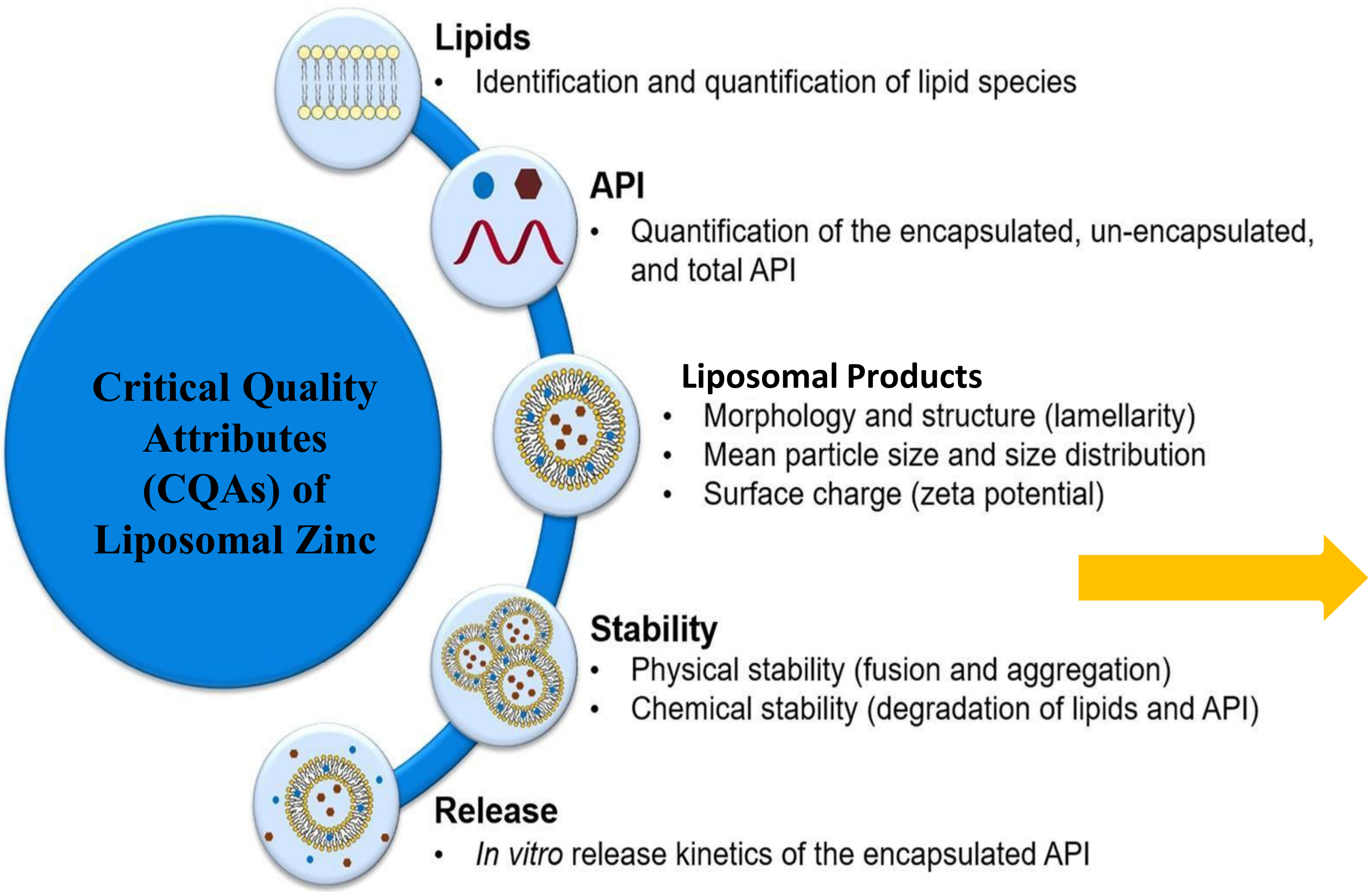
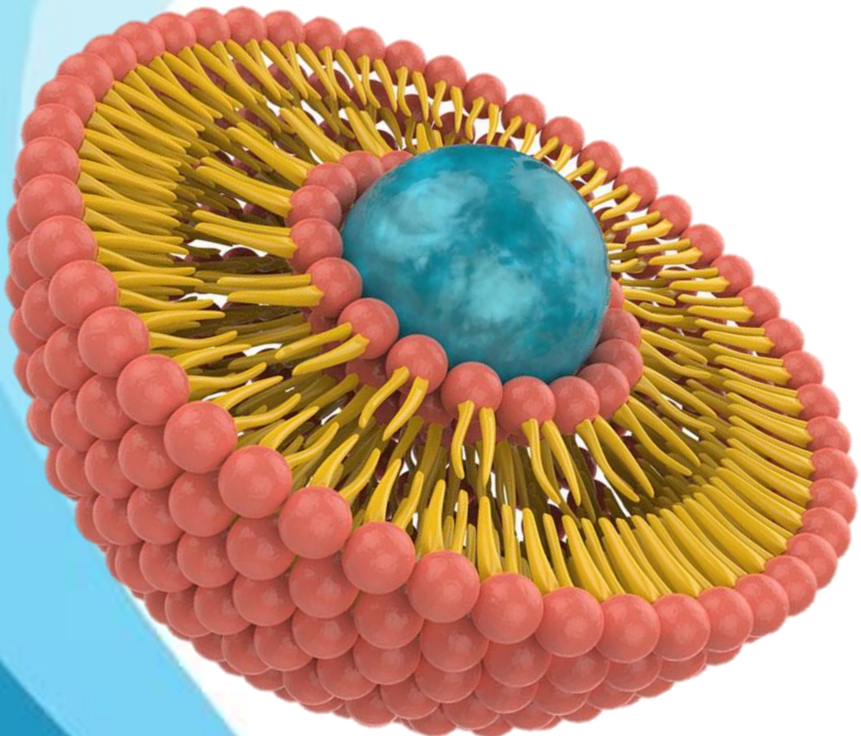
West Bengal Chemical Industries Limited

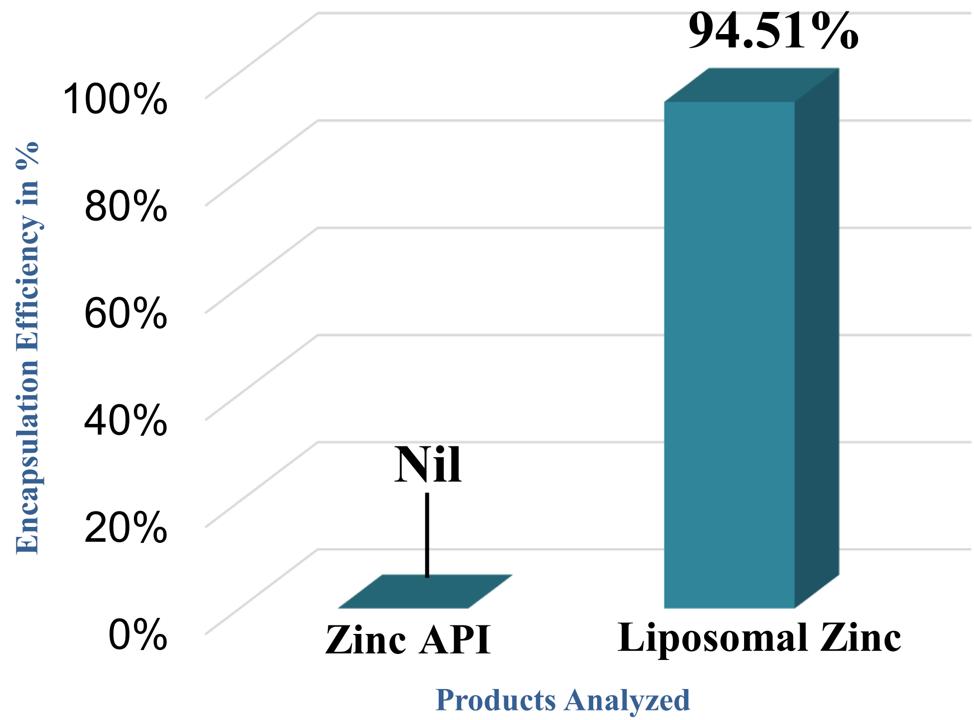
Summary of Characterizations Performed on Liposomal Zinc



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 composition*
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^{o} *C temperatures*
9. *Mineral Loading Capacity*
10. *Particle Specifications*

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 **94.51% 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

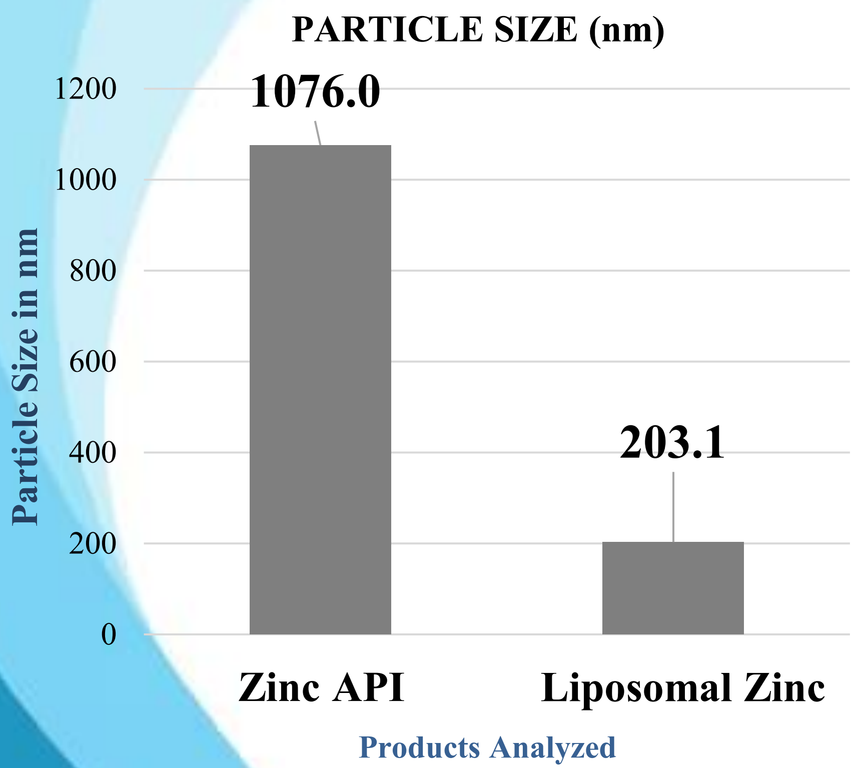


Figure 1 – Chart showing the particle size of Zinc API with Liposomal Zinc

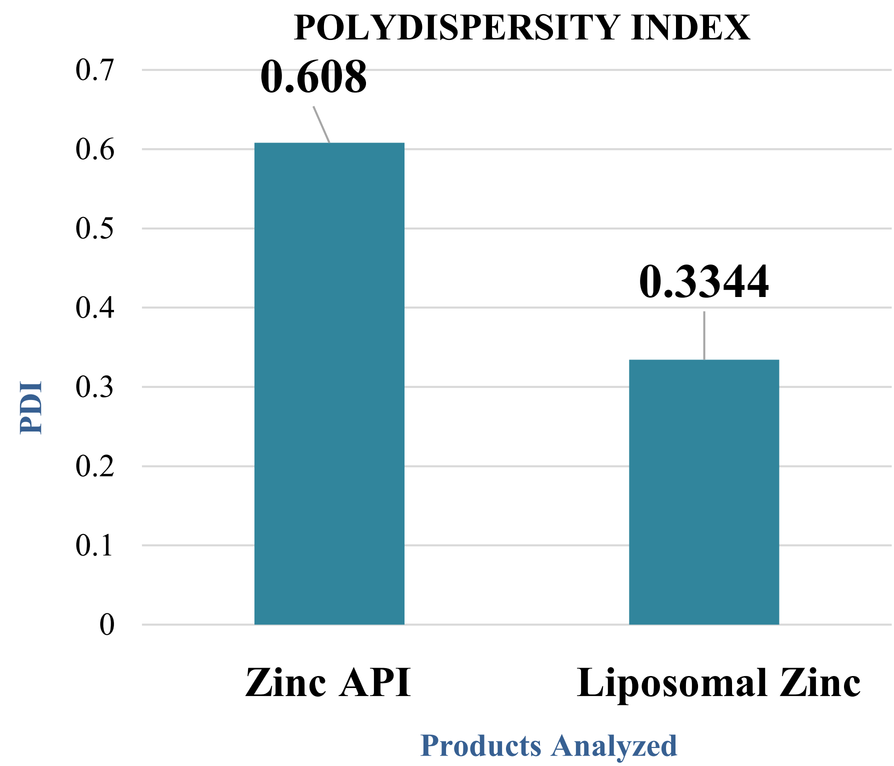


Figure 2 – Chart comparing the Polydispersity Index of Zinc API and Liposomal Zinc in solution

- 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

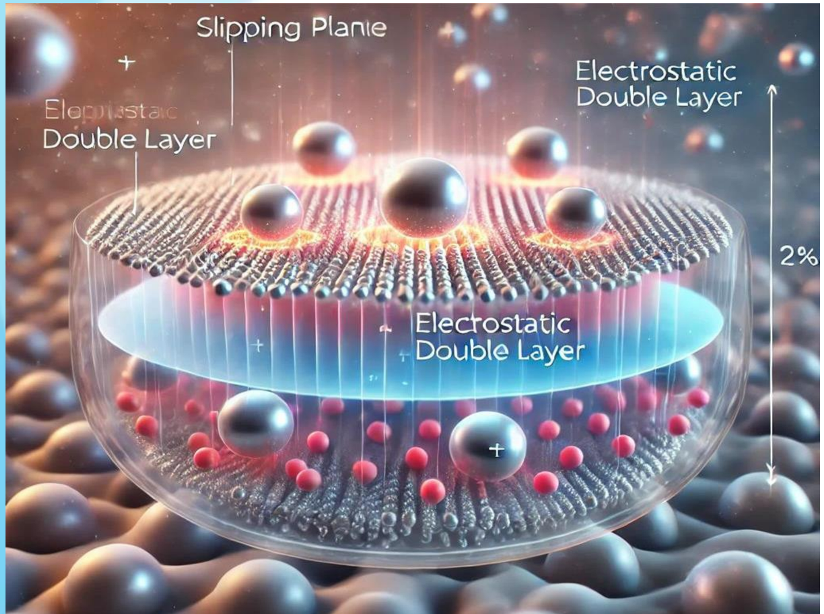


Figure 1 – A figure showing the balance of attractive and repelling forces which determine how particles behave in a medium.

- 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.

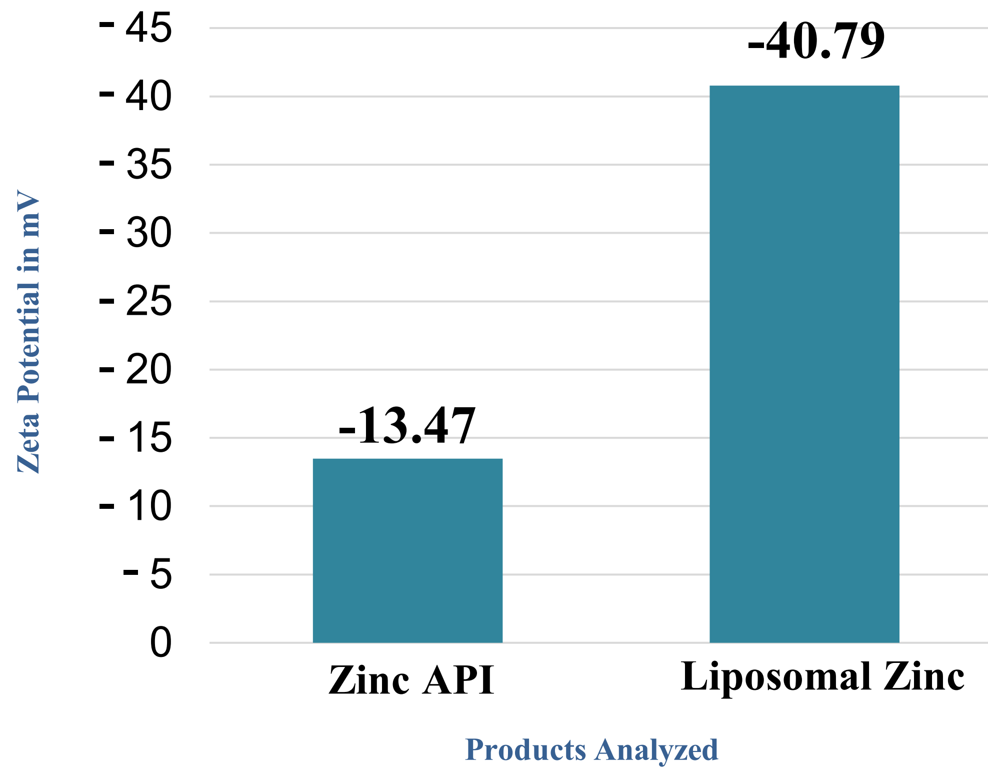
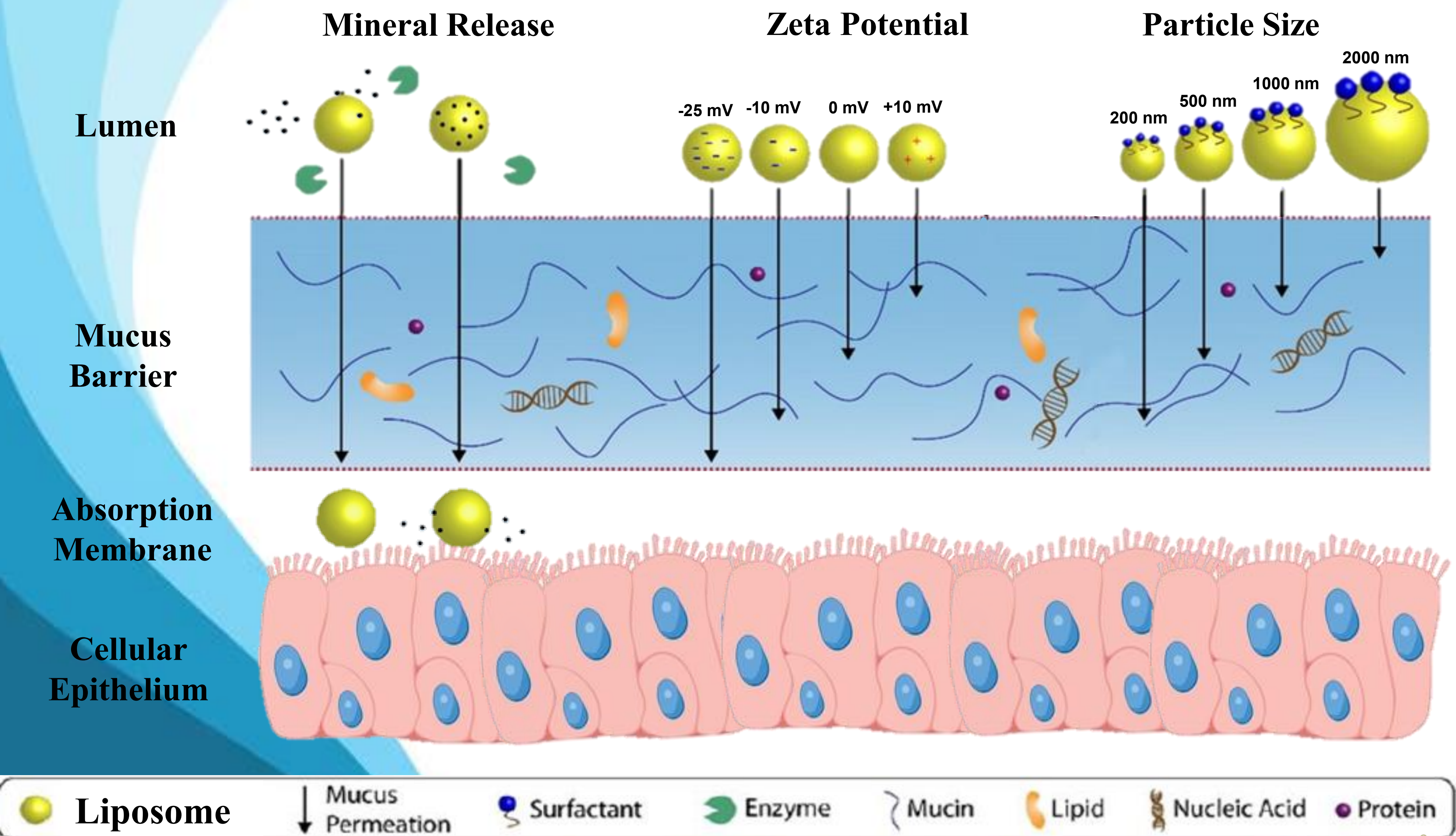


Figure 2 – Chart comparing the zeta potential of Zinc API and Liposomal Zinc showing Zinc in Liposomal form is stable and unlikely to agglomerate

❖ **Acceptance criteria:**

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

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



Adapted from: https://www.sciencedirect.com/science/article/pii/S0169409X21004907?via%3Dihub

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

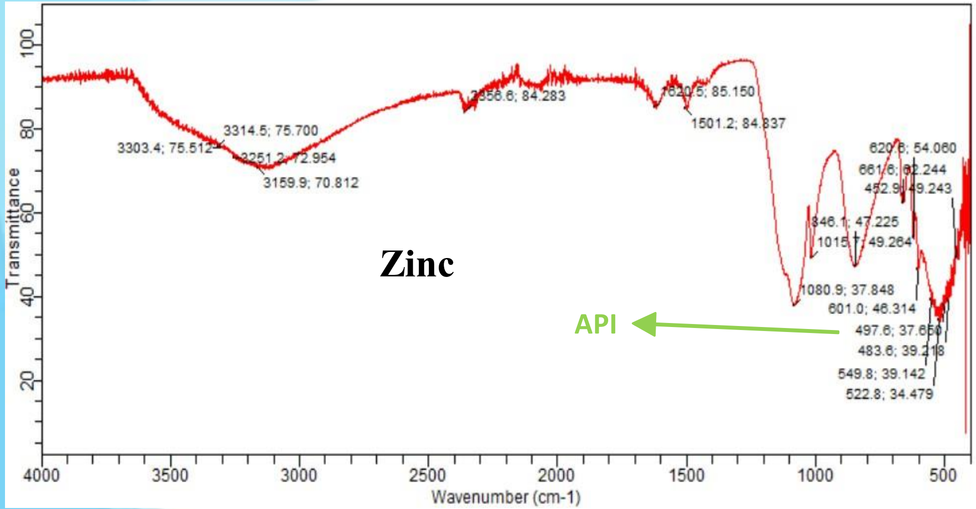


Figure 1: IR Transmission spectrum showing bands at different wavelengths of Zinc Oxide API

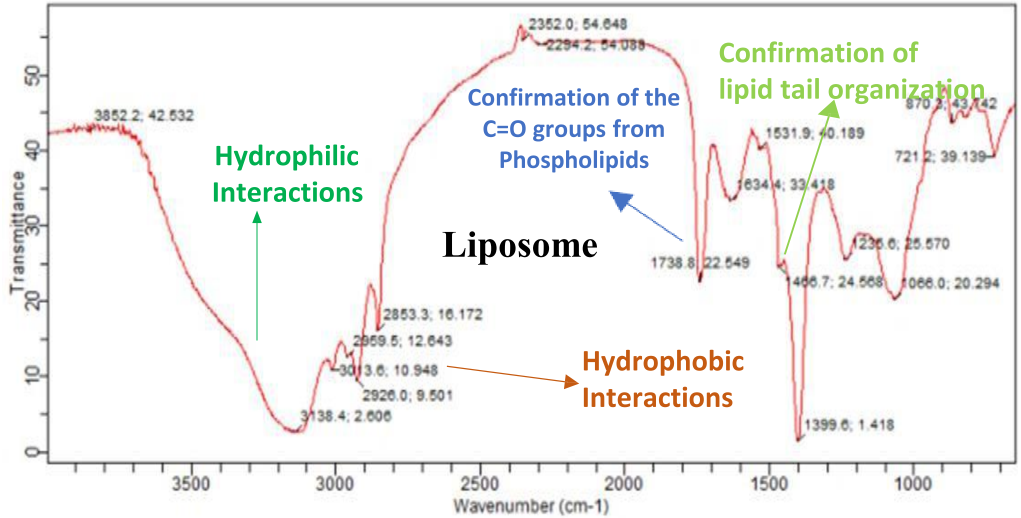


Figure 2: Hydrophobic and Hydrophilic interactions within Empty Liposome

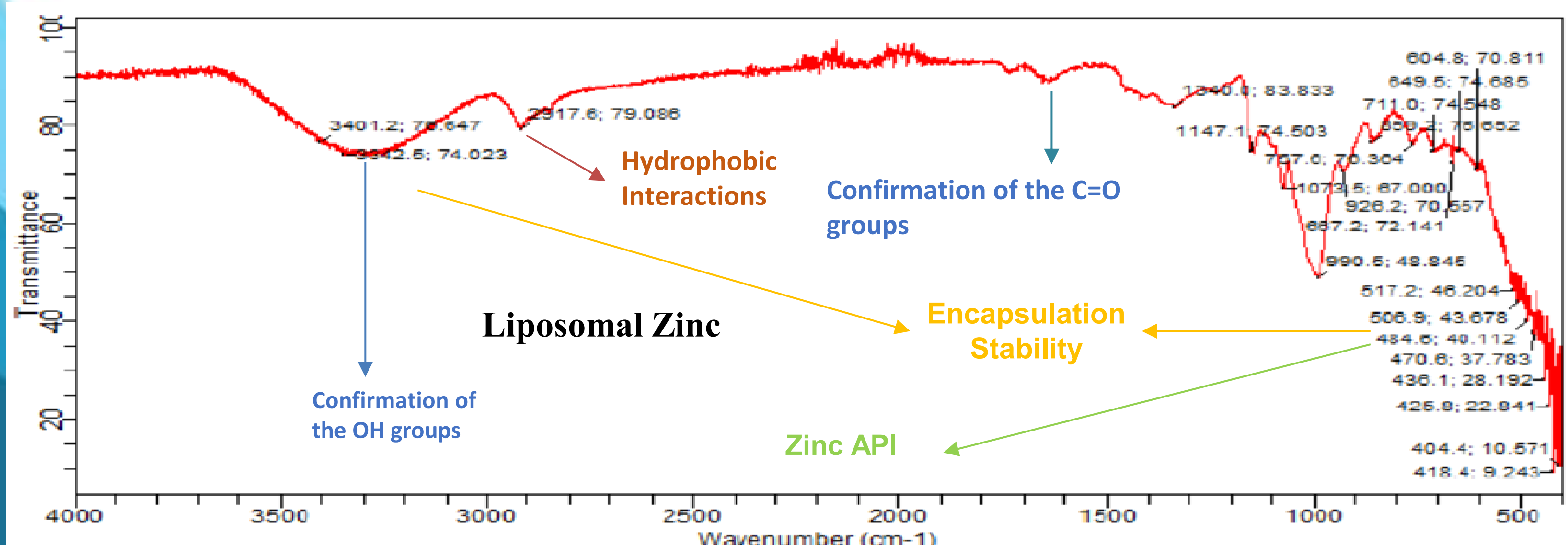


Figure 3: Successful integration of Zinc into Liposome

4b. Summary of FTIR Analysis of Liposomal Zinc

1. **Confirmation of the OH groups -** Broad **-OH (~3401.2 cm^{-1})** confirm structural stability.
2. **Hydrophobic Interactions** - Distinct CH_2 peaks **(~2920 and 2850 cm^{-1})** confirm lipid tail packing, ensuring bilayer stability.
3. **Hydrophilic Interactions -** Broad **-OH peaks (~3401.2 cm^{-1})** indicate strong hydrogen bonding between Zinc ions and phospholipid headgroups.
4. **API-** Shifts in **Zn-O (~484.4 cm^{-1})** and well-defined **CH_2 peaks (2920, 2850 cm^{-1})** confirm strong integration of Zinc ions in the lipid bilayer.
5. **Encapsulation Stability -** Distinct CH_2 peaks **(~2920 and 2850 cm^{-1})** and **484.4 cm^{-1} Zn-O** confirm successful interaction between API and lipid bilayer.

5. Elemental Analysis of Liposomal Zinc

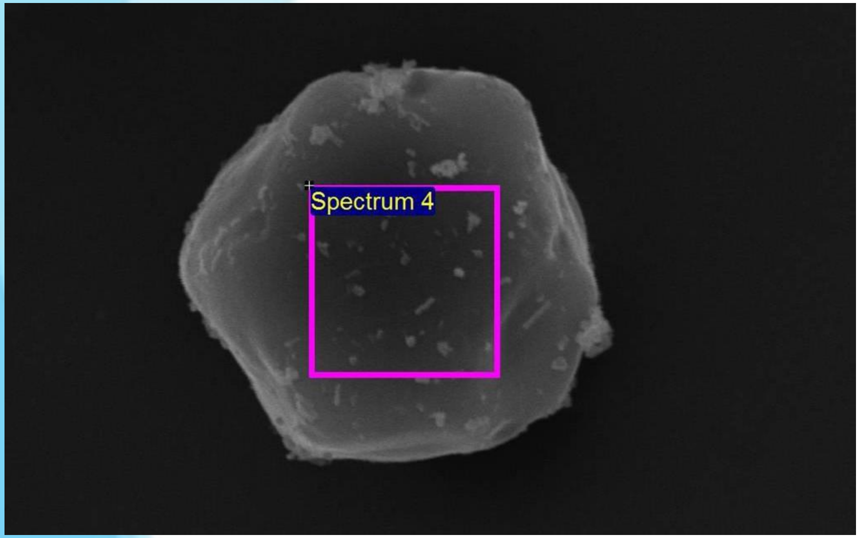
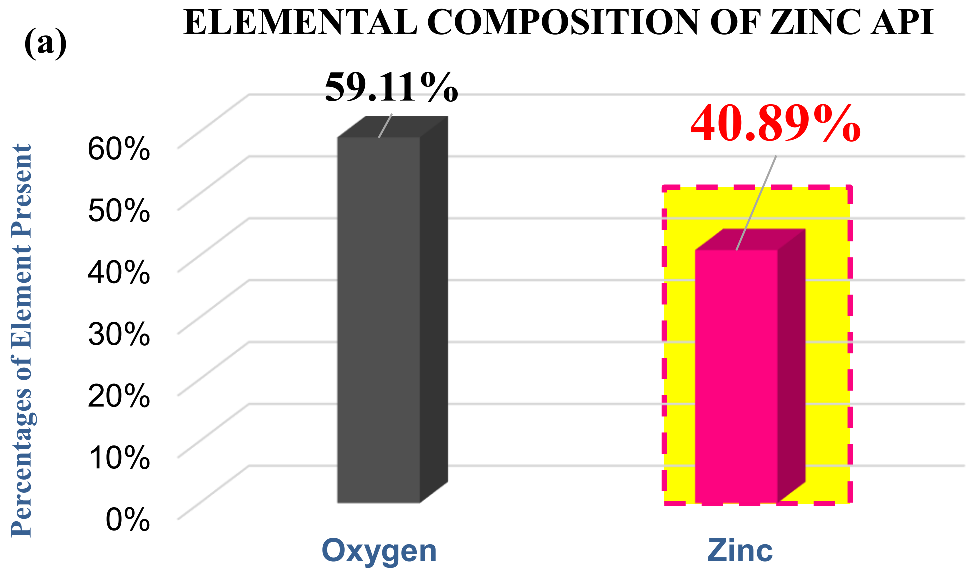


Figure 1 – SEM image of Liposomal Zinc showing the area scanned using Energy Dispersive X-Ray Spectroscopy (EDAX)

- **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.



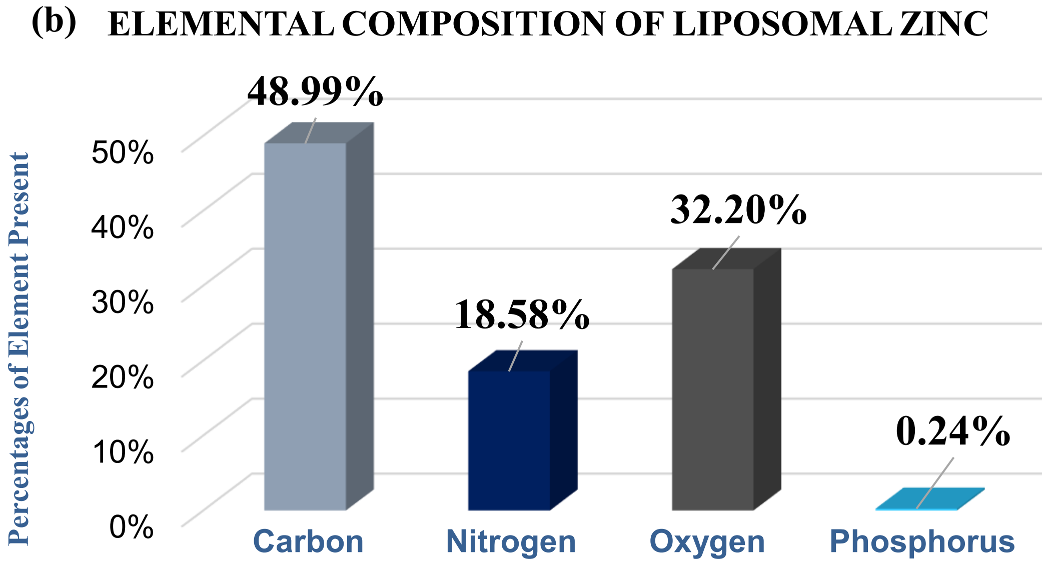


Figure 2 – A graphical representation of the percentages of elements composing (a) Zinc API and (b) Liposomal Zinc

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

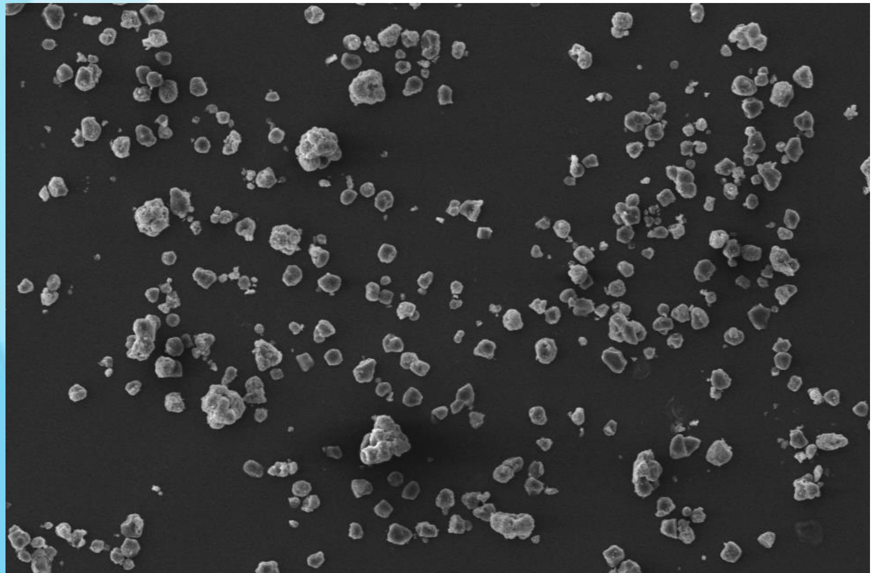


Figure 1 – SEM image of non-encapsulated Zinc API

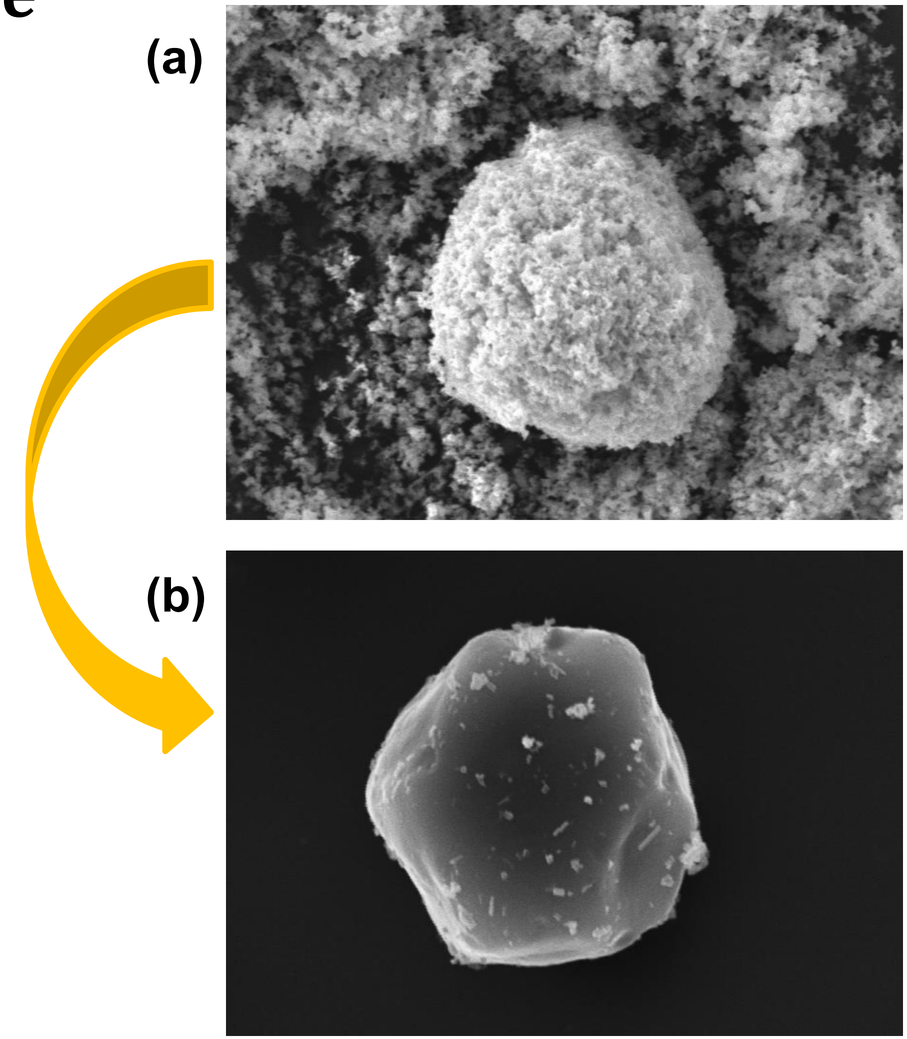


Figure 2 – SEM panels showing transformation from (a) Zinc API to (b) Liposomal Zinc after encapsulation.

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

7. Leakage of Zinc from Liposomes

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

- **Encapsulation efficiency remains high (~ 94%)** throughout 6 months of storage, indicating stable liposome structure.
- **Assay values for free Zinc remain low (~20%)**, showing minimal leakage over time.
- The formulation shows **excellent retention of Zinc**, confirming its suitability for long-term shelf storage.

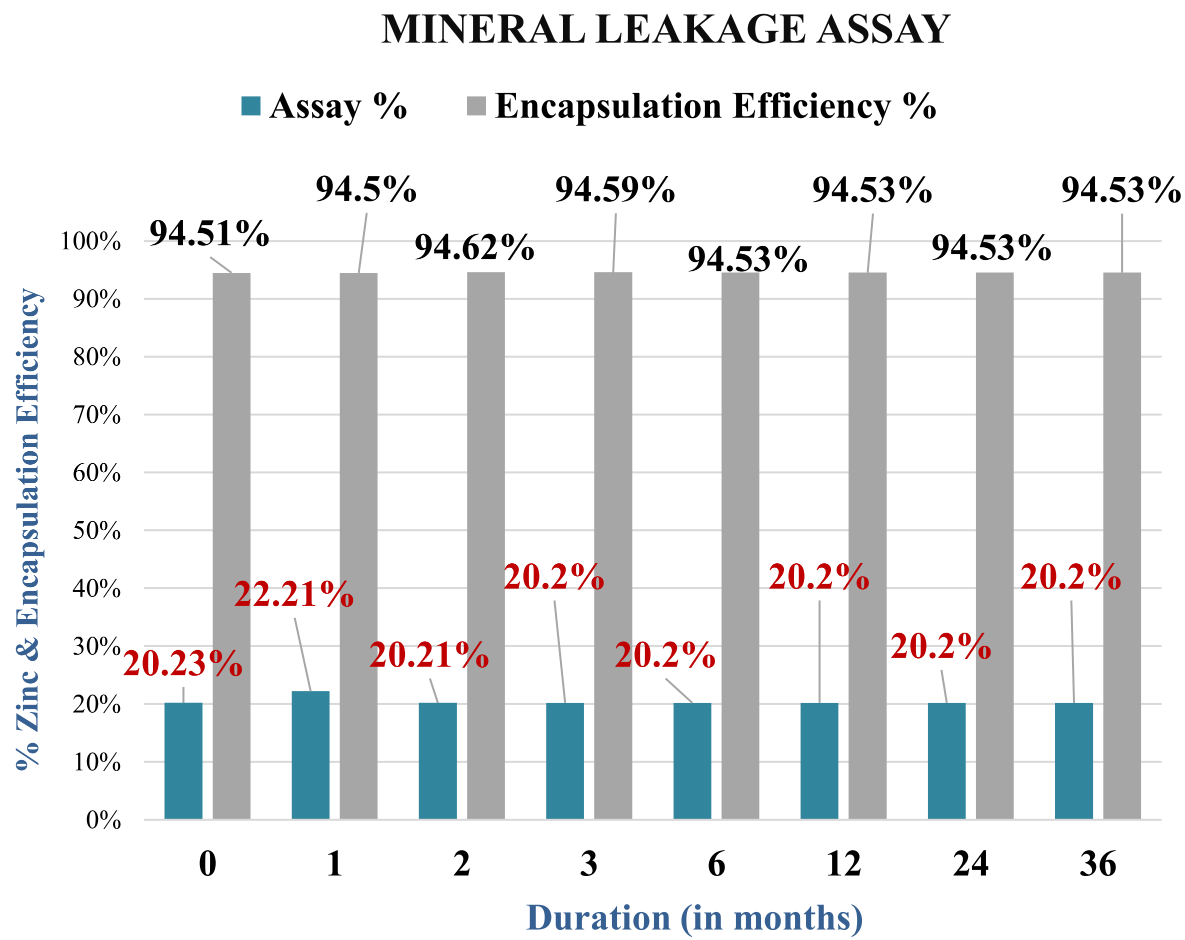


Figure 2 – Chart comparing the stability of Liposomal Zinc stored over a period of 6 months at 40°C ± 2 °C and a relative humidity of 75% ± 5%.

8. Stability of Zinc Liposomes at Elevated Temperatures

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

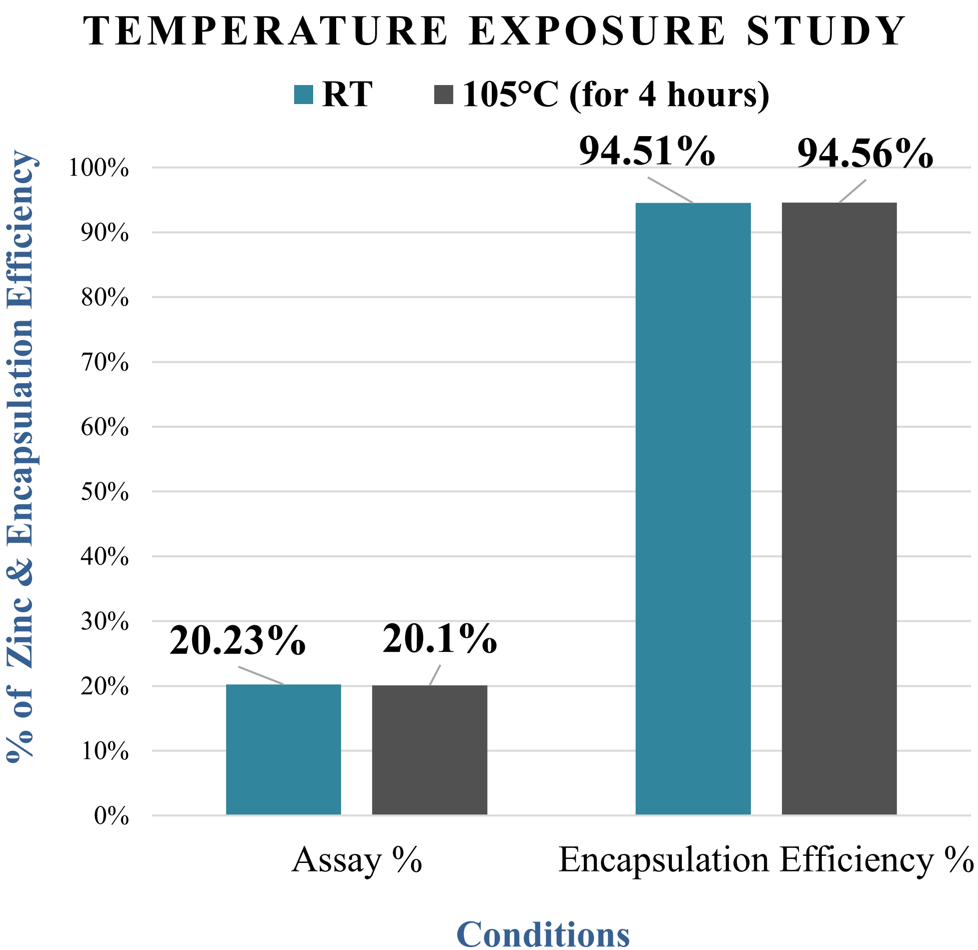
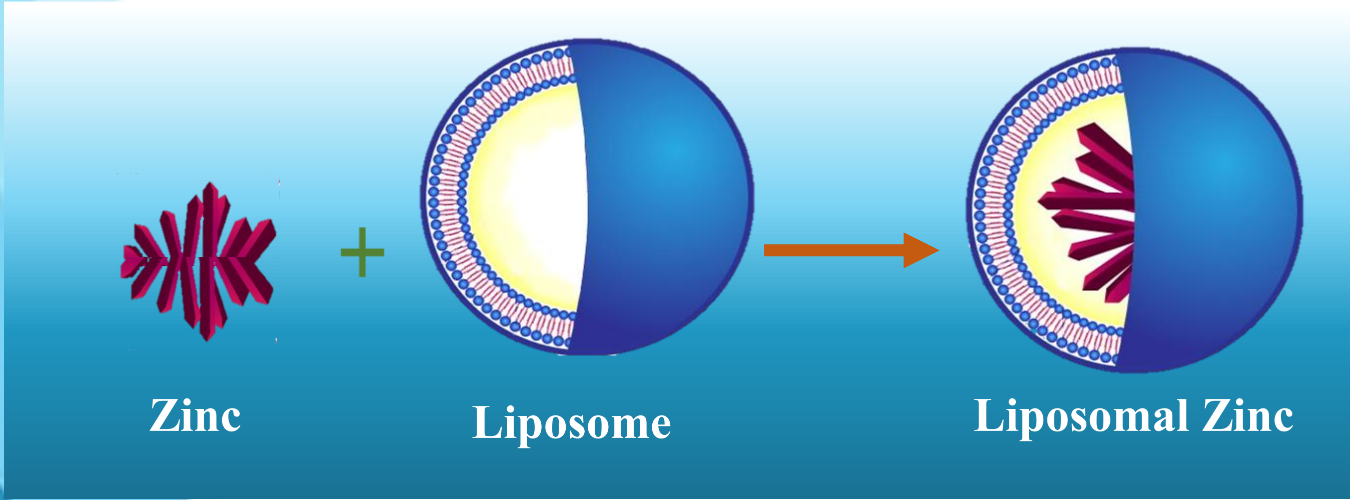


Figure 2 – Chart comparing the stability of Liposomal Zinc both at room temperature (RT) and at 105°C (exposure for 4 hours).

- **Encapsulation efficiency remains high (≈94%)** 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.

9. Mineral Loading Capacity



Formulation of Zinc in Liposomes

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

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

10. Particle Specifications

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

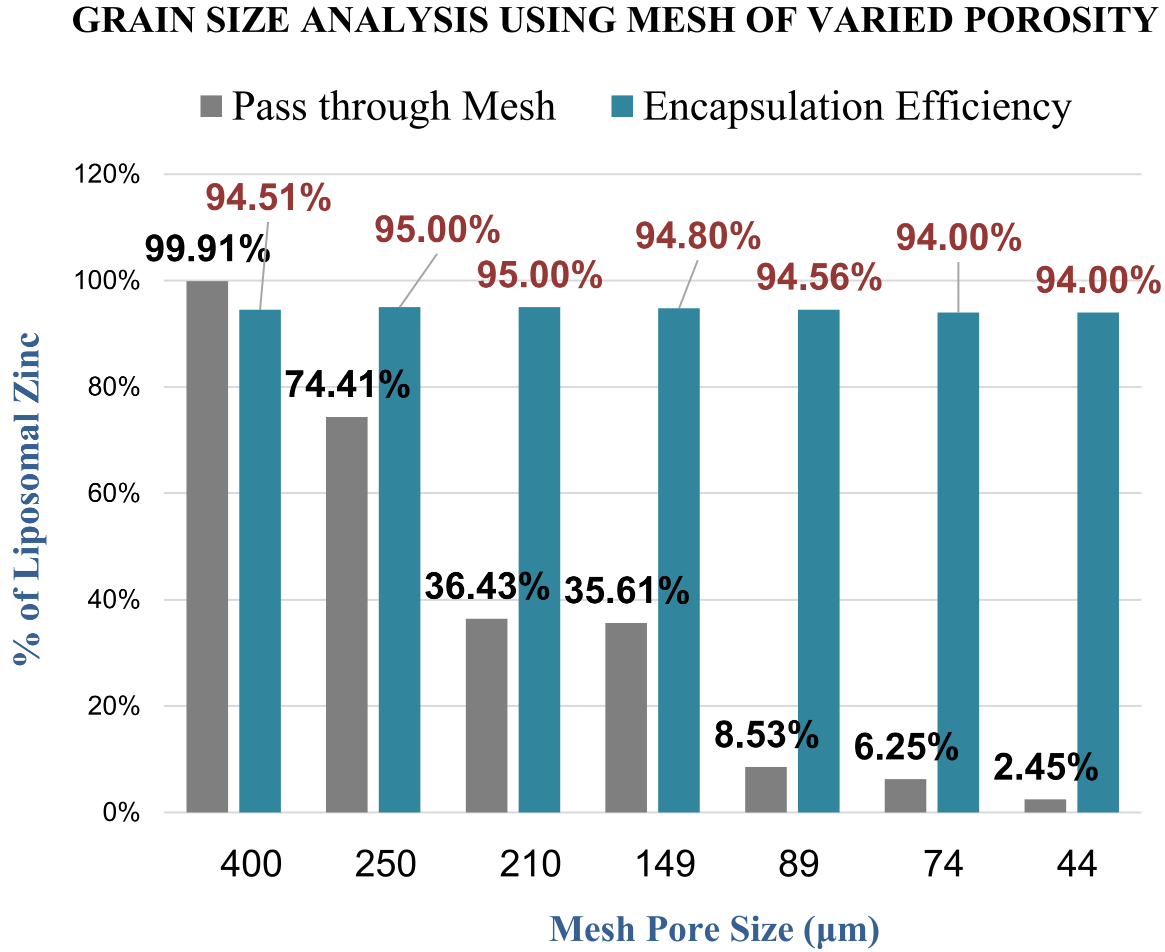


Figure 2 – Chart comparing the % of Liposomal Zinc products that can pass through mesh of varied porosity with their respective encapsulation efficiency percentages.

Thank You!!!

WEST BENGAL CHEMICAL INDUSTRIES LIMITED
(A Joint Venture with Government of West Bengal | A cGMP & ISO 9001 : 2015 Certified Company)
145/1, Jessore Road, Lake Town, Kolkata - 700 089, India.
wbcil@wbcil.com | www.wbcil.com | +91 (033) 4025 1555 / 1539