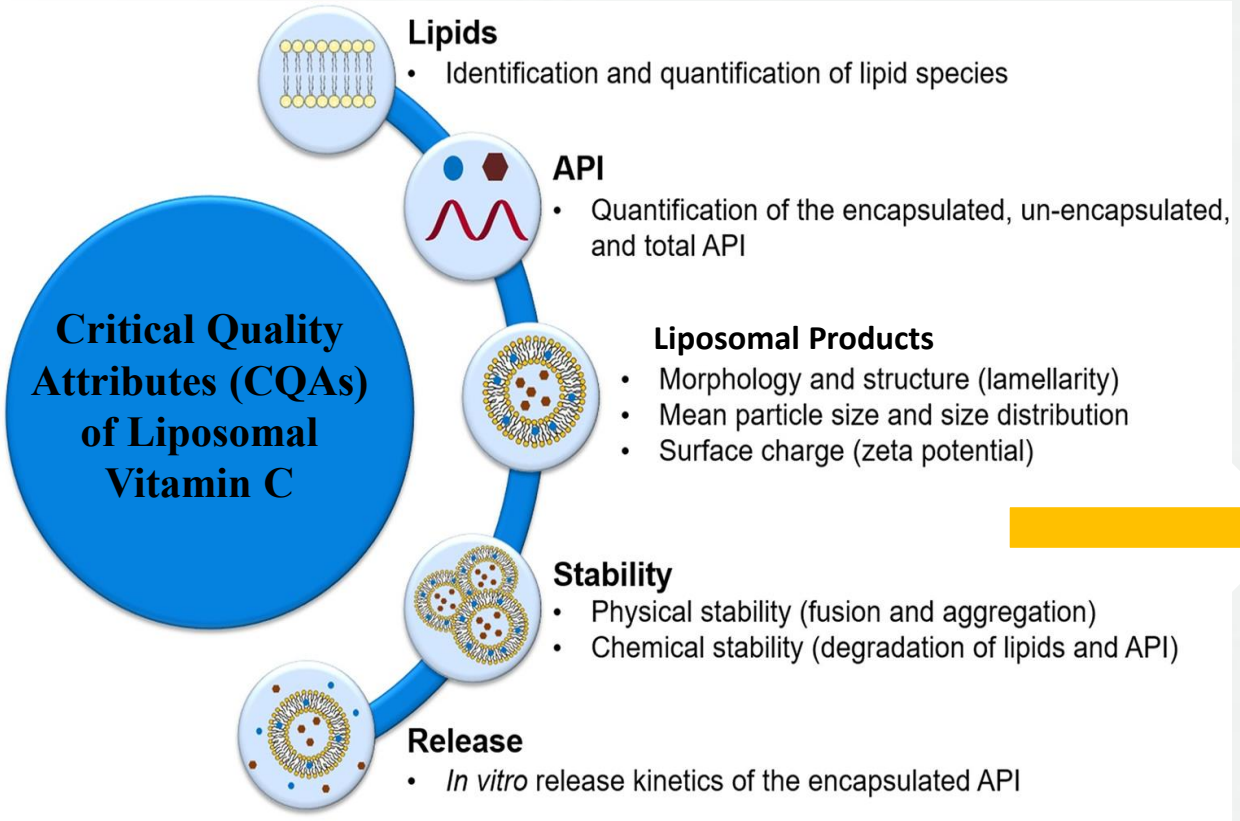
West Bengal Chemical Industries Limited

Summary of Characterizations Performed on Liposomal Glutathione



1. ***Encapsulation efficiency of Liposomal Glutathione***
2. ***Analysis of particle size and uniformity of Liposomal Glutathione using DLS***
3. ***Behavior of Liposomal Glutathione particles in liquid medium using DLS Zeta-sizer***
4. ***FTIR analysis of Liposomal Glutathione composition***
5. ***Elemental analysis of Liposomal Glutathione***
6. ***Morphology analysis of Liposomal Glutathione using SEM***
7. ***Analysis of leakage from Liposomal Glutathione***
8. ***Stability analysis of Liposomal Glutathione at 105^{o} C temperatures***
9. ***Mineral Loading Capacity***

1. Encapsulation Efficiency of 92.23% Liposomal Glutathione

- **Acceptance criteria:**
  - **Assay : NLT 90%**
  - **Encapsulation efficiency : NLT 70%**

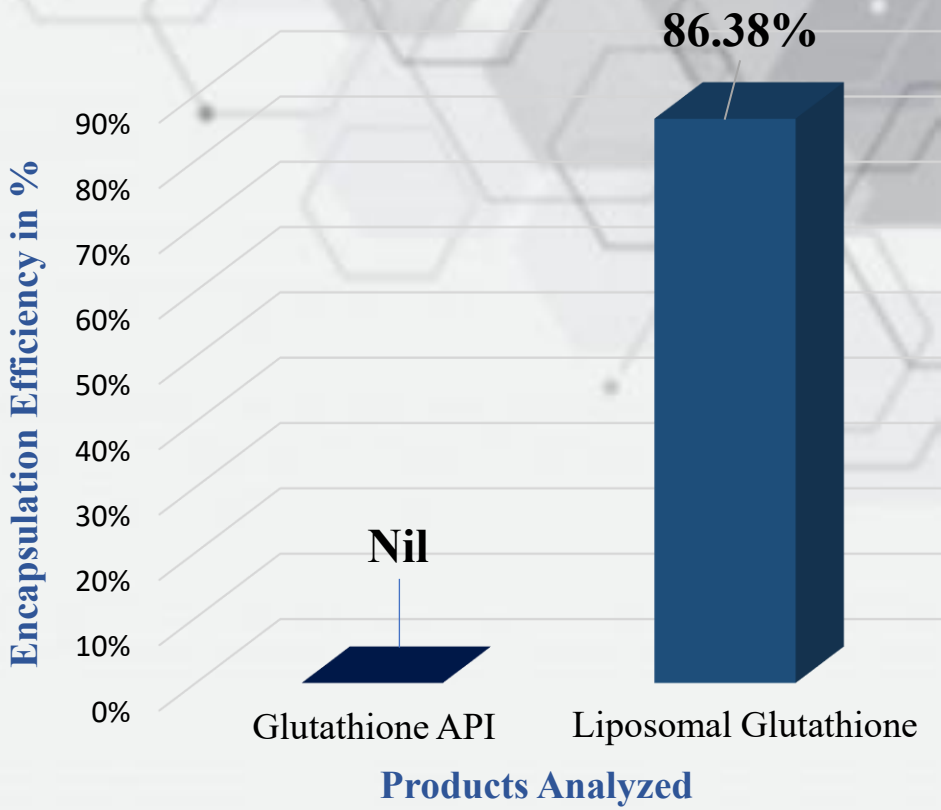


Figure 2 – Encapsulation Efficiency measured by validated HPLC analytical data

- Liposomal encapsulation ensures **86.38% 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 Glutathione forms.

2. Dynamic Light Scattering Analysis of Liposomal Glutathione

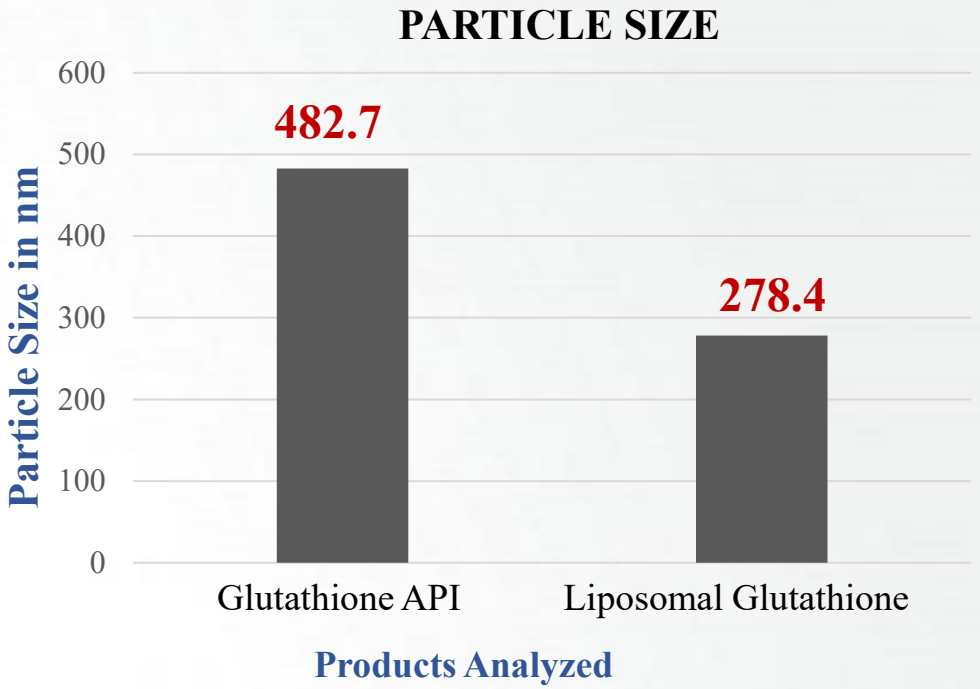


Figure 1 – Chart comparing the particle size of Glutathione API with Liposomal Glutathione

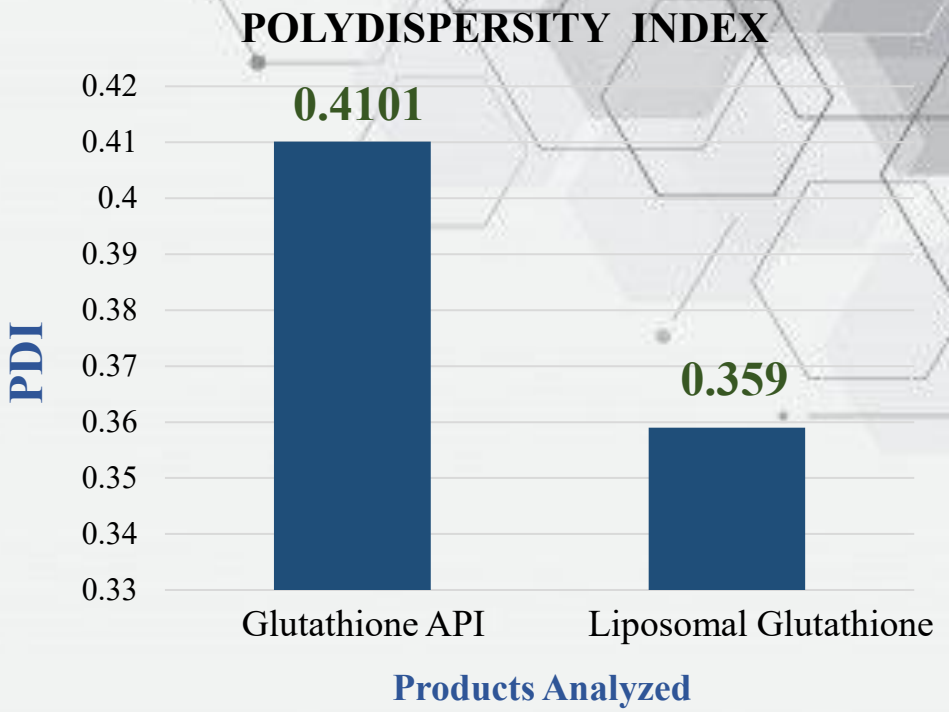


Figure 2 – Polydispersity Index (PDI) of Liposomal Glutathione in solution

- Nanosized, uniform particles offer greater colloidal stability and improved shelf life.
- Smaller particles (**Particle size: 278.4** and **PDI 0.359)** 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 Glutathione

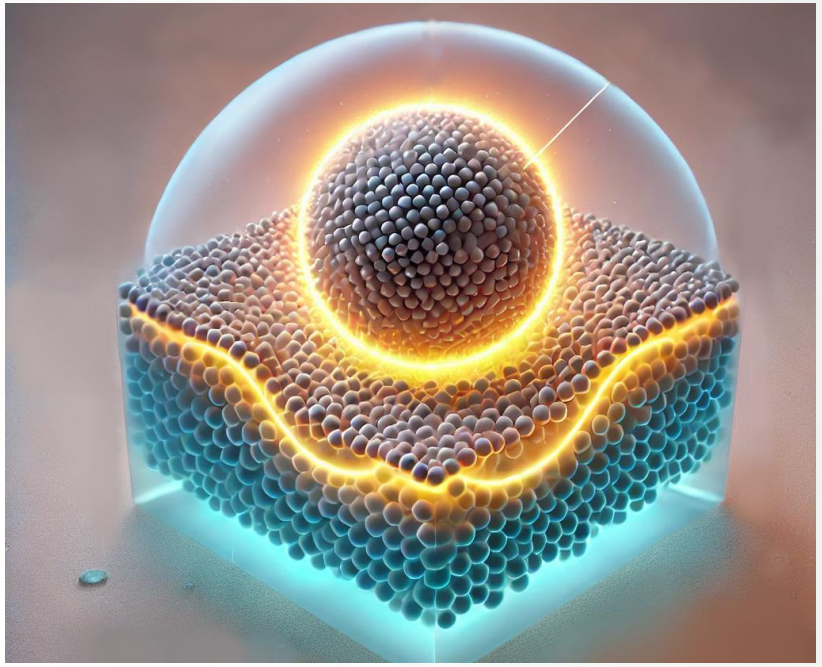


Figure 1 – Zeta potential visualization showing a negatively charged particle suspended in a liquid solution, surrounded by a well-defined electric double layer of positively charged ions.

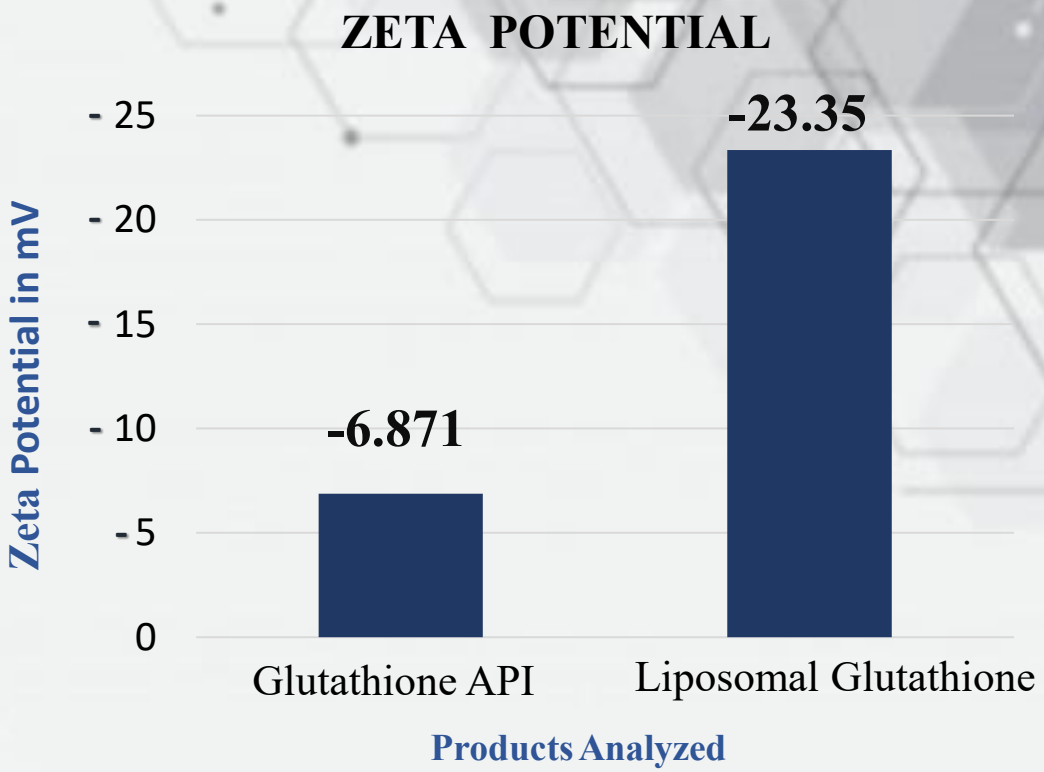


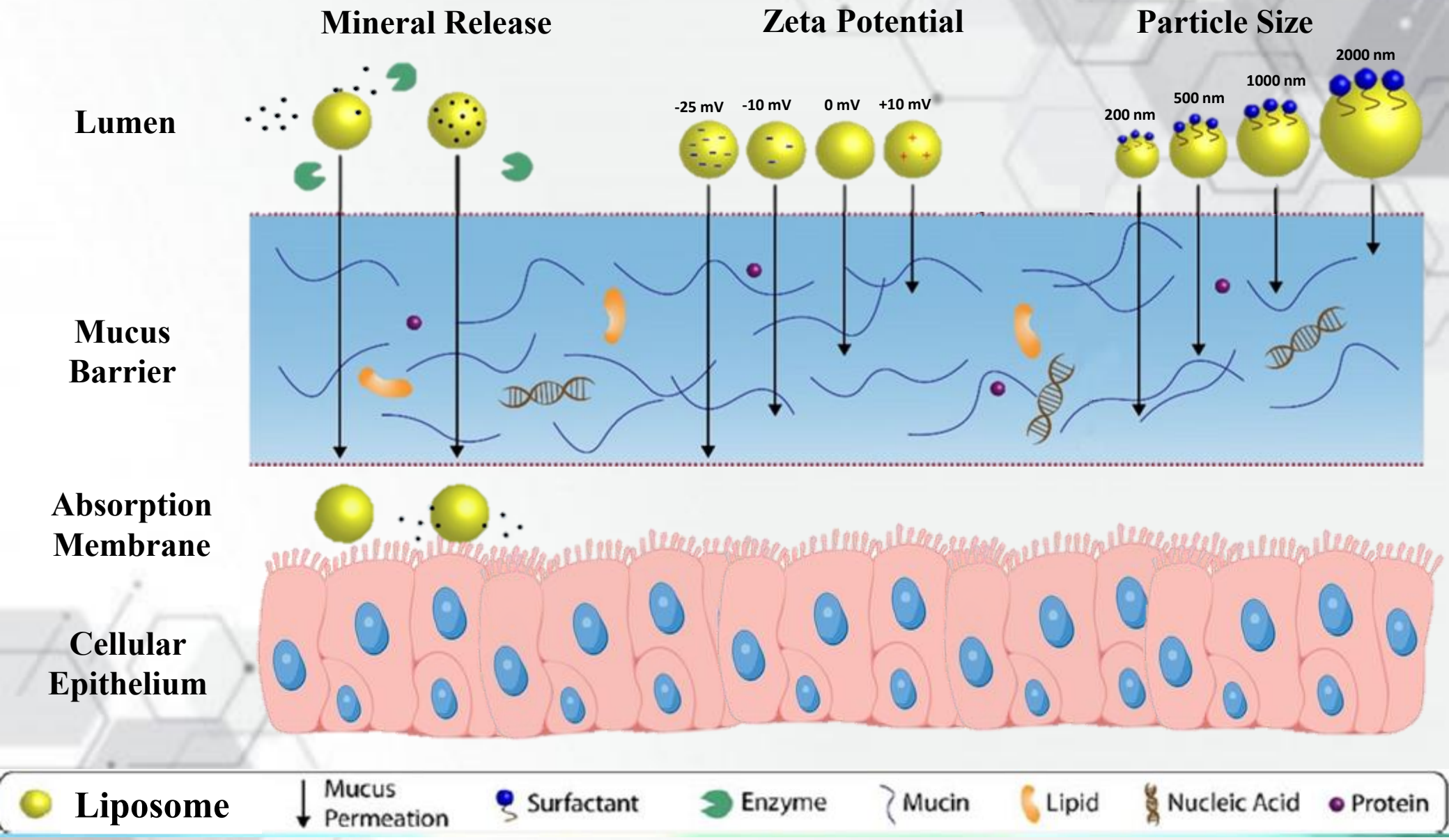
Figure 2 – Chart comparing the zeta potential of Glutathione API and Liposomal Glutathione showing Glutathione in Liposomal form is stable and less prone to agglomerate in solution.

- Liposomal Glutathione shows **high zeta potential (-23.35 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 Glutathione 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 Glutathione

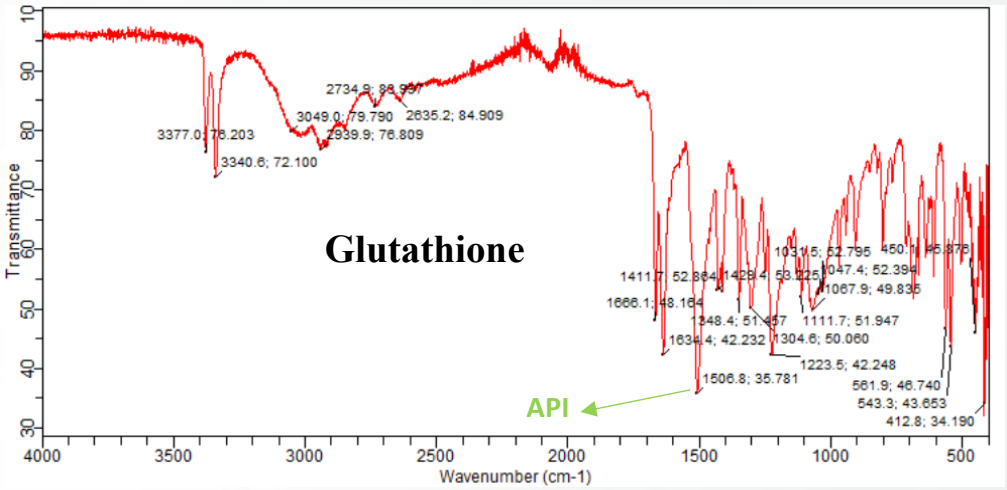


Figure 1: IR Transmission spectrum showing bands at different wavelengths of Glutathione API

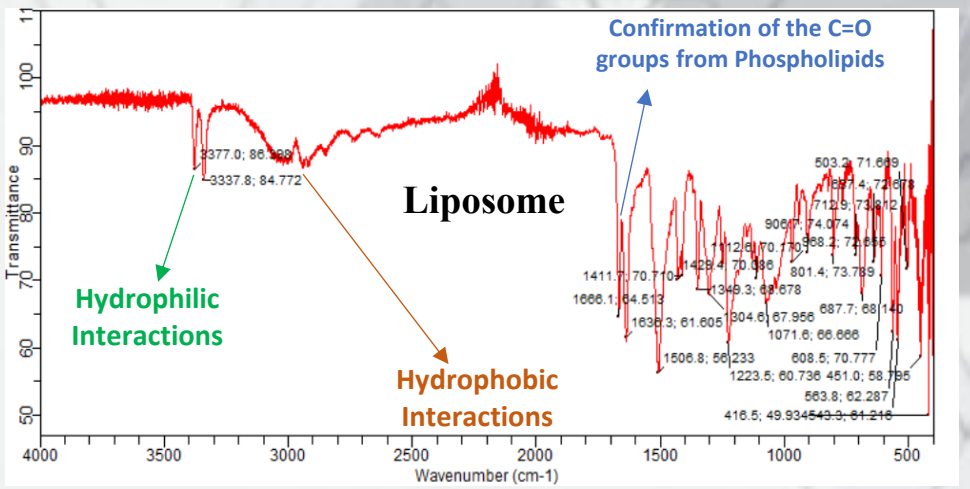


Figure 2: Hydrophobic and Hydrophilic interactions within Empty Liposome

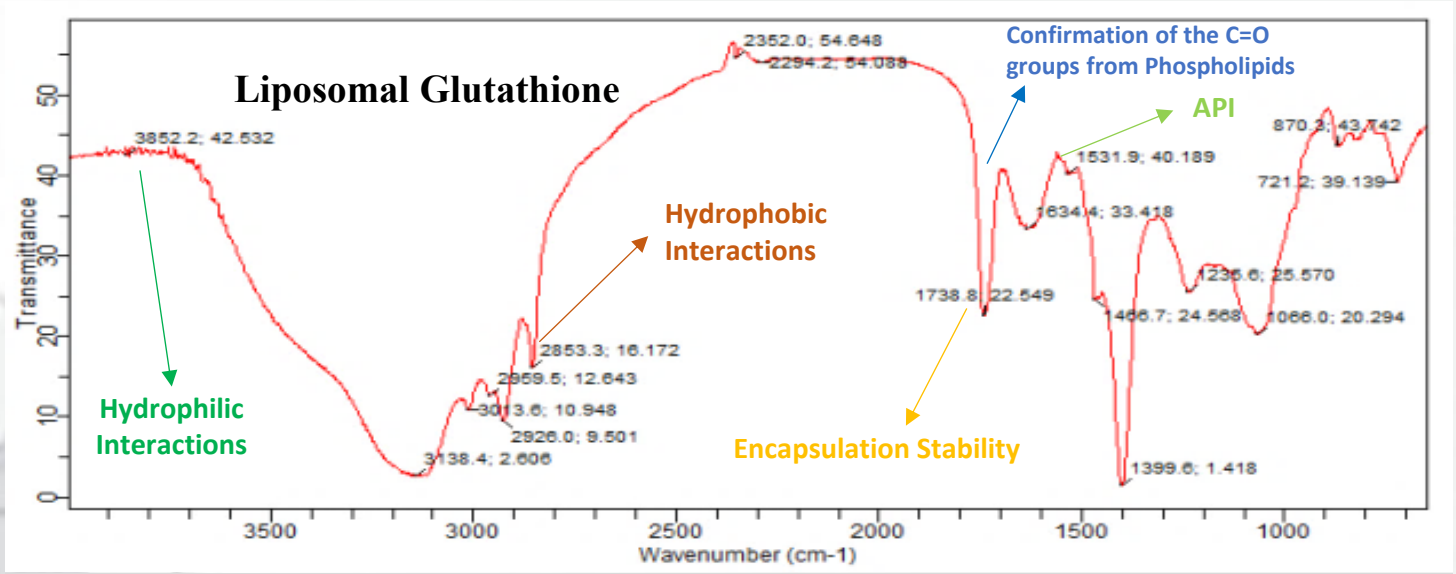


Figure 3: IR Transmission spectrum of Liposomal Glutathione is shown

4b. Summary of FTIR Analysis of Liposomal Glutathione

1. **Confirmation of the C=O and O-H Groups -** Ester carbonyl stretching peak at 1738.8 cm^{-1}, and broad O-H stretching peak near 3600 cm^{-1} confirms the ester bond.

2. **Hydrophobic Interaction -** C-H stretching vibrations of aliphatic –CH2– groups near 2853 cm^{-1} reflects the hydrophobic interactions within the lipid bilayer of the liposome.

3. **Hydrophilic Interaction -** Broad peak at ~3852 cm^{-1} corresponds to O-H stretching vibrations, indicating significant hydrophilic interactions through hydrogen bonding within the liposomal glutathione system.

4. **Encapsulation Stability -** Peak at 1738.8 cm^{-1} confirms the presence of ester carbonyl (C=O) stretching vibrations, which represent the structural integrity and encapsulation stability of the phospholipid-based liposome.

5. **API Presence -** The Amide II band observed at 1531.9 cm^{-1}, arising from N-H bending and C-N stretching, confirms the presence of the glutathione API encapsulated within the liposomal carrier.

5. Elemental Analysis of Liposomal Glutathione

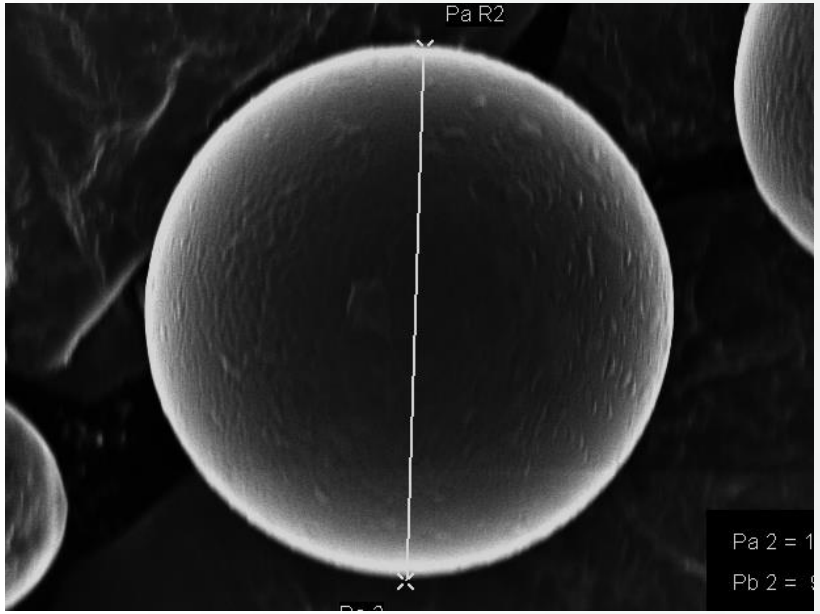


Figure 1 – SEM imaging showing the area scanned using Energy Dispersive X-Ray Spectroscopy (EDAX)

- The liposomal formulation shows a relative **increase in oxygen content** and **decrease in sulfur**, suggesting integration with phospholipid bilayers and possible shielding of sulfur-containing groups from surface detection.
- The presence of phosphorus in liposomal glutathione (absent in the API) indicates successful incorporation of phospholipids, validating liposomal encapsulation.

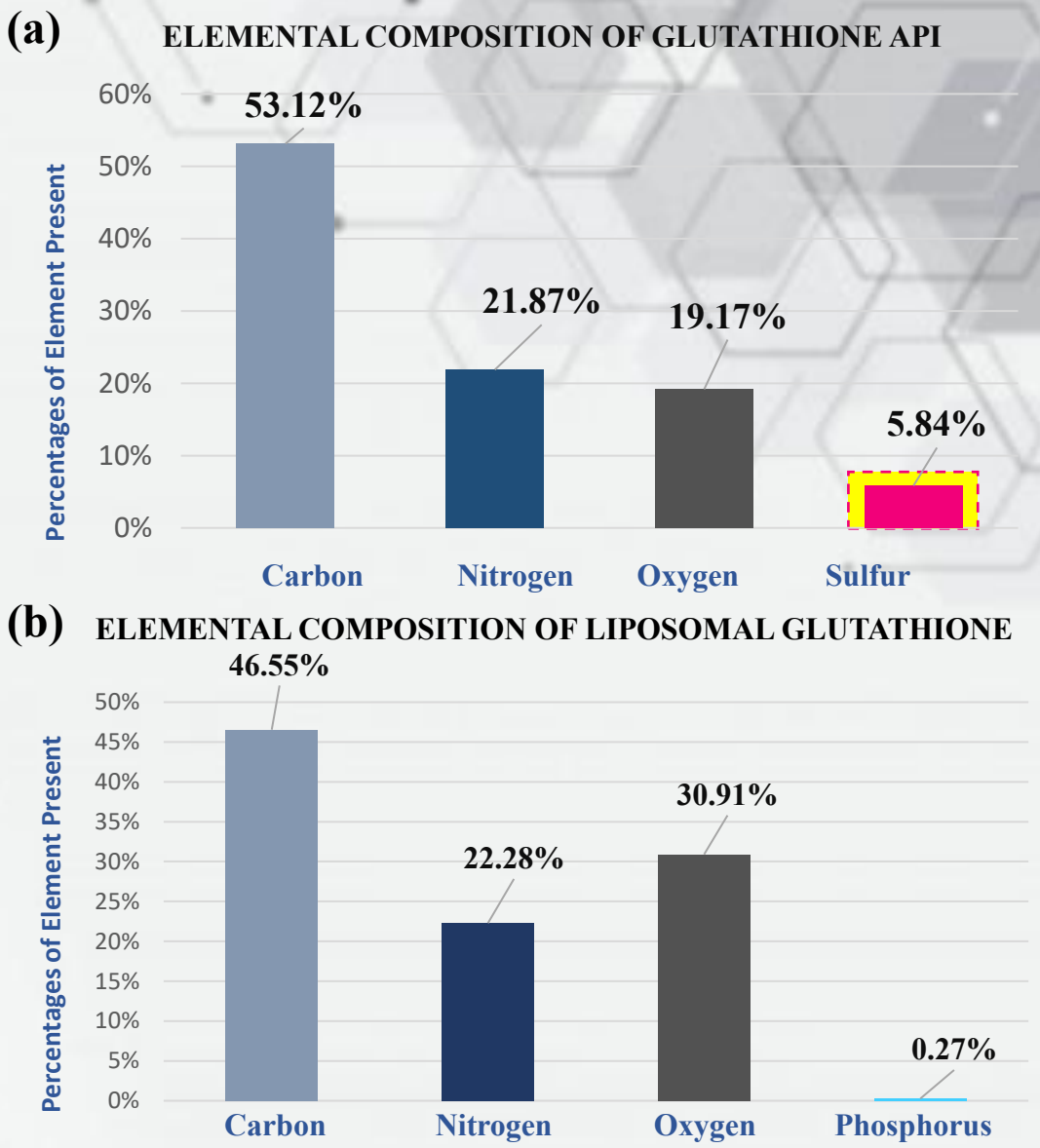


Figure 2 – A graphical representation of the percentages of elements composing (a) Glutathione API and (b) Liposomal Glutathione

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

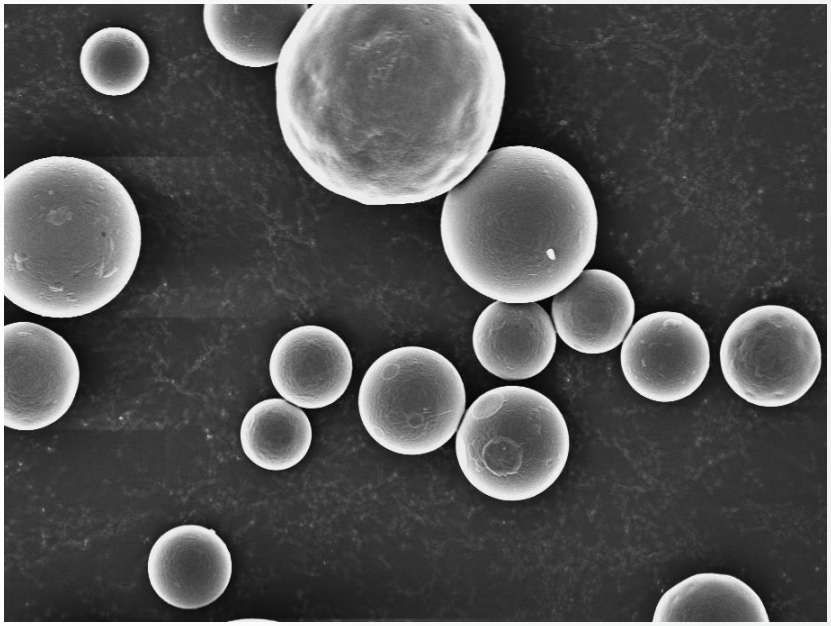


Figure 1 – SEM image showing several Glutathione Liposomes scattered within the field of view under observation

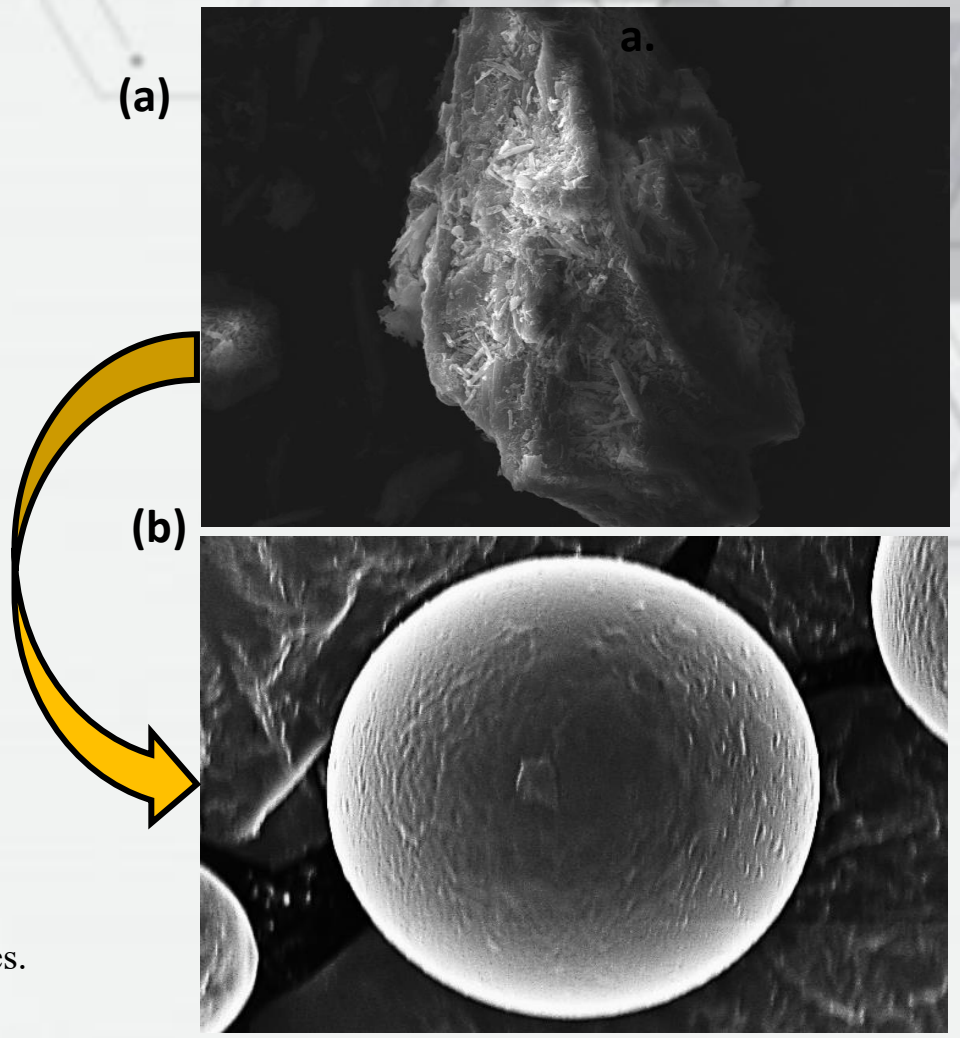


Figure 2 – SEM panels showing transformation from (a) Glutathione API, to (b) Liposomal Glutathione after encapsulation.

- Spherical morphology observed in liposomal Glutathione 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 Glutathione

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

- **Encapsulation efficiency remains high (~86%)** throughout 3 years of storage, indicating stable liposome structure.
- **Assay values for free Glutathione remain un same range (~92%)**, showing minimal leakage over time.
- The formulation shows **excellent retention of Glutathione**, confirming its suitability for long-term shelf storage.

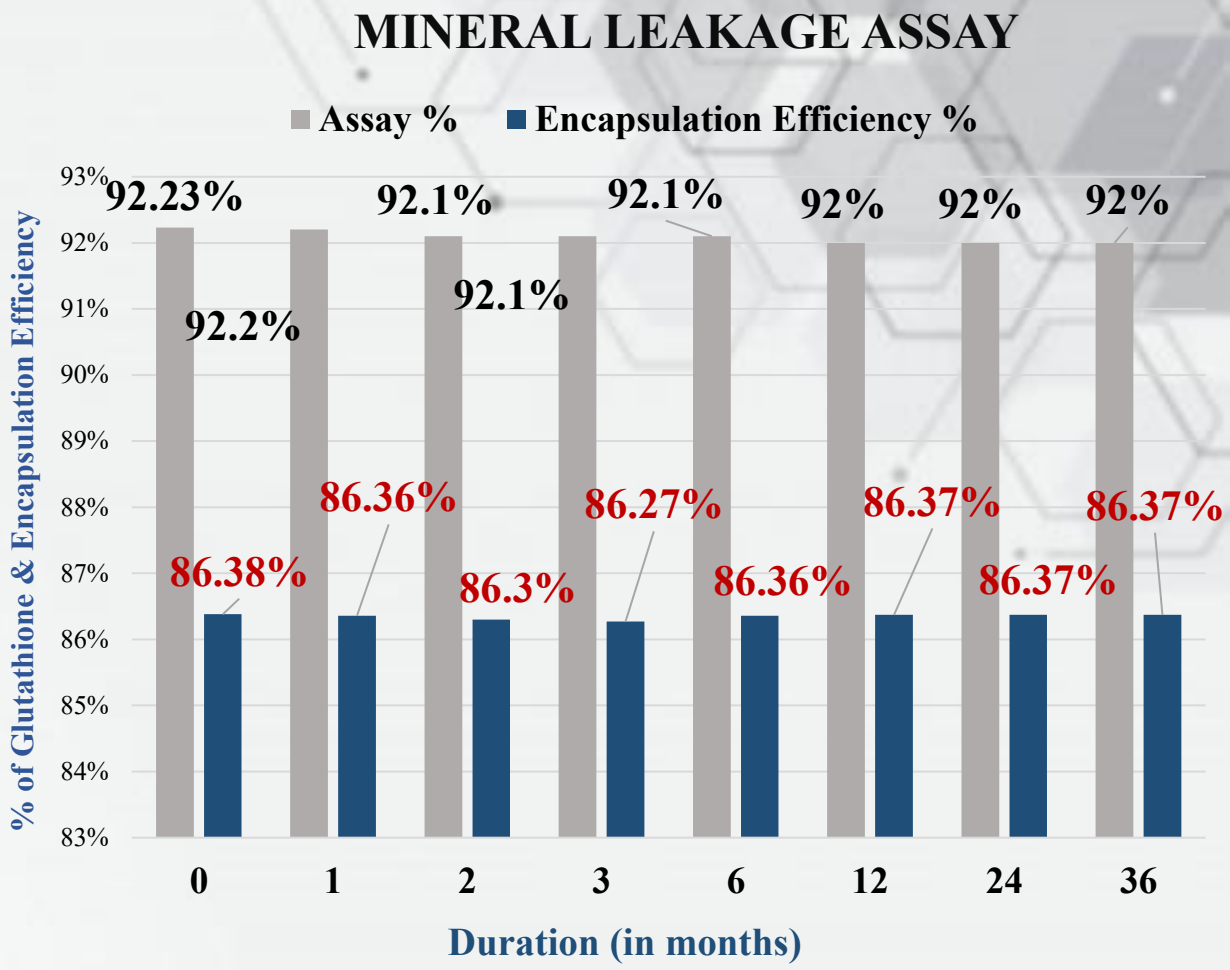


Figure 2 – Chart comparing the stability of Liposomal Glutathione stored over a period of 3 years at 40°C ± 2 °C and a relative humidity of 75% ± 5%.

8. Stability of Liposomal Glutathione at Elevated Temperatures

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

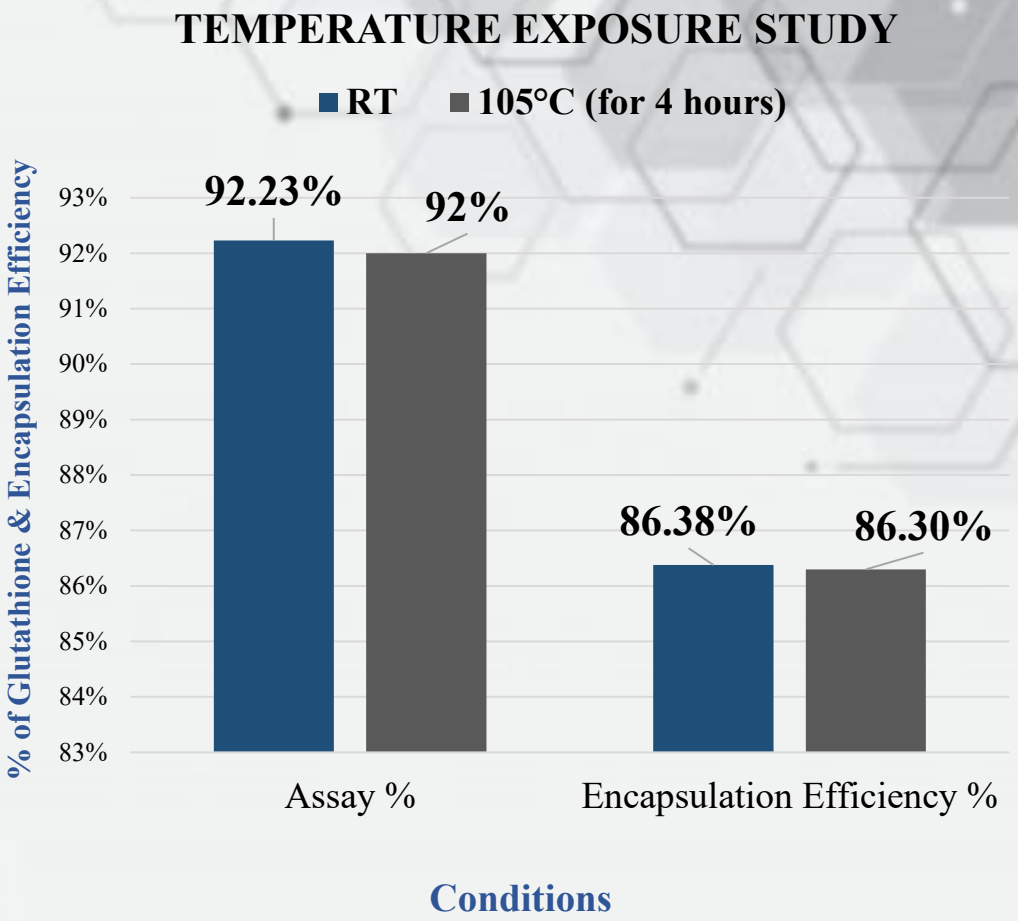
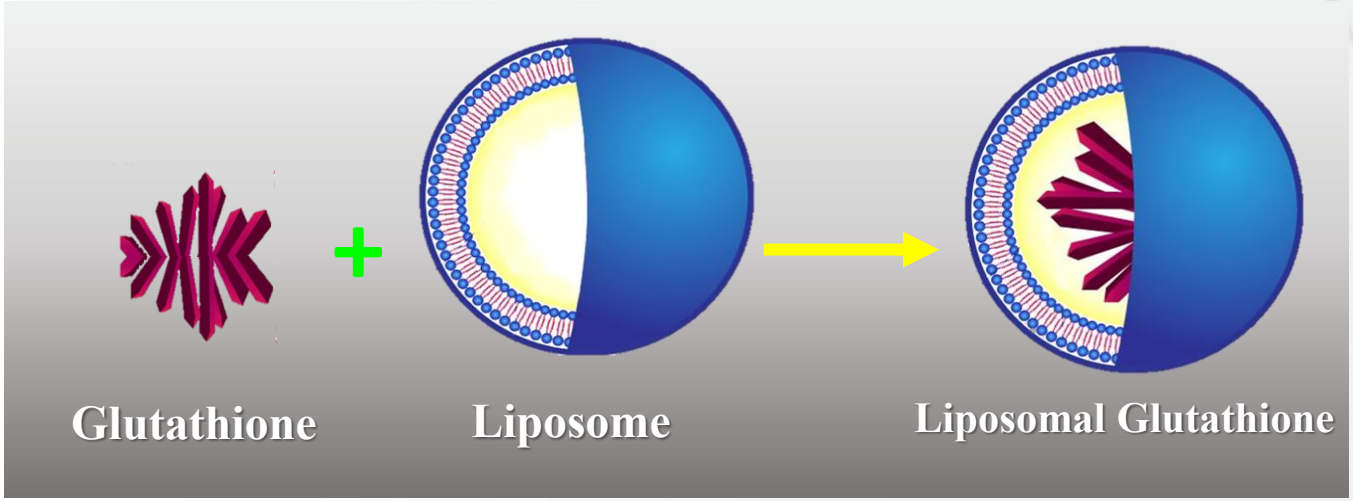


Figure 2 – Chart comparing the stability of Glutathione API and the encapsulation efficiency of liposomal Glutathione both at room temperature (RT) and at 105°C exposure for 4 hours.

- **Encapsulation efficiency remains high (≈86%)** even after exposure to 105°C for 4 hours.
- **Assay values (92.23% at RT vs. 92% at 105°C)** show minimal variation, indicating **negligible Glutathione leakage**.
- Demonstrates **thermal robustness**, making the formulation suitable for transport and storage in hot climates.

9. Mineral Loading Capacity



Formulation of Glutathione in Liposomes

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

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

Thank You!!!

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