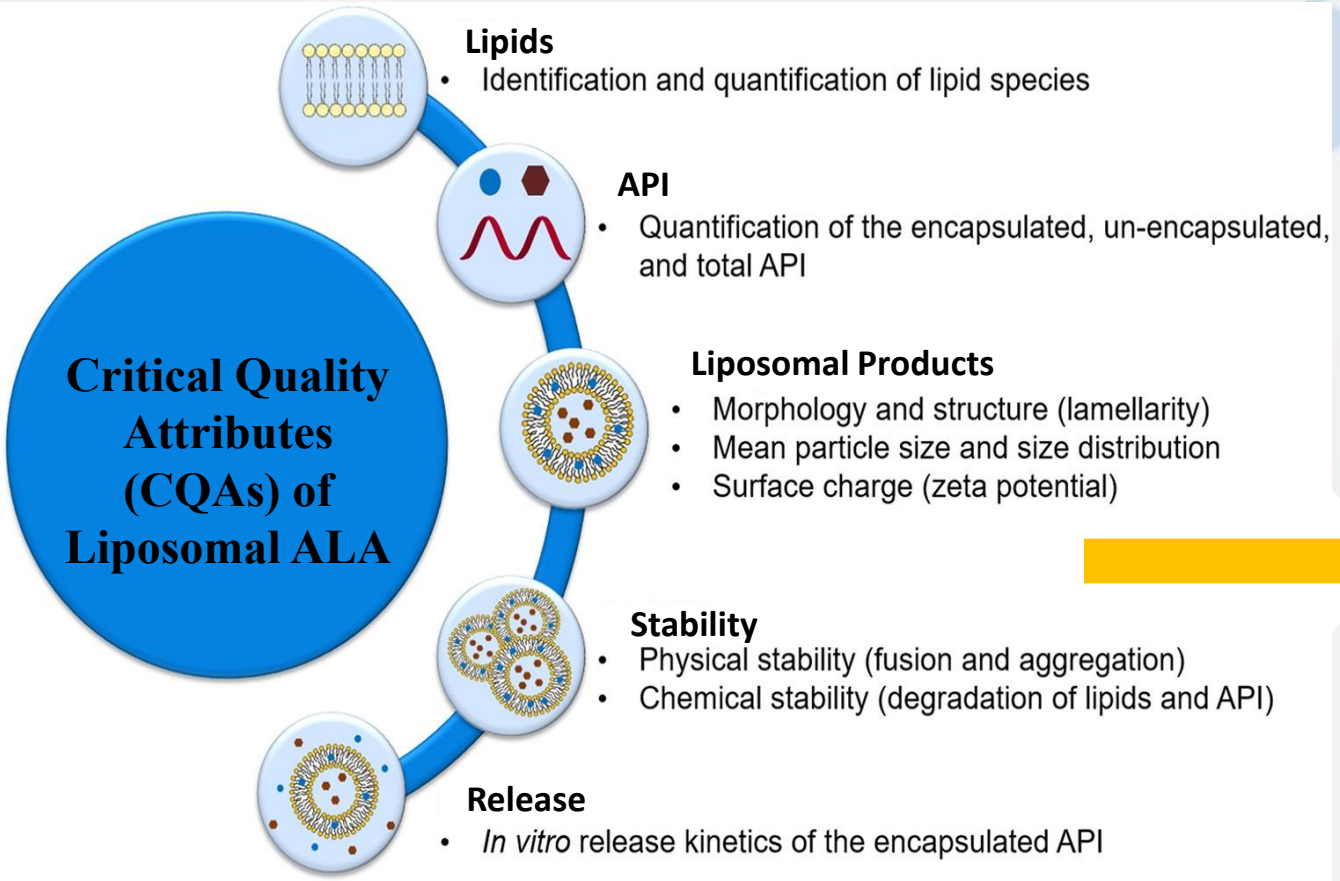
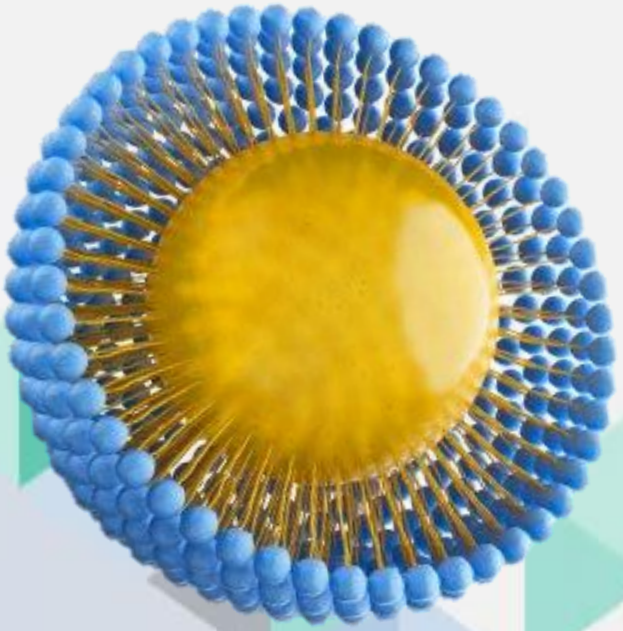
West Bengal Chemical Industries Limited

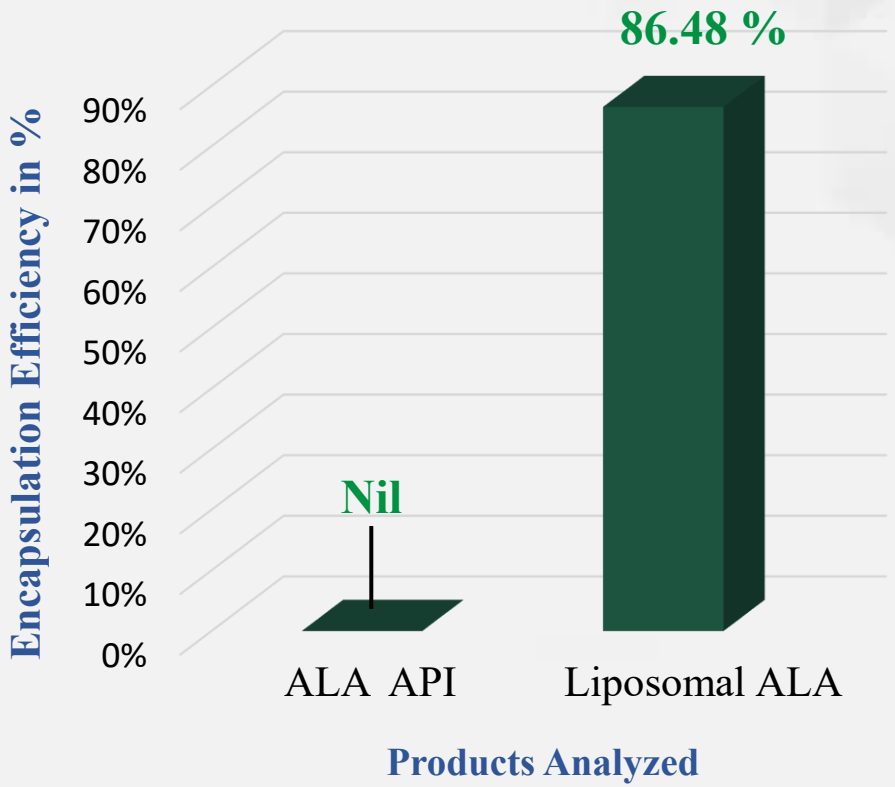
Summary of Characterizations Performed on Liposomal ALA



1. ***Encapsulation efficiency of Liposomal ALA***
2. ***Analysis of particle size and uniformity of Liposomal ALA using DLS***
3. ***Behavior of Liposomal ALA particles in liquid medium using DLS Zeta-sizer***
4. ***FTIR analysis of Liposomal ALA composition***
5. ***Elemental analysis of Liposomal ALA***
6. ***Morphology analysis of ALA Liposomes using SEM***
7. ***Analysis of ALA leakage from Liposomes***
8. ***Stability analysis of Liposomes at 105° C temperatures***
9. ***Endothermic study of Liposomal ALA using DSC analysis***
10. ***Thermogravimetric Analysis (TGA) of Liposomal Iron***
11. ***Correlation between DSC & TGA***
12. ***Mineral loading capacity***

1. Encapsulation Efficiency of 35.86% Liposomal ALA





- **Acceptance criteria:**
  - **Assay : NLT 30%**
  - **Encapsulation efficiency : NLT 70%**

Encapsulation Efficiency measured via validated HPLC data

- Liposomal encapsulation ensures **86.48% 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 ALA forms.

2. Dynamic Light Scattering Analysis of Liposomal ALA

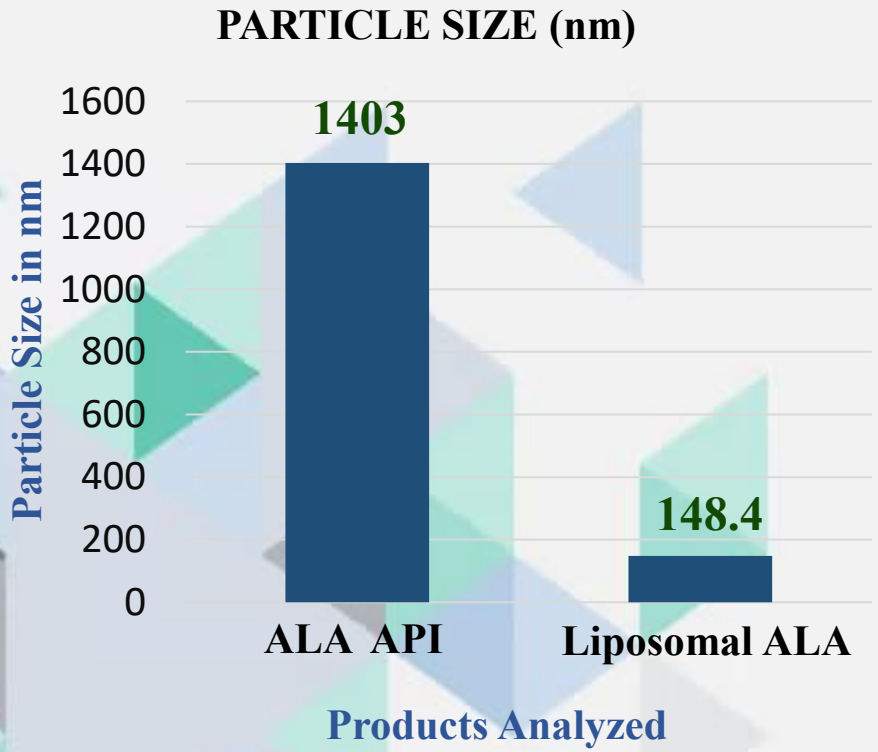


Figure 1 – Chart showing the particle size of ALA API with Liposomal ALA

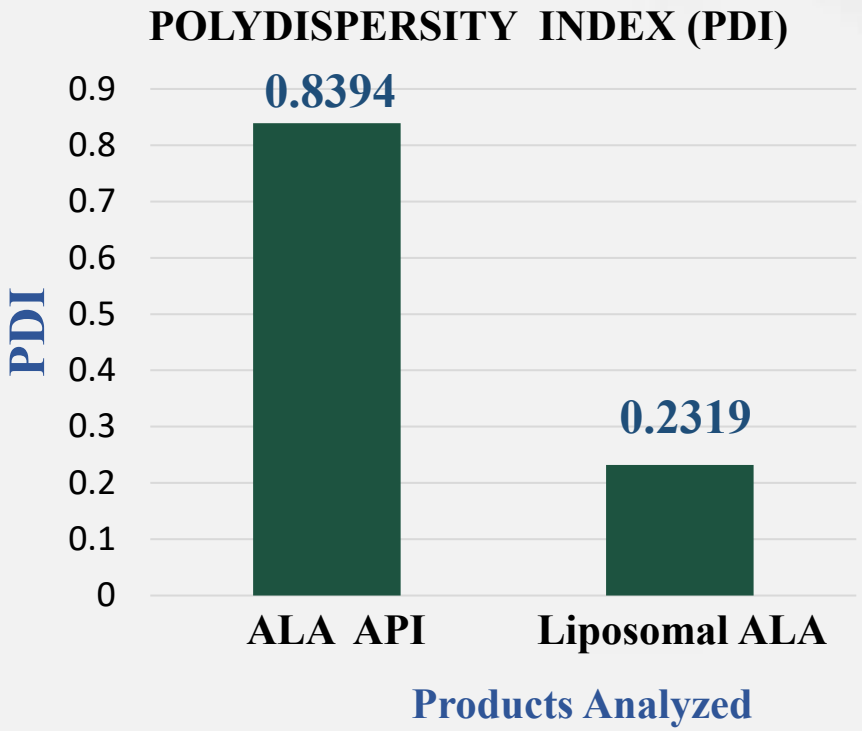


Figure 2 – Polydispersity Index (PDI) of Liposomal ALA in solution

- Nanosized, uniform particles offer greater colloidal stability and improved shelf life.
- Smaller particles (**Particle size: 148.4 and PDI 0.2319)** 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 ALA

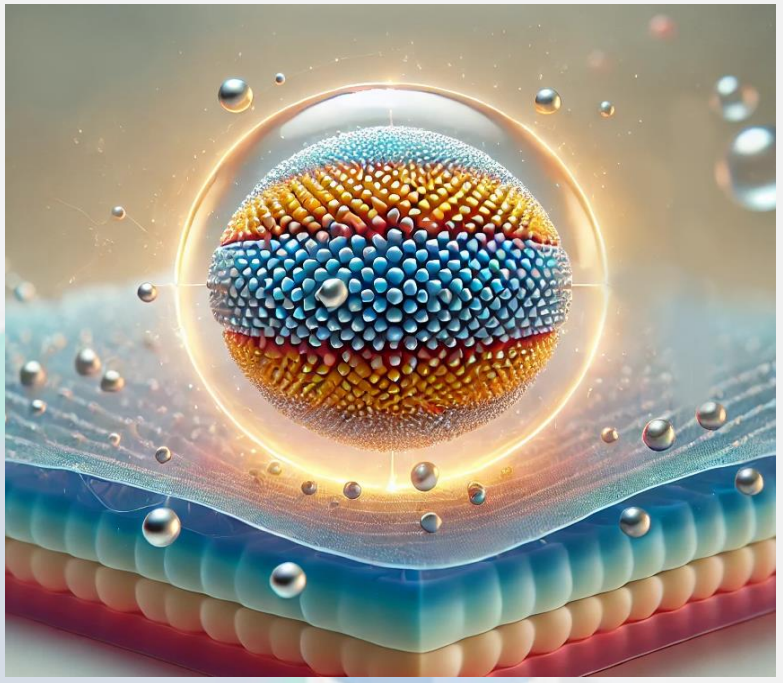


Figure 1 – Illustration of zeta potential, showing the electrostatic interactions of particles in suspension

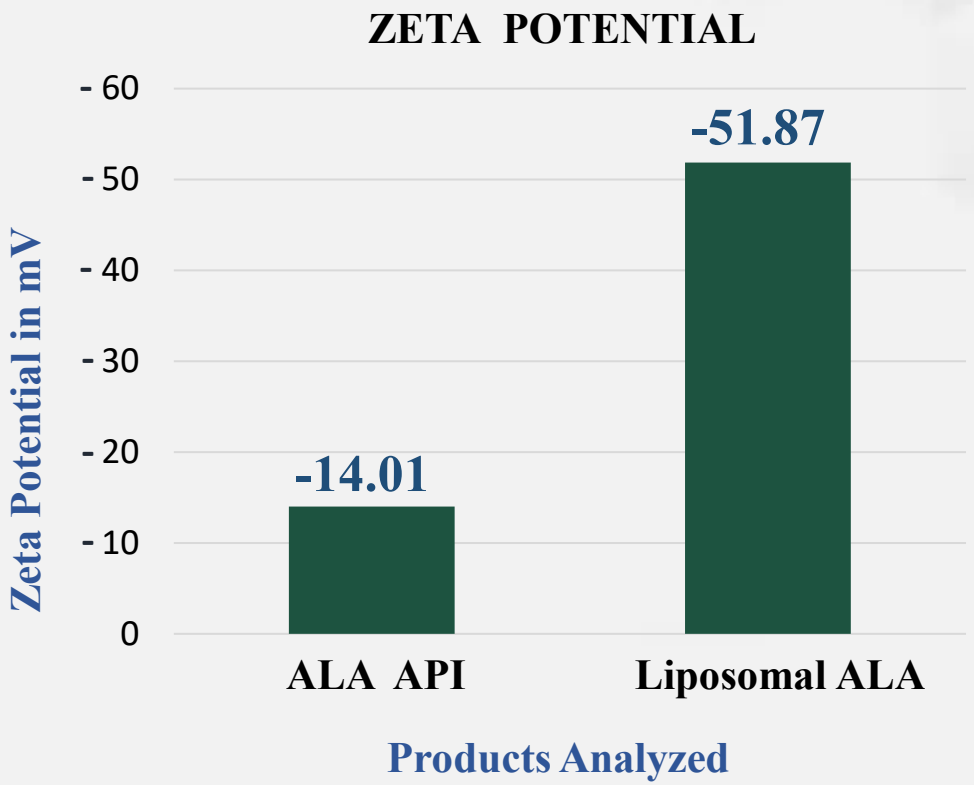


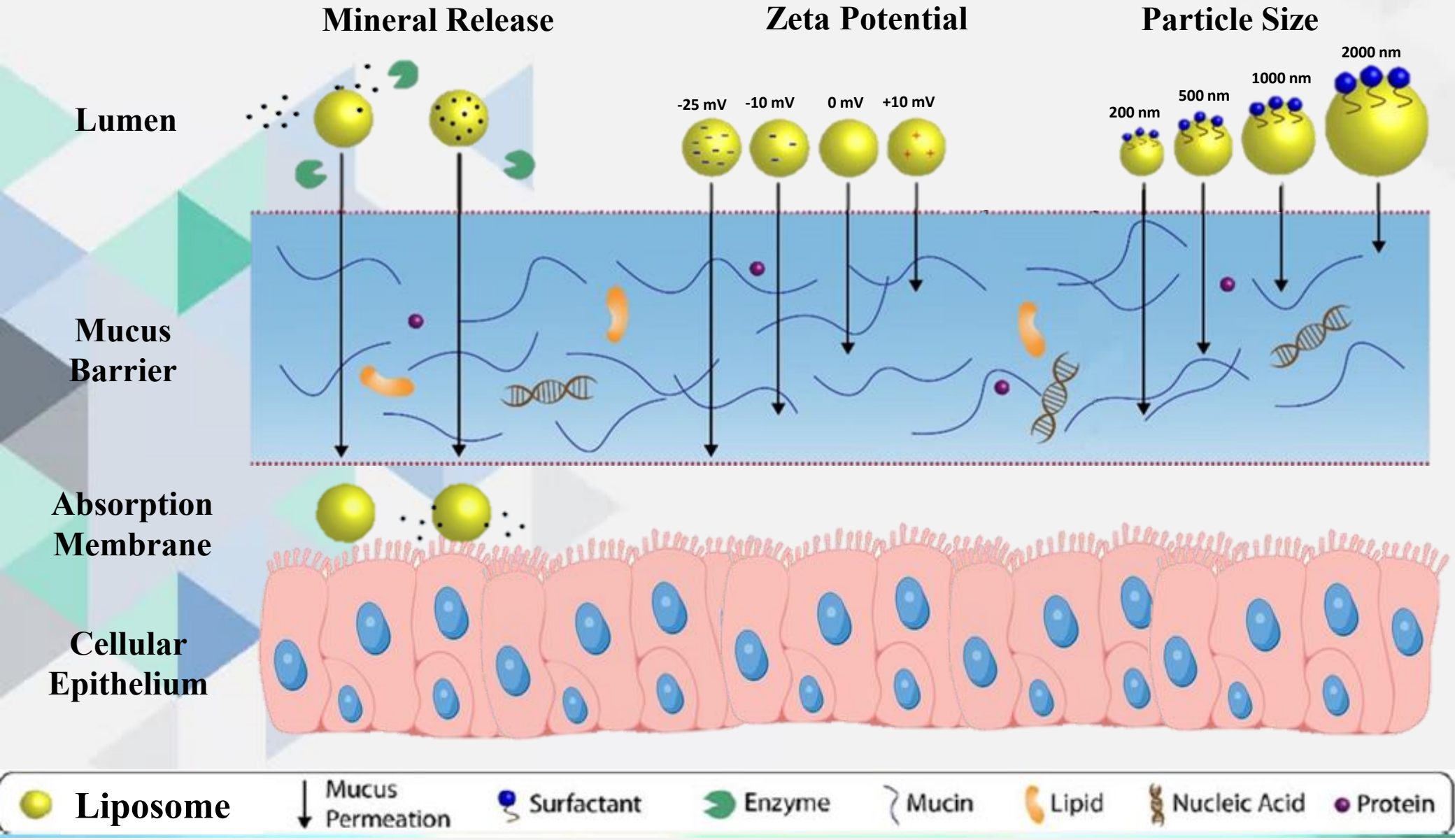
Figure 2 – Chart comparing the zeta potential of ALA API and Liposomal ALA showing ALA in Liposomal form is stable and unlikely to agglomerate in solution.

- Liposomal ALA shows **high zeta potential (-51.87 mV)** → excellent colloidal stability.
- Prevents particle aggregation → ensures **uniform suspension**.
- Enhances **product shelf life** and **bioavailability** in liquid form.

❖ **Acceptance criteria:**

➢ **Zeta Potential : < -30 mV**

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



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

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

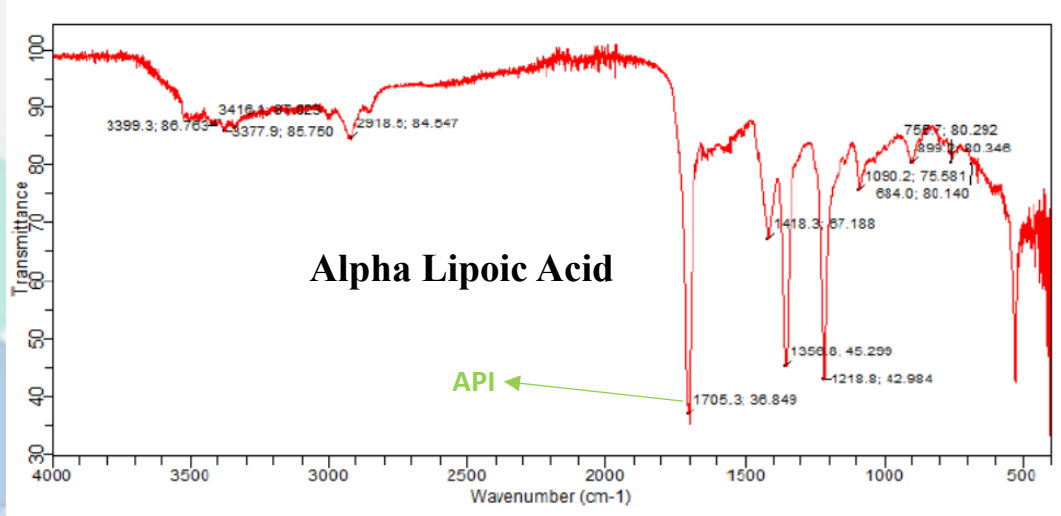


Figure 1: IR Transmission spectrum showing bands at different wavelengths of Alpha Lipoic Acid API

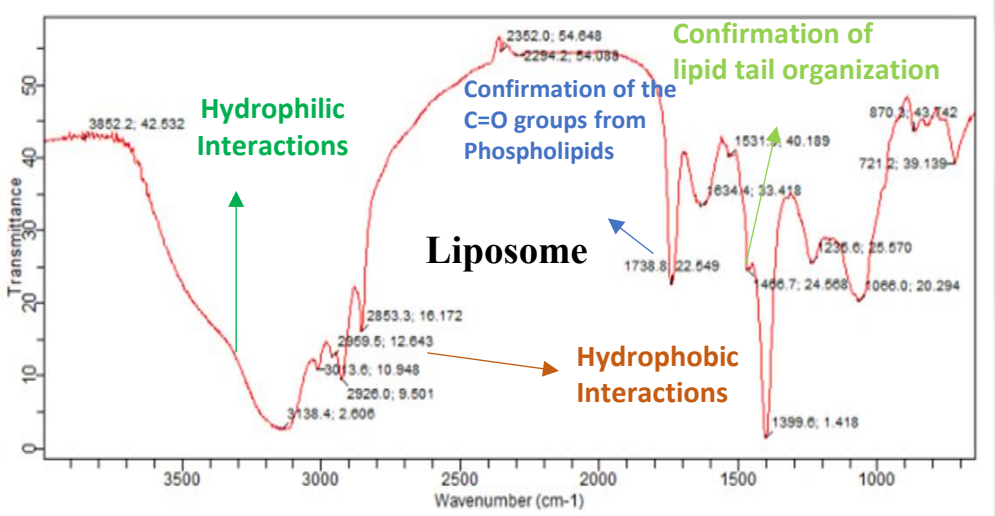


Figure 2: Hydrophobic and Hydrophilic interactions within Empty Liposome

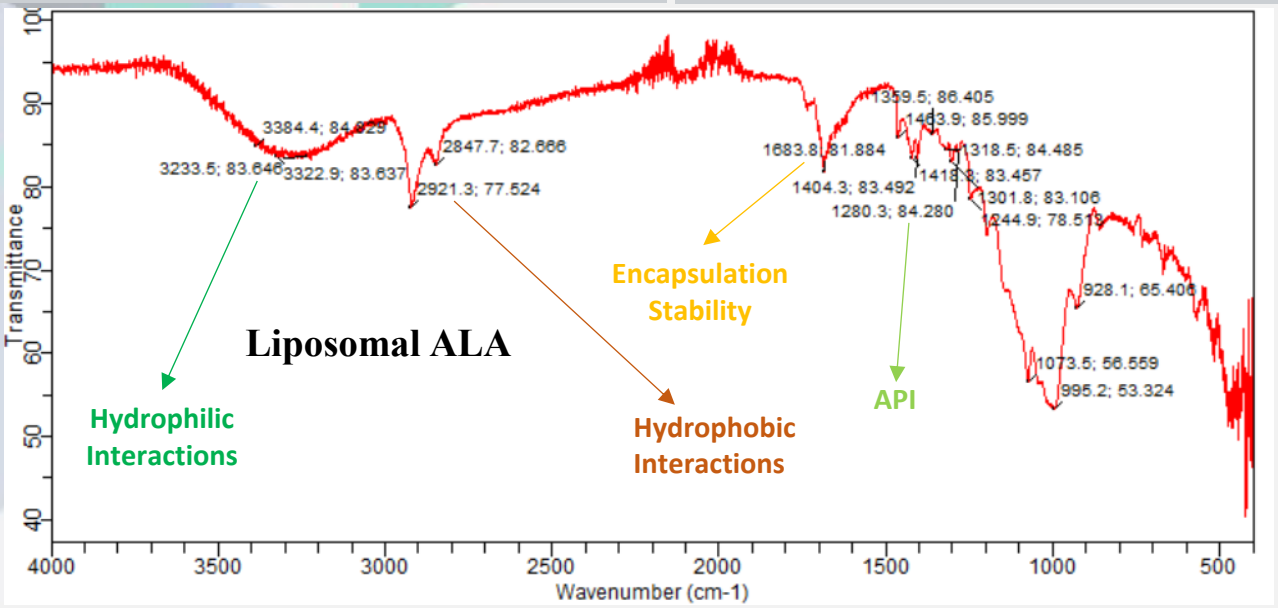


Figure 3: IR Transmission spectrum of Liposomal ALA is shown

4b. Summary of FTIR Analysis of Liposomal ALA

1. **Confirmation of the C=O and O-H Groups -** The C=O stretching at **1740.7 cm^{-1}** confirms the ester bond.

2. **Hydrophobic Interactions -** The C-H stretching at **2921.3 cm^{-1}** and **2847.7 cm^{-1}** reflect the hydrophobic interactions.

3. **Hydrophilic Interactions -** O-H stretching at **3233.5 cm^{-1}, 3322.9 cm^{-1}**, and **3384.4 cm^{-1}** suggest hydrogen bonding interactions between water or hydrophilic groups.

4. **Encapsulation Stability -** Peaks at **1683.8 cm^{-1}** and **1593.5 cm^{-1}**, corresponding to **C=O stretching** and amide bonds show complete encapsulation.

5. **API -** This peak corresponds to the **C-H bending at 1418.9 cm^{-1}** confirms presence of ALA.

5. Elemental Analysis of Liposomal ALA

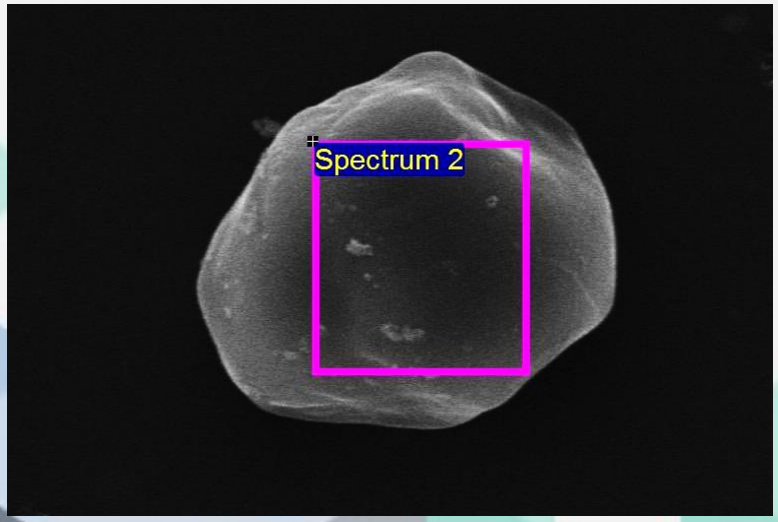


Figure 1 – SEM image of Liposomal ALA showing the area scanned using Energy Dispersive X-Ray Spectroscopy (EDAX)

- ALA API shows high carbon (74.60%) **and notable sulfur content (11.10%), consistent with its thiol structure**; sulfur is absent in liposomal ALA, indicating encapsulation.
- In liposomal ALA, increased oxygen (35.41%), nitrogen (17.70%), and phosphorus (0.52%) confirm the presence of phospholipids and successful encapsulation.

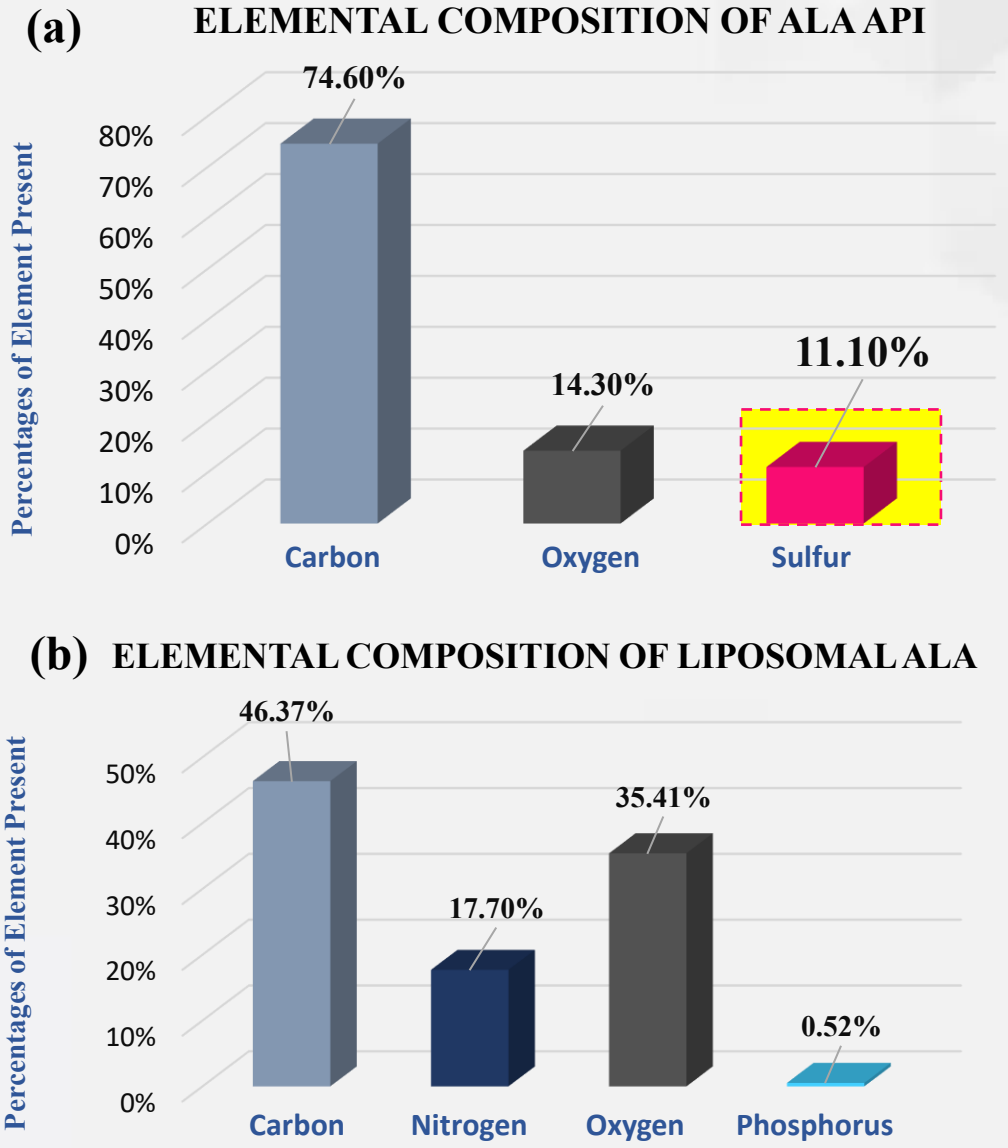


Figure 2 – A graphical representation of the percentages of elements composing (a) ALA API and (b) Liposomal ALA

6. Morphology of Liposomal ALA As Viewed Under a Scanning Electron Microscope

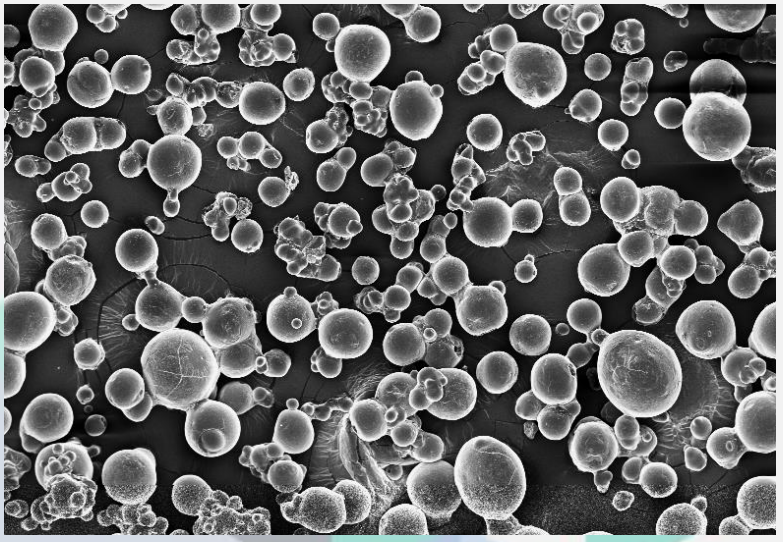


Figure 1 – SEM image of few Liposomal ALA scattered within the field of view under observation

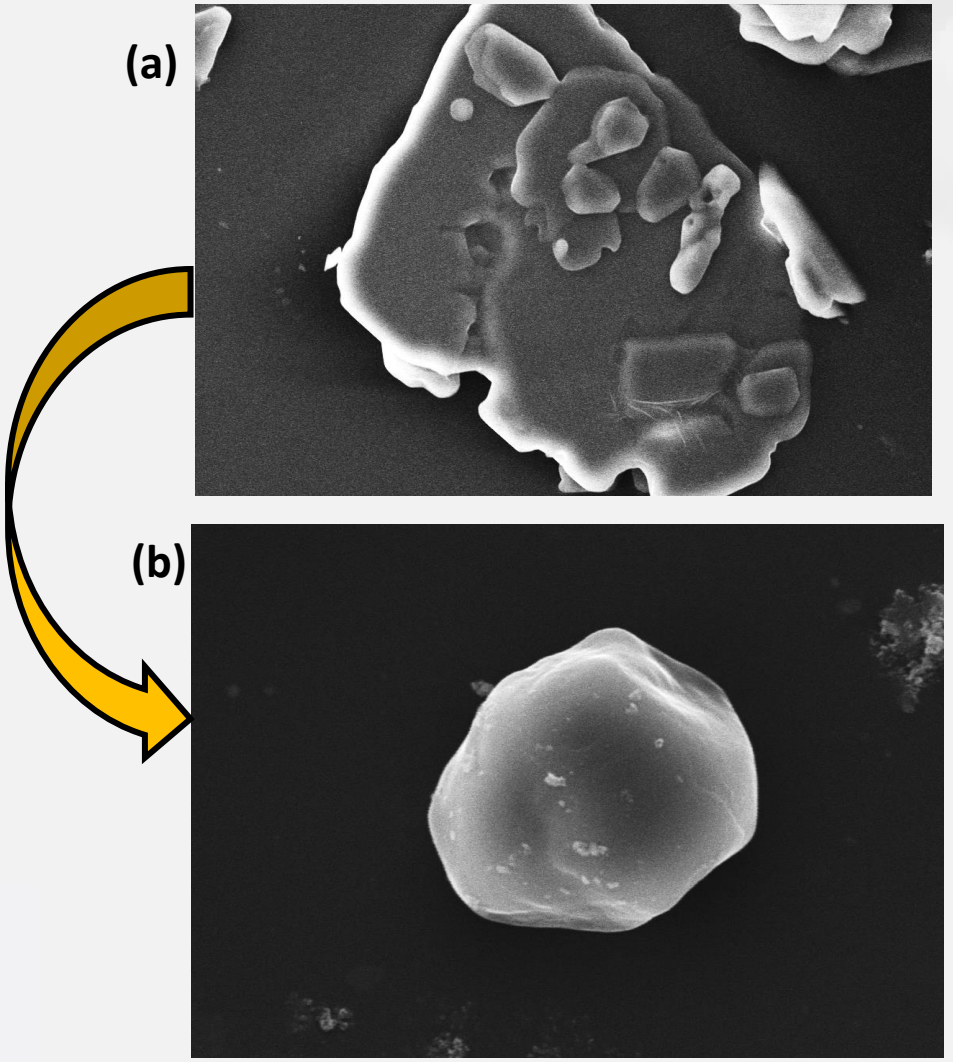


Figure 2 – SEM panels showing transformation from (a) ALA API to (b) Liposomal ALA after encapsulation.

- Spherical morphology observed in liposomal ALA 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 Liposomal ALA

Figure 1 – An image representing the storage of formulations in shelves

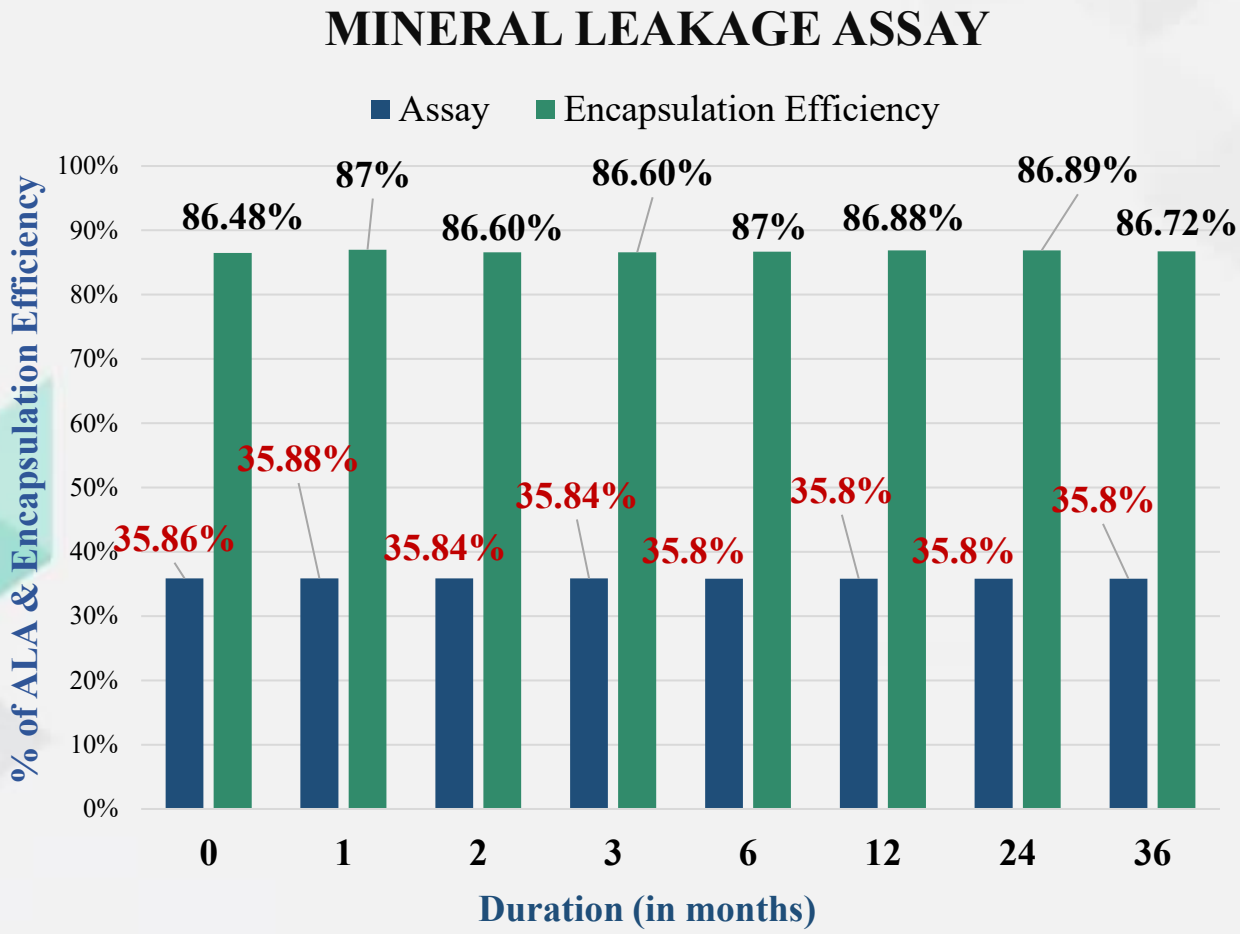


Figure 2 – Chart comparing the stability of Liposomal ALA stored over a period of 3 years at 40°C ± 2 °C and a relative humidity of 75% ± 5%.

- **Encapsulation efficiency remains high (~86%)** throughout 3 years of storage, indicating stable liposome structure.
- **Assay values for free ALA remain in a range (~38%)**, showing minimal leakage over time.
- The formulation shows **excellent retention of ALA**, confirming its suitability for long-term shelf storage.

8. Stability of Liposomal ALA at Elevated Temperatures

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

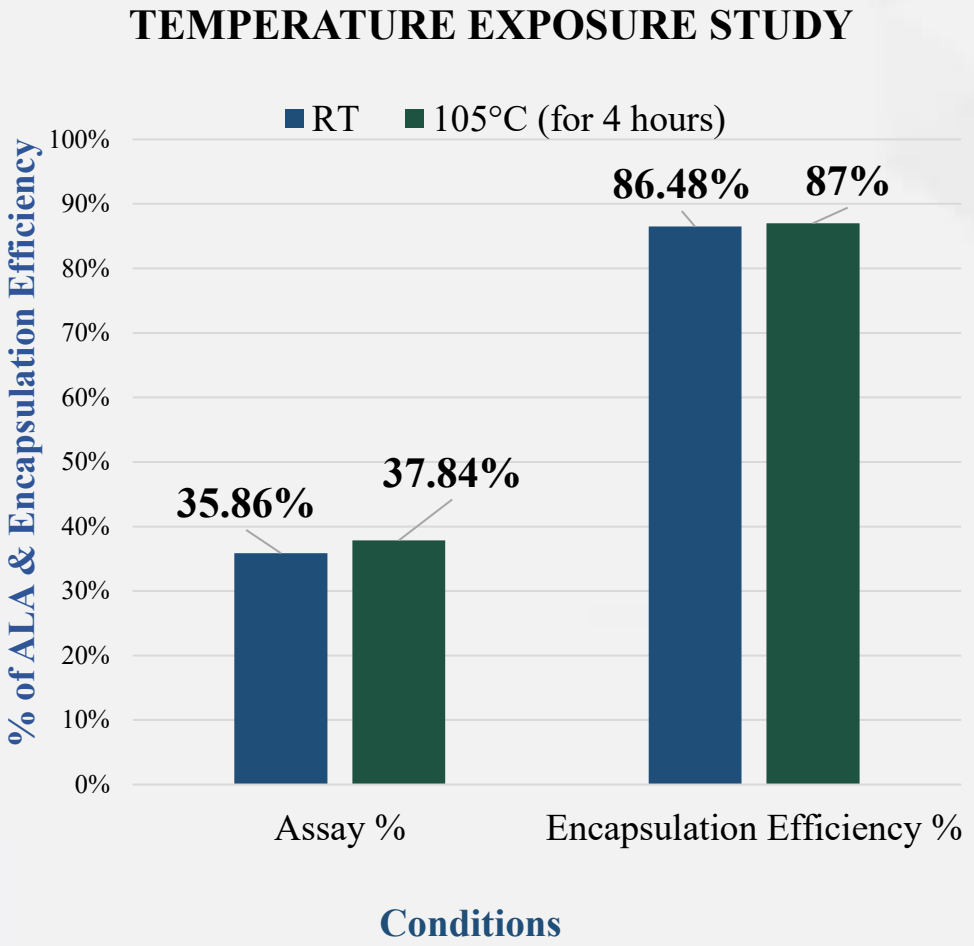


Figure 2 – Chart comparing the stability of Liposomal ALA both at room temperature (RT) and at 105°C for 4 hours of exposure.

- **Encapsulation efficiency remains high (≈86%)** even after exposure to 105°C for 4 hours.
- **Assay values (38% at RT vs. 37.79% 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. Endothermic study of Liposomal ALA using Differential Scanning Calorimetry Analysis

An illustration showing how the phospholipid bilayer is preventing the heat to ALA API from getting disintegrated due to the applied heat.

Sample	Thermal Events (°C)	Inference
ALA API	63.24, 284.32	Distinct thermal transitions indicate phase changes or melting points associated with ALA*.
Liposome	136.85, 212.78, 278.42	Exhibits multiple transitions related to phospholipid structural changes and thermal stability*.
Liposomal ALA	88.34, 275.05, 299.11	Thermal stability of ALA is increased due to encapsulation within liposome to 299.11°C, indicating a high stability of the formulation before degradation*.

**Thermograms available for reference*

10. Thermogravimetric Analysis of Liposomal ALA

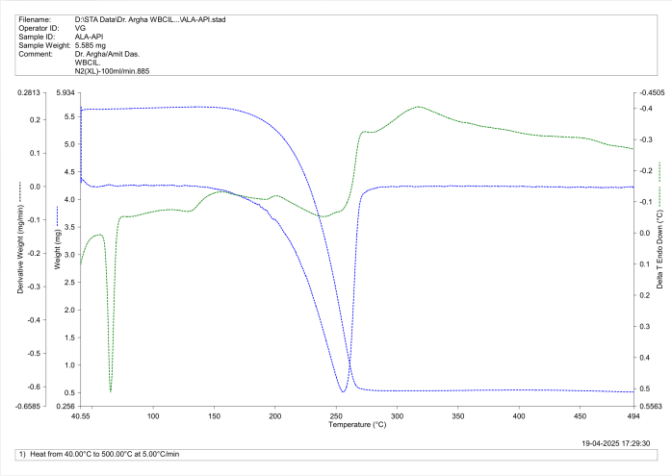


Figure 1 – A Thermogravimetric Analysis (TGA▬) and Differential Thermogravimetric Analysis (DTA - -) plots showing mass loss pattern from iron API upon application of heat up to 830°C.

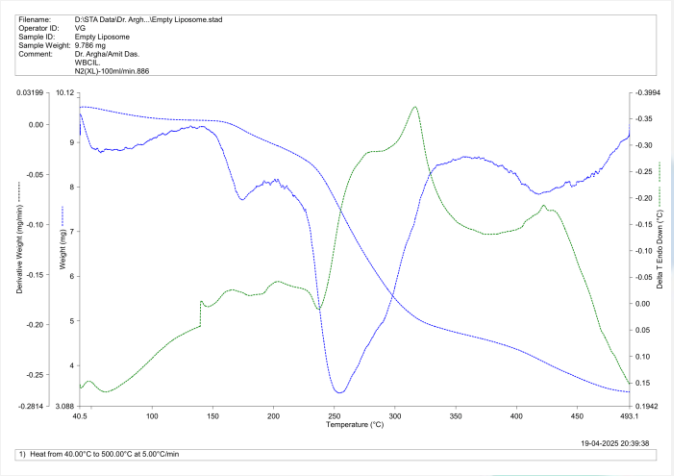


Figure 1 – A Thermogravimetric Analysis (TGA▬) and Differential Thermogravimetric Analysis (DTA - -) plots showing mass loss pattern from empty Liposomes upon application of heat up to 830°C.

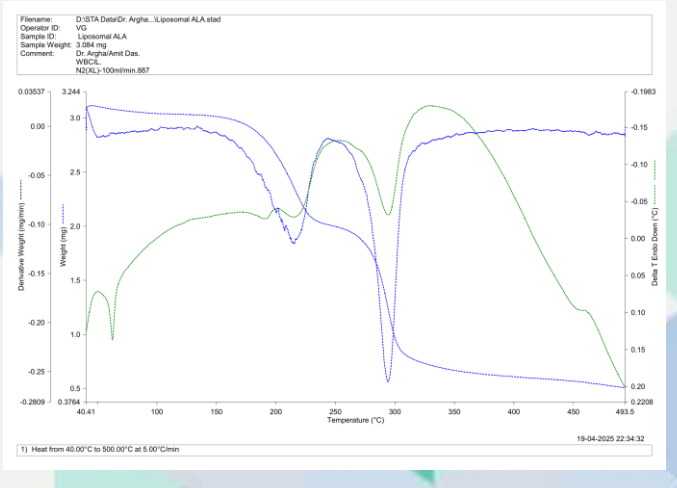


Figure 2 – Here mass loss analysis from (TGA ▬) and (DTA----) plots showing mass loss pattern from Liposomal Iron upon identical levels on heat application.

Sample	Total Weight Loss (%)	Major Degradation Temperature (°C)
ALA API	90-95 (near-complete decomposition)	150-200 (sharp initial loss due to instability)
Liposome	70-80 (gradual organic loss)	200-250 (slow lipid bilayer breakdown)
Liposomal ALA	80-85 (controlled and efficient loss)	200-250 (with early ALA phase, offering protective stability)

The total weight loss of Liposomal ALA (80-85%) is more controlled and efficient compared to ALA API (90-95%) and Liposome (70-80%), indicating better stability.

The major degradation temperature of Liposomal ALA (200-250°C with an early ALA contribution) is optimized for a protective effect, surpassing the lower threshold of ALA API (150-200°C) and the gradual loss of Liposome (200-250°C), highlighting its superior thermal resilience.

11. Correlating DSC and TGA of Liposomal ALA

Parameter	Analysis purpose	Analogy
DSC (Differential Scanning Calorimetry)	Measuring how much heat a material absorbs or releases as it's heated	Burning a log and seeing how much ash is left.
TGA (Thermogravimetric Analysis)	Checking if the material is losing weight as it's heated — from things like evaporation or breakdown.	Melting chocolate and measuring how much heat is needed.

🔗 Why Correlation Matters:

If both DSC and TGA respond at the same temperature, it means a real change is happening — both inside the material (DSC) and on the outside (TGA).

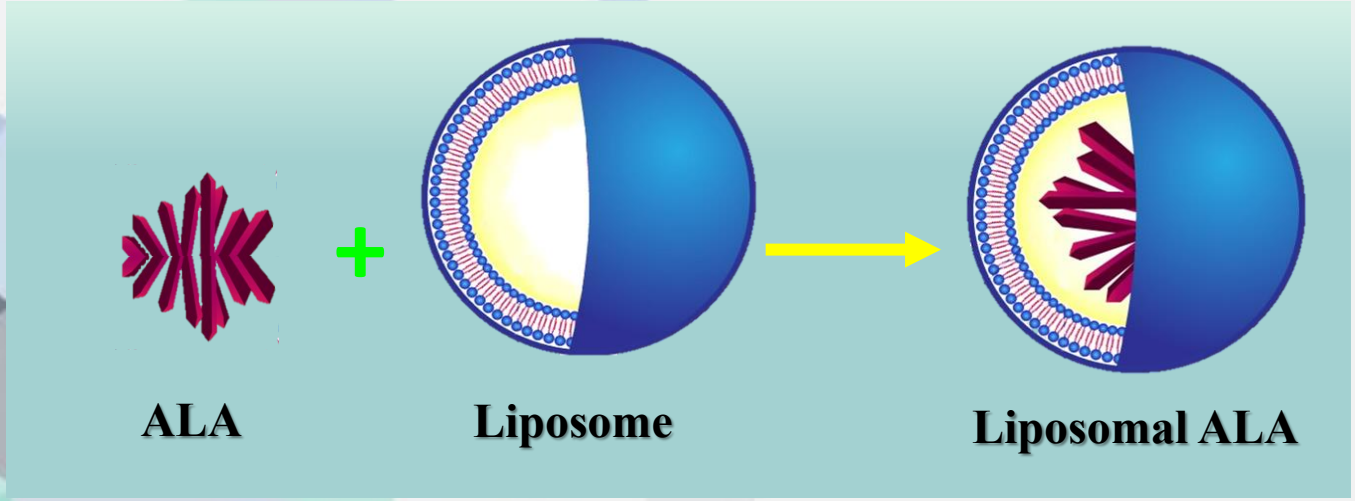
The material is **physically changing** (e.g., losing water or breaking down)

And it's also **thermally active** (taking in or releasing energy)

Correlation Summary

Temp (°C)	DSC Observation	TGA Observation	Correlation Interpretation	
63.24	Clear DSC Peak	No significant mass loss	🔶 Phase transition associated with ALA (e.g., polymorphic or melting transition)	ALA API
284.32	Yes	Yes (~200–330°C, 8.28% mass loss)	✅ Strong correlation — main decomposition of ALA	
136.85	Yes	Not clearly observable	🔶 Lipid phase transition or structural rearrangement	Empty Liposome
212.78	Yes	Minor weight change (~200°C)	✅ Partial correlation — initial thermal degradation onset	
275.05	Yes	Yes (~150–300°C, 8.34% mass loss)	✅ Correlates with multi-stage decomposition	Liposomal ALA
299.11	Yes	Yes (~300°C tail of decomposition)	✅ Final decomposition phase; enhanced thermal stability via encapsulation	
88.34	Yes	Possibly minor loss (~80–100°C)	✅ Likely moisture or loosely bound water loss	

12. Mineral Loading Capacity



Formulation of ALA in Liposomes

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

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

Thank You !

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