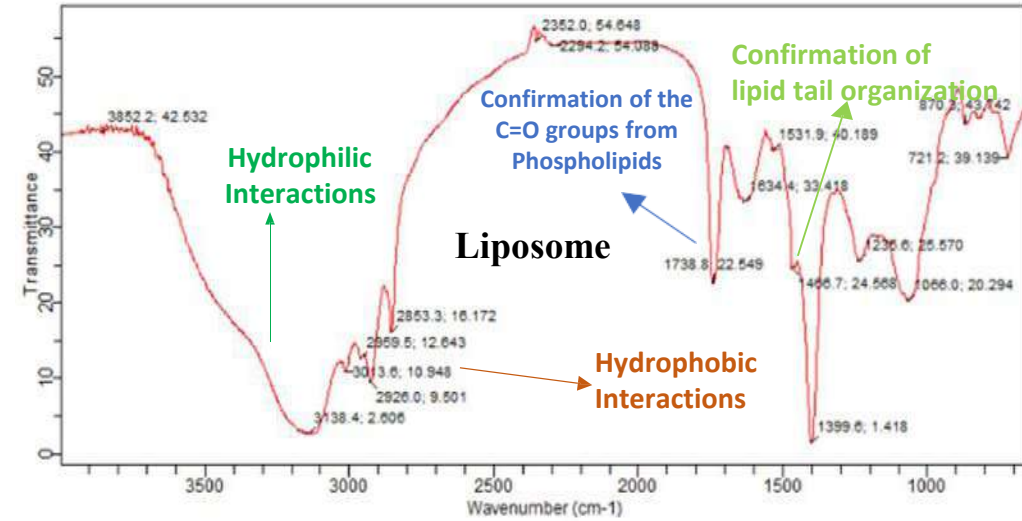
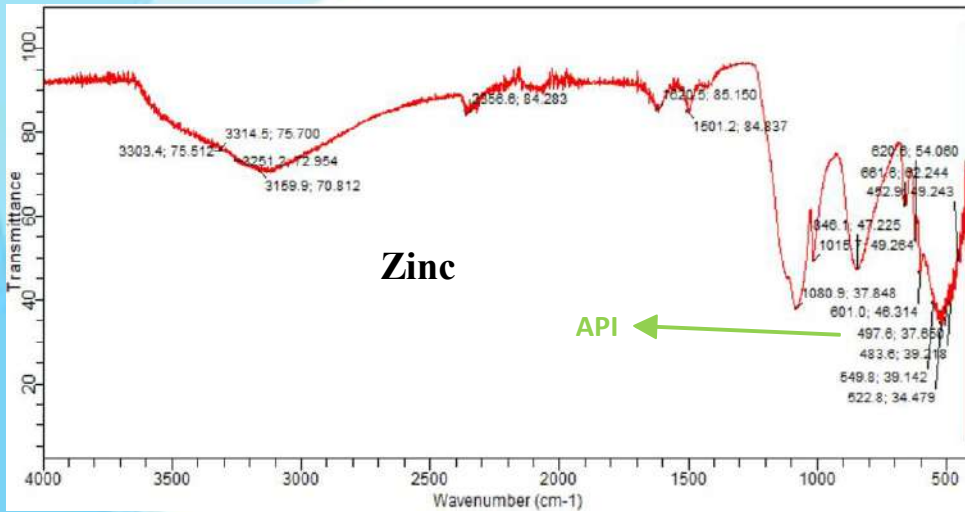


Figure 1: IR Transmission spectrum showing bands at different wavelengths of Zinc Oxide API

Figure 2: Hydrophobic and Hydrophilic interactions within Empty Liposome

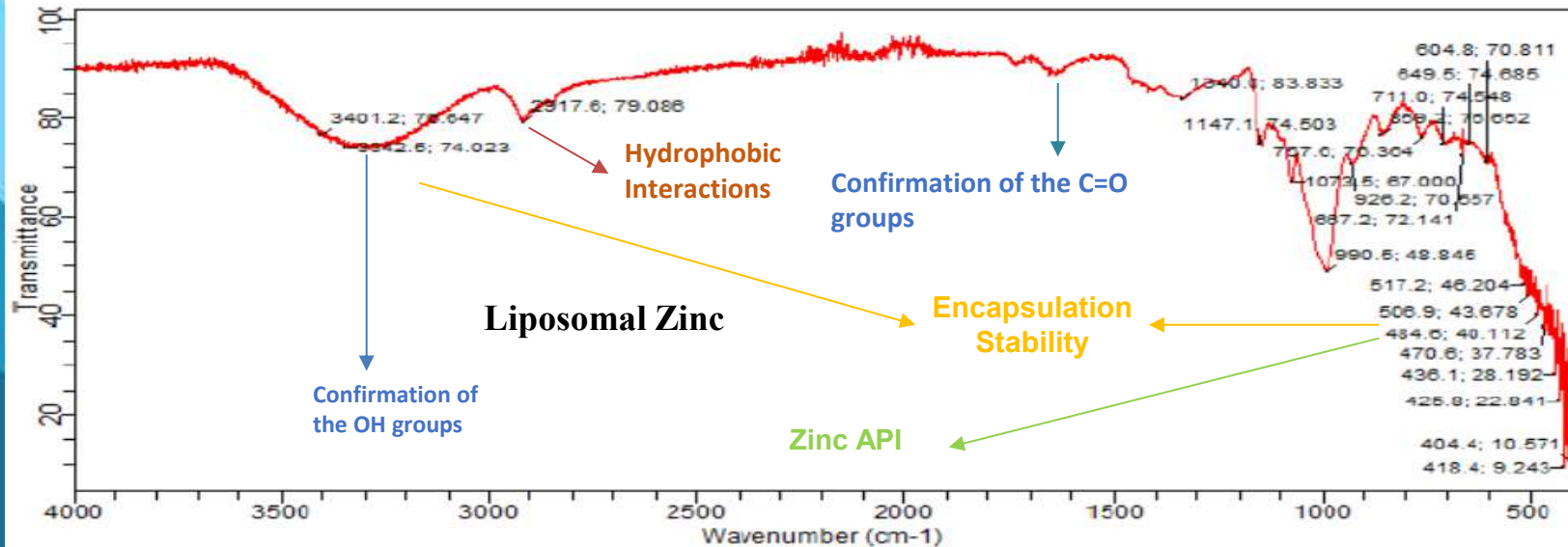


Figure 3: Successful integration of Zinc into Liposome



4b. Summary of FTIR Analysis of Liposomal Zinc

1. **Confirmation of the OH groups** - Broad -OH ($\sim 3401.2 \text{ cm}^{-1}$) confirm structural stability.
2. **Hydrophobic Interactions** - Distinct CH₂ peaks (~ 2920 and 2850 cm^{-1}) confirm lipid tail packing, ensuring bilayer stability.
3. **Hydrophilic Interactions** - Broad -OH peaks ($\sim 3401.2 \text{ cm}^{-1}$) indicate strong hydrogen bonding between Zinc ions and phospholipid headgroups.
4. **API** - Shifts in Zn-O ($\sim 484.4 \text{ cm}^{-1}$) and well-defined CH₂ peaks ($2920, 2850 \text{ cm}^{-1}$) confirm strong integration of Zinc ions in the lipid bilayer.
5. **Encapsulation Stability** - Phosphate headgroup region ($\sim 1240\text{--}1250$ and $1090\text{--}1060 \text{ cm}^{-1}$) shows **reduced intensity and slight shifts/broadening** vs. empty liposome, with Zn²⁺ coordination to PO₂⁻/P-O-C groups, distinct CH₂ peaks (~ 2920 and 2850 cm^{-1}) and 484.4 cm^{-1} Zn-O confirm successful interaction between the lipid bilayer and API.

5. Elemental Analysis of Liposomal Zinc

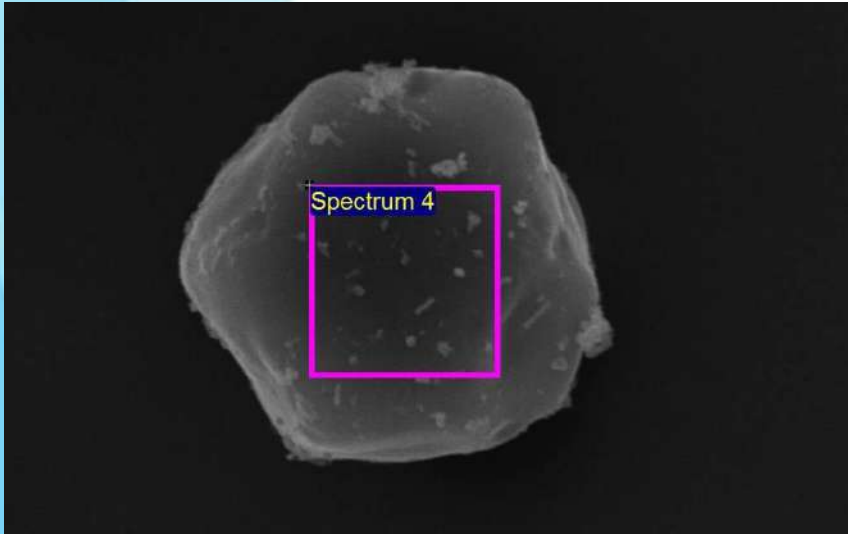


Figure 1 – SEM image of Liposomal Zinc showing the area scanned using Energy Dispersive X-Ray Spectroscopy (EDAX)

- **Zinc Composition:** Zinc makes up 40.89% of the Zinc API.
- **SEM Image:** EDAX analysis shows the Liposomal Zinc structure.
- **Encapsulation Conclusion:** Zinc is fully encapsulated within the liposome, as seen in the elemental composition.

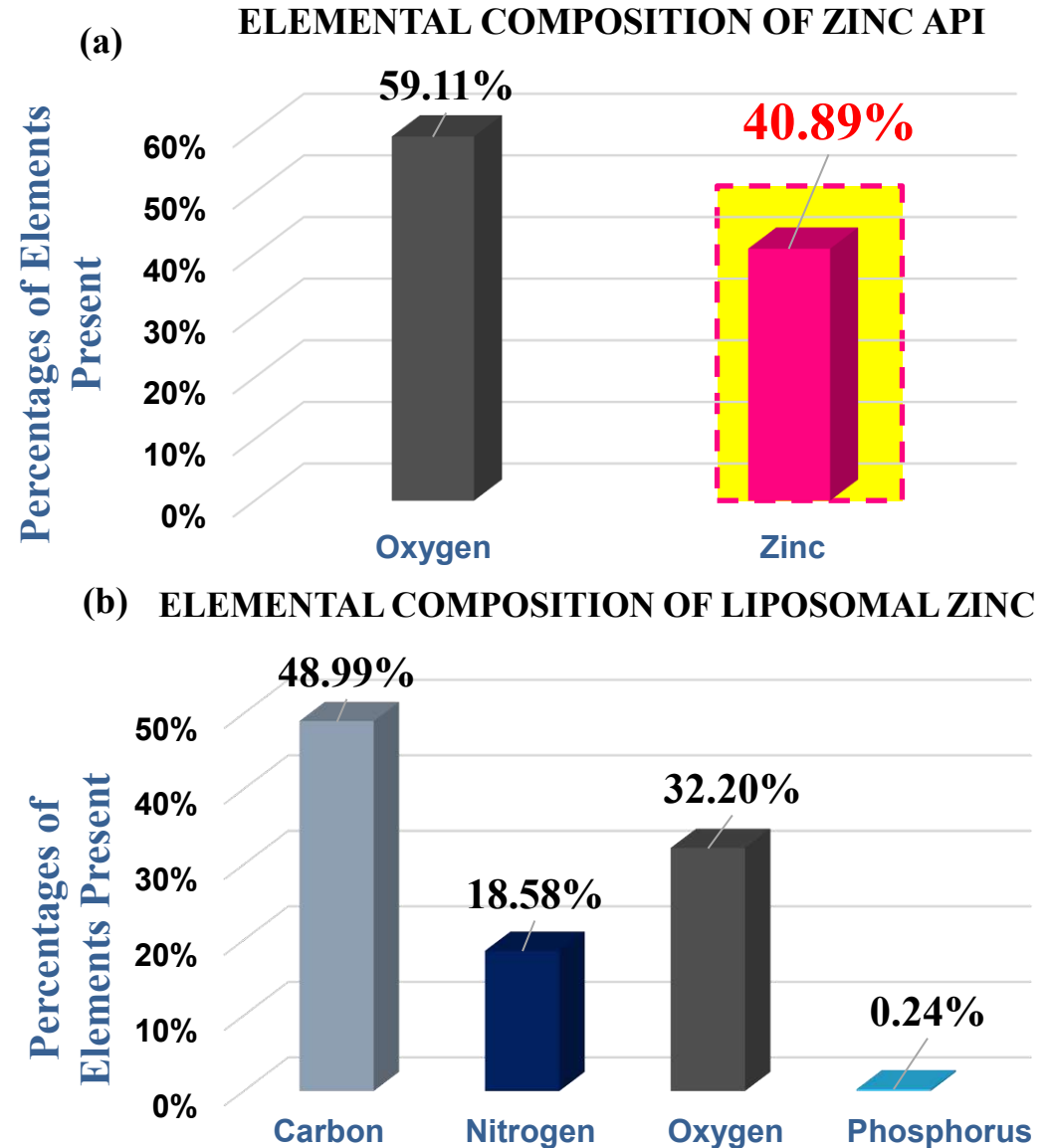


Figure 2 – A graphical representation of the percentages of elements composing (a) Zinc API and (b) Liposomal Zinc

6. Morphology of Zinc Liposomes as viewed under a Scanning Electron Microscope

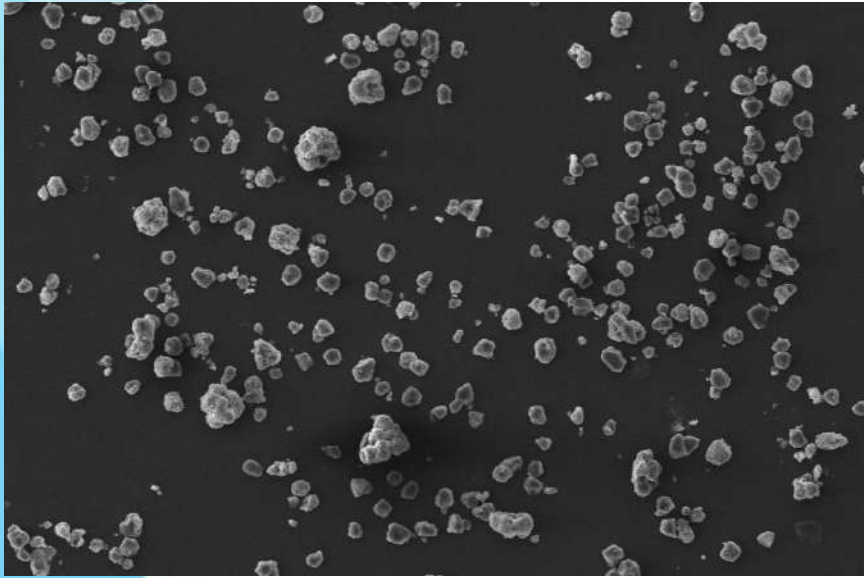


Figure 1 – SEM image of non-encapsulated Zinc API

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

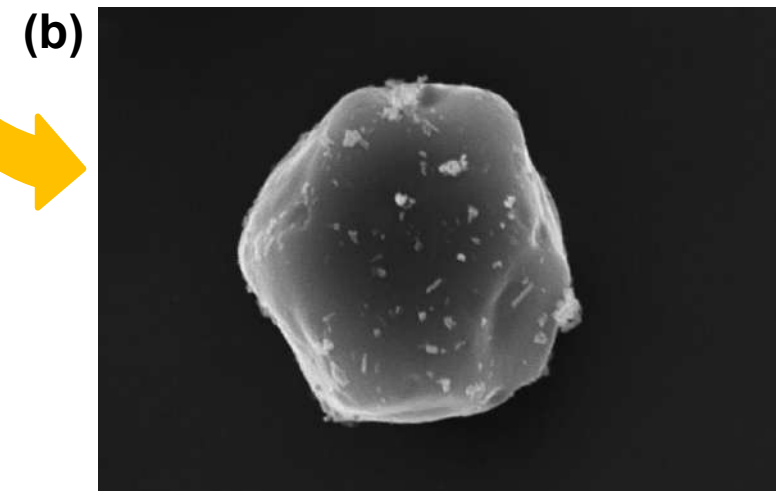
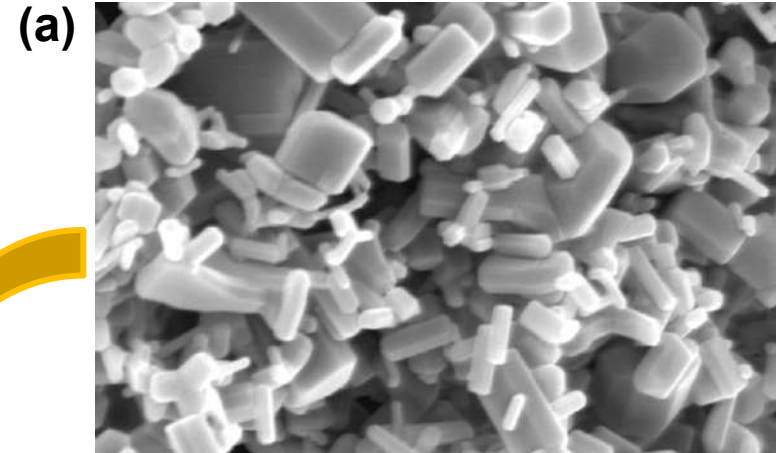


Figure 2 – SEM panels showing transformation from (a) Zinc API to (b) Liposomal Zinc after encapsulation.

7. Leakage of Zinc from Liposomes



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

- **Encapsulation efficiency remains high (~ 94%)** throughout 3years of storage, indicating stable liposome structure.
- **Assay values for Liposomal Zinc remain low (~20%),** showing minimal leakage over time.
- The formulation shows **excellent retention of Zinc**, confirming its suitability for long-term shelf storage.

MINERAL LEAKAGE ASSAY

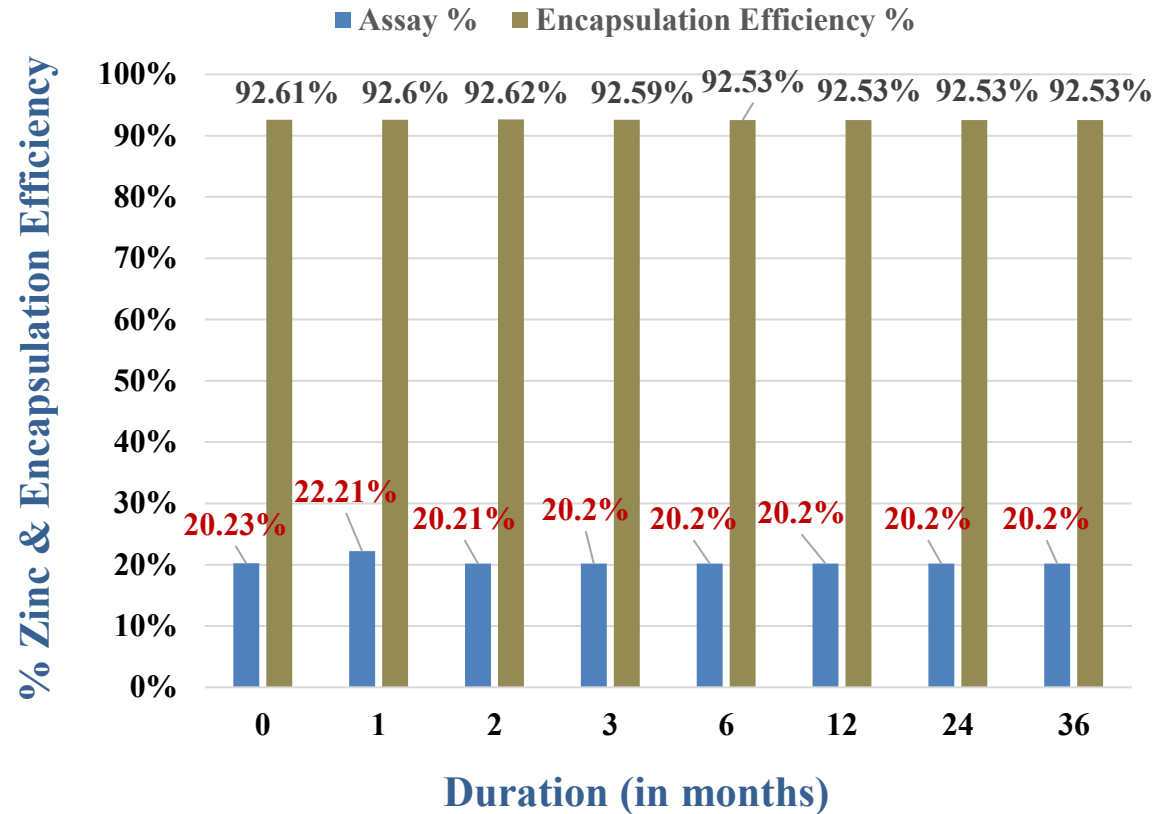


Figure 2 – Chart comparing the stability of Liposomal Zinc stored over a period of 3 years at 40°C ± 2 °C and a relative humidity of 75% ± 5%.

8. Stability of Zinc Liposomes at Elevated Temperatures



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

- Encapsulation efficiency remains high ($\approx 92\%$) even after exposure to 105°C for 4 hours.
- Assay values (20.23% at RT vs. 20.1% at 105°C) show minimal variation, indicating negligible ALA leakage.
- Demonstrates **thermal robustness**, making the formulation suitable for transport and storage in hot climates.

TEMPERATURE EXPOSURE STUDY

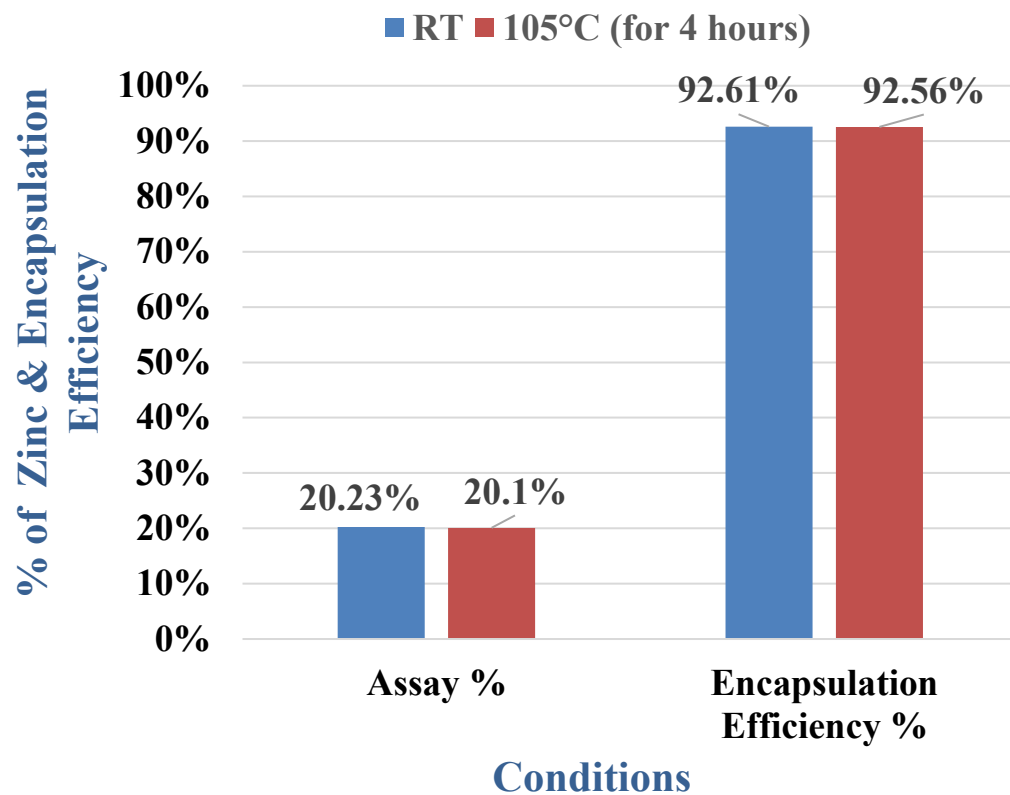


Figure 2 – Chart comparing the stability of Liposomal Zinc both at room temperature (RT) and at 105°C (exposure for 4 hours).



9. Product Uniformity – Mesh Size vs EE%

GRAIN SIZE ANALYSIS USING MESH OF VARIED POROSITY



Figure 1 – A representative image showing Zinc formulation kept in a bowl.

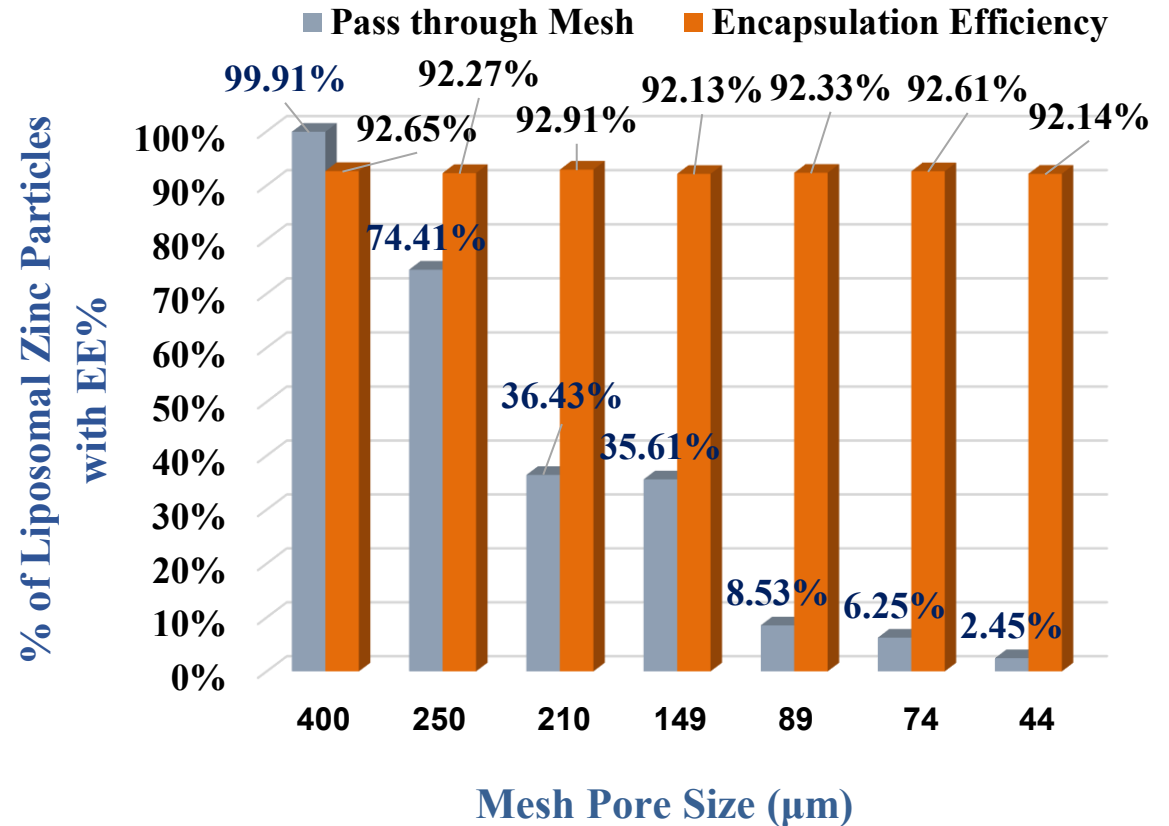


Figure 2 – Chart comparing the % of Liposomal Zinc products that can pass through mesh of varied porosity with their respective encapsulation efficiency percentages.



Thank You!!!

WEST BENGAL CHEMICAL INDUSTRIES LIMITED

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