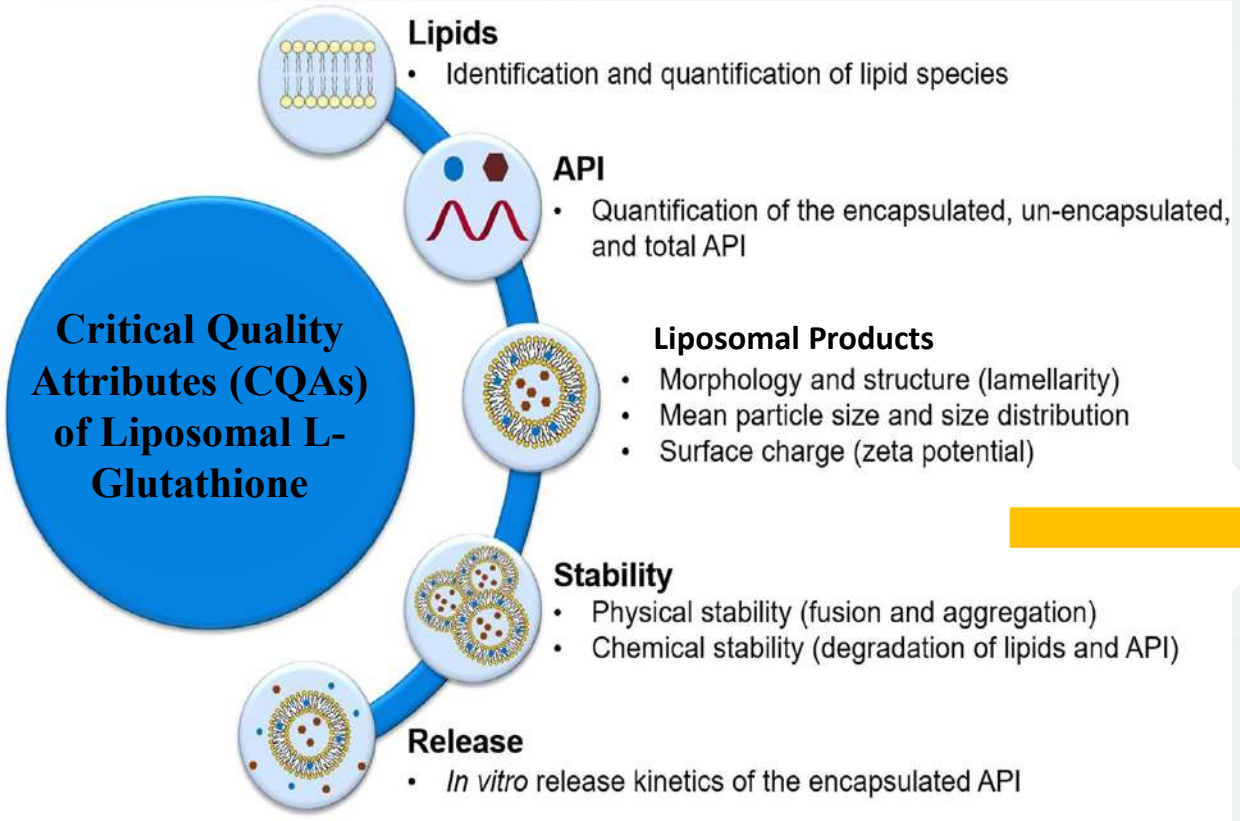
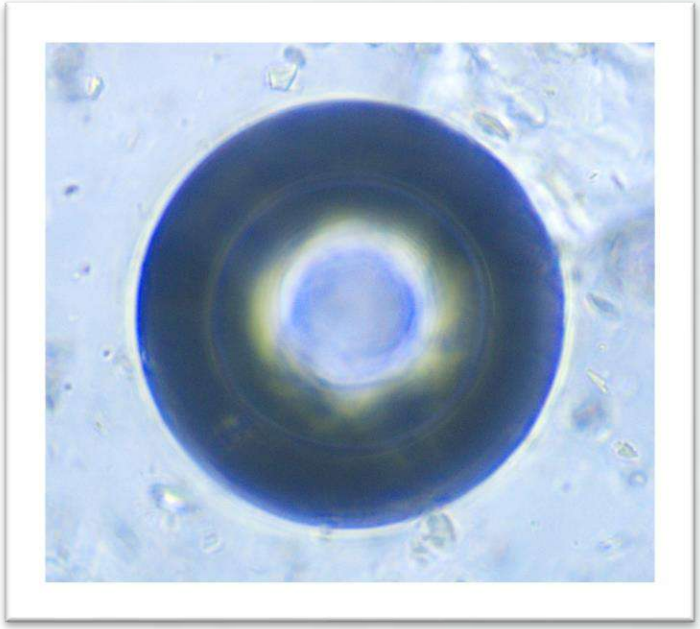
West Bengal Chemical Industries Limited

Summary of Characterizations Performed on Liposomal L-Glutathione



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

1. Encapsulation Efficiency of 92.23% Liposomal Glutathione



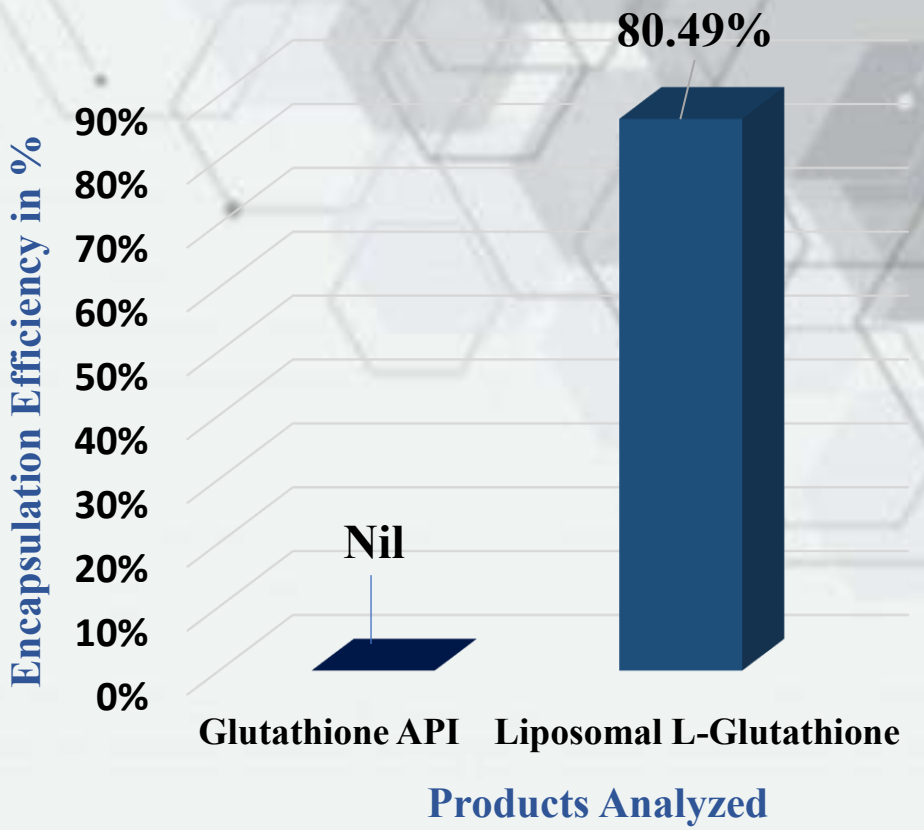


Figure 2 – Encapsulation Efficiency measured by validated HPLC analytical data

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

- **Liposomal encapsulation ensures 80.49% 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 L-Glutathione

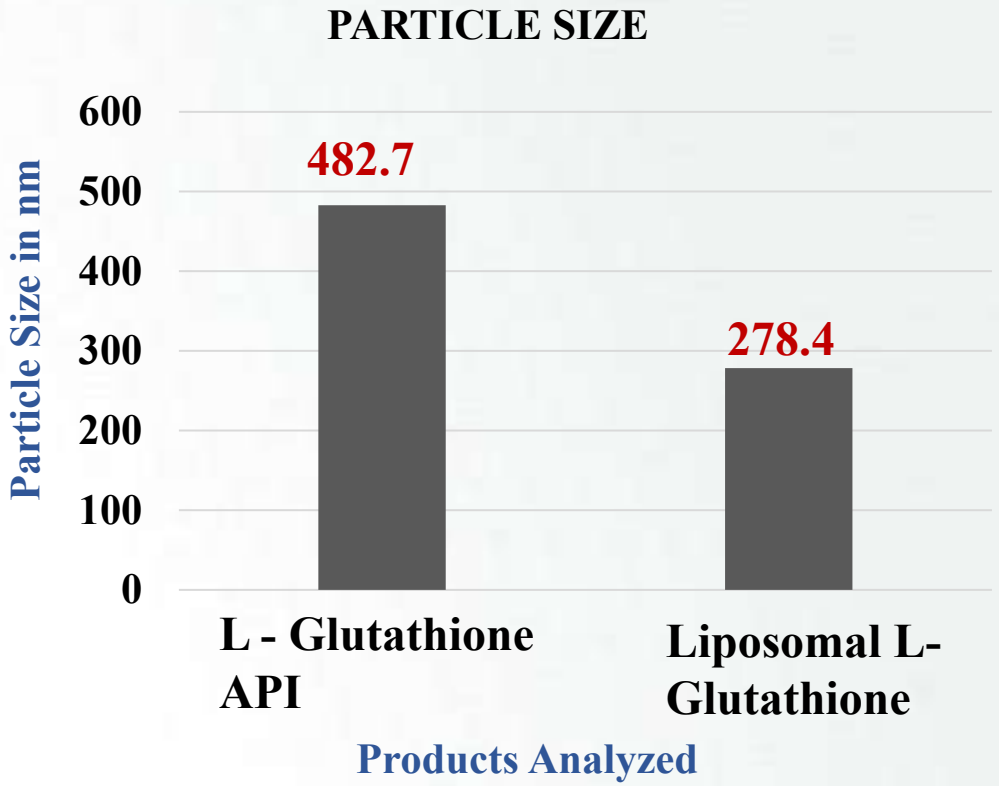


Figure 1 – Chart comparing the particle size of Glutathione API with Liposomal Glutathione

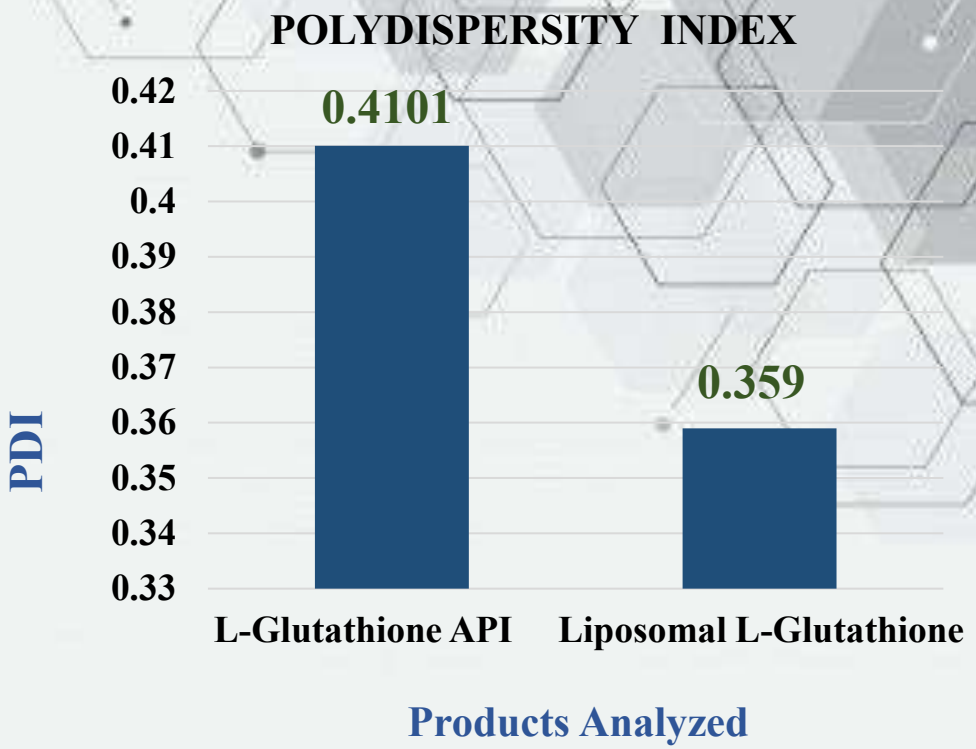


Figure 2 – Polydispersity Index (PDI) of Liposomal Glutathione in solution

❖ **Acceptance criteria:**

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

- Nanosized, uniform particles offer greater colloidal stability and improved shelf life.
- Smaller particles (**Particle size: 278.4** and **PDI 0.359)** support **increased mucosal permeability** and cellular uptake.
- DLS characterization confirms high formulation control and **batch-to-batch reproducibility**.

3a. Behavior of Liposomal Glutathione

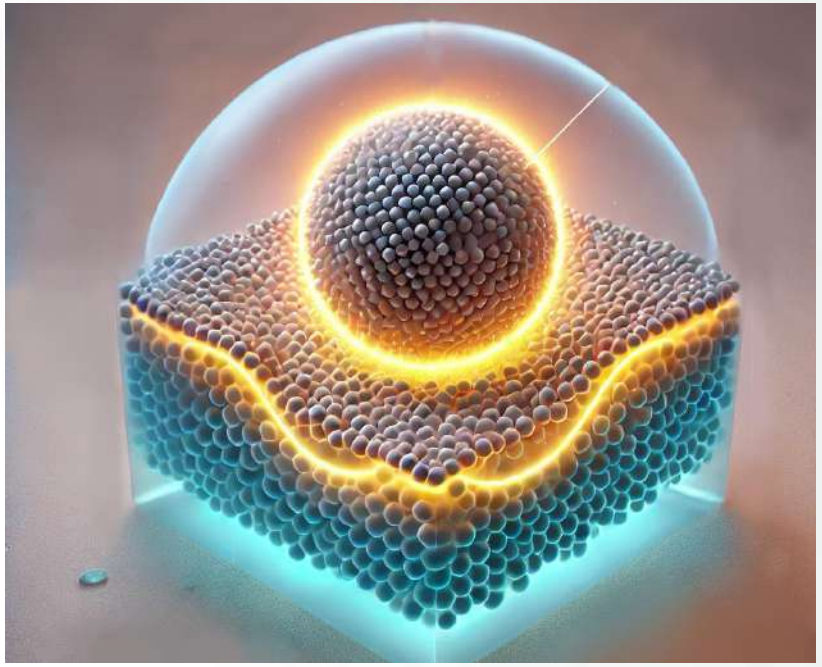


Figure 1 – Zeta potential visualization showing a negatively charged particle suspended in a liquid solution, surrounded by a well-defined electric double layer of positively charged ions.

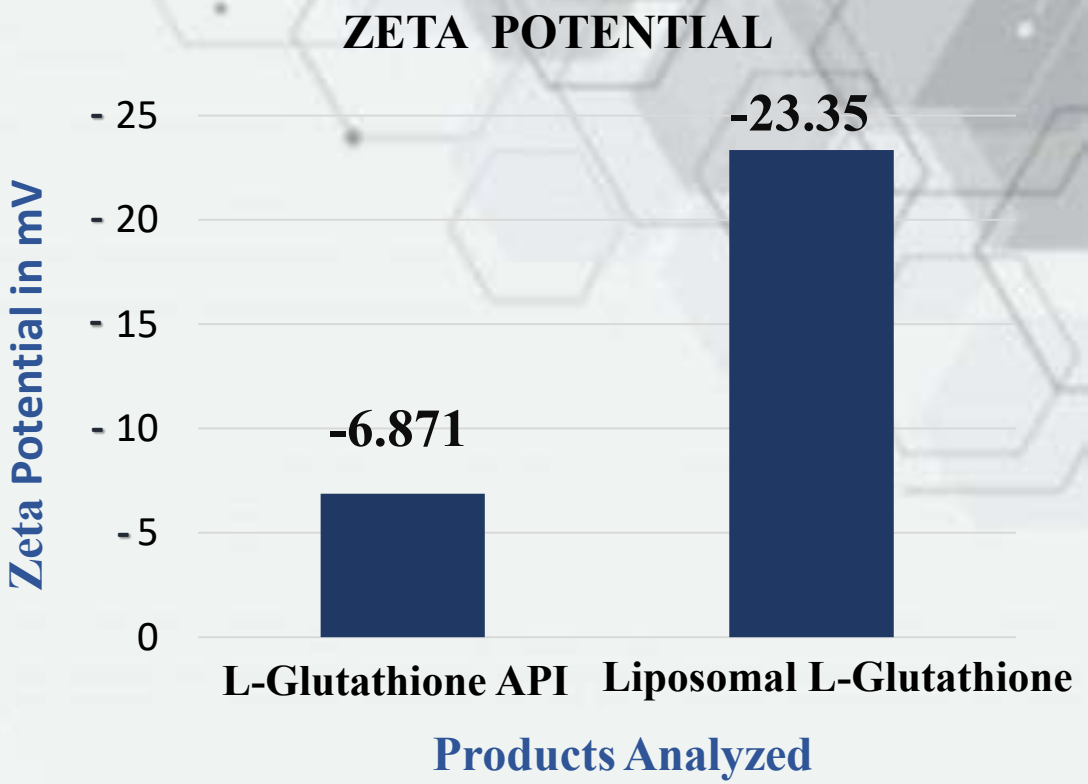


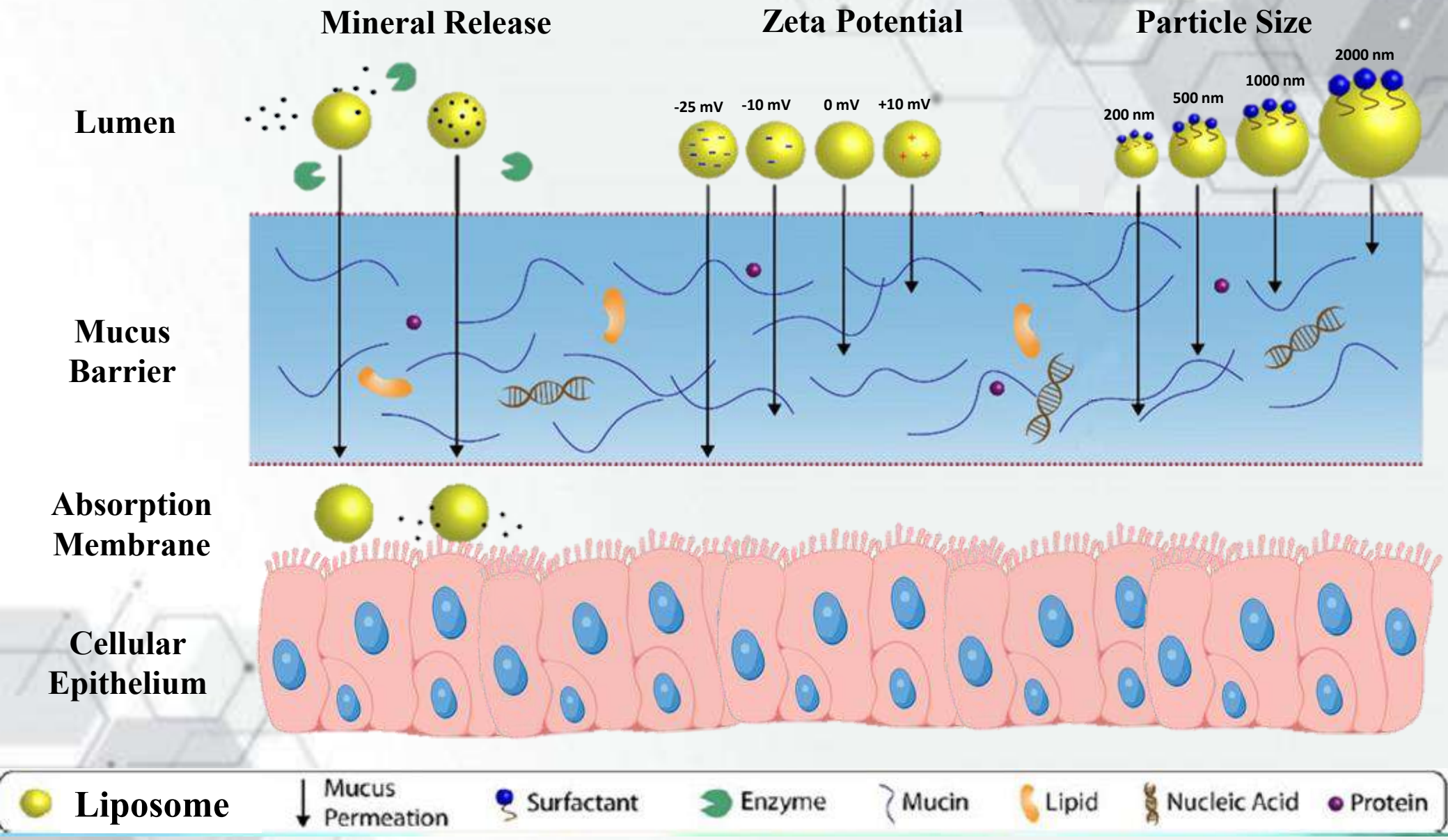
Figure 2 – Chart comparing the zeta potential of L-Glutathione API and Liposomal L-Glutathione showing L-Glutathione in Liposomal form is stable and less prone to agglomerate in solution.

- Liposomal L-Glutathione shows **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 : < -20 mV**

3b. Absorption of Liposomal L-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

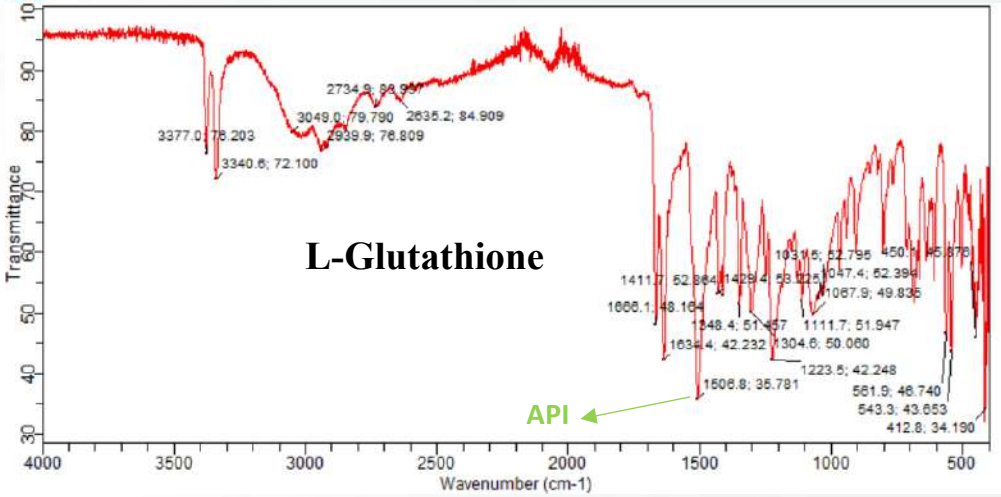


Figure 1: IR Transmission spectrum showing bands at different wavelengths of Glutathione API

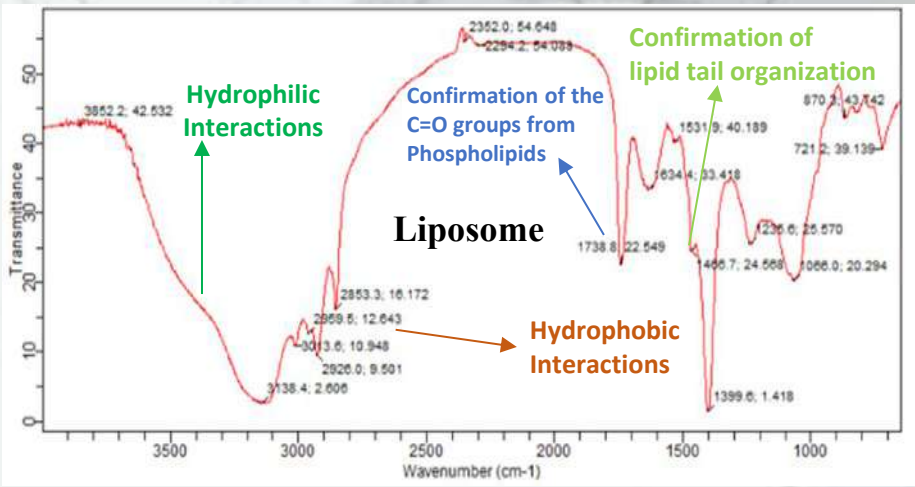


Figure 2: Hydrophobic and Hydrophilic interactions within Empty Liposome

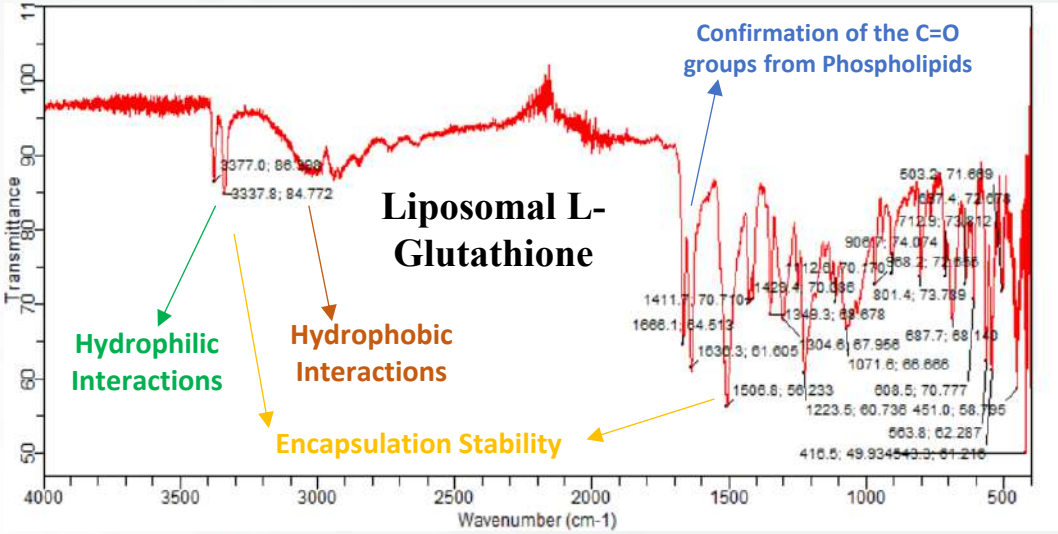


Figure 3: IR Transmission spectrum of Liposomal L-Glutathione is shown

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 1506.8 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

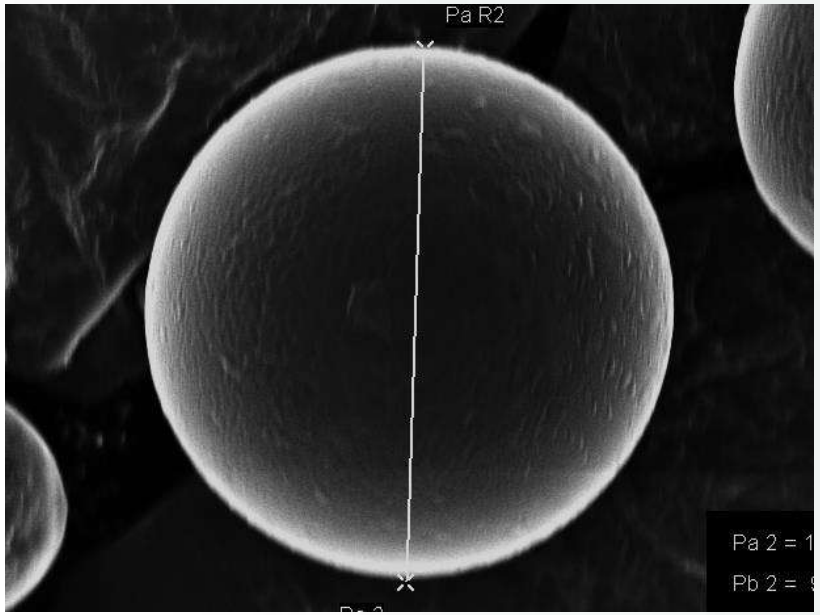


Figure 1 – SEM imaging showing the area scanned using Energy Dispersive X-Ray Spectroscopy (EDAX)

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

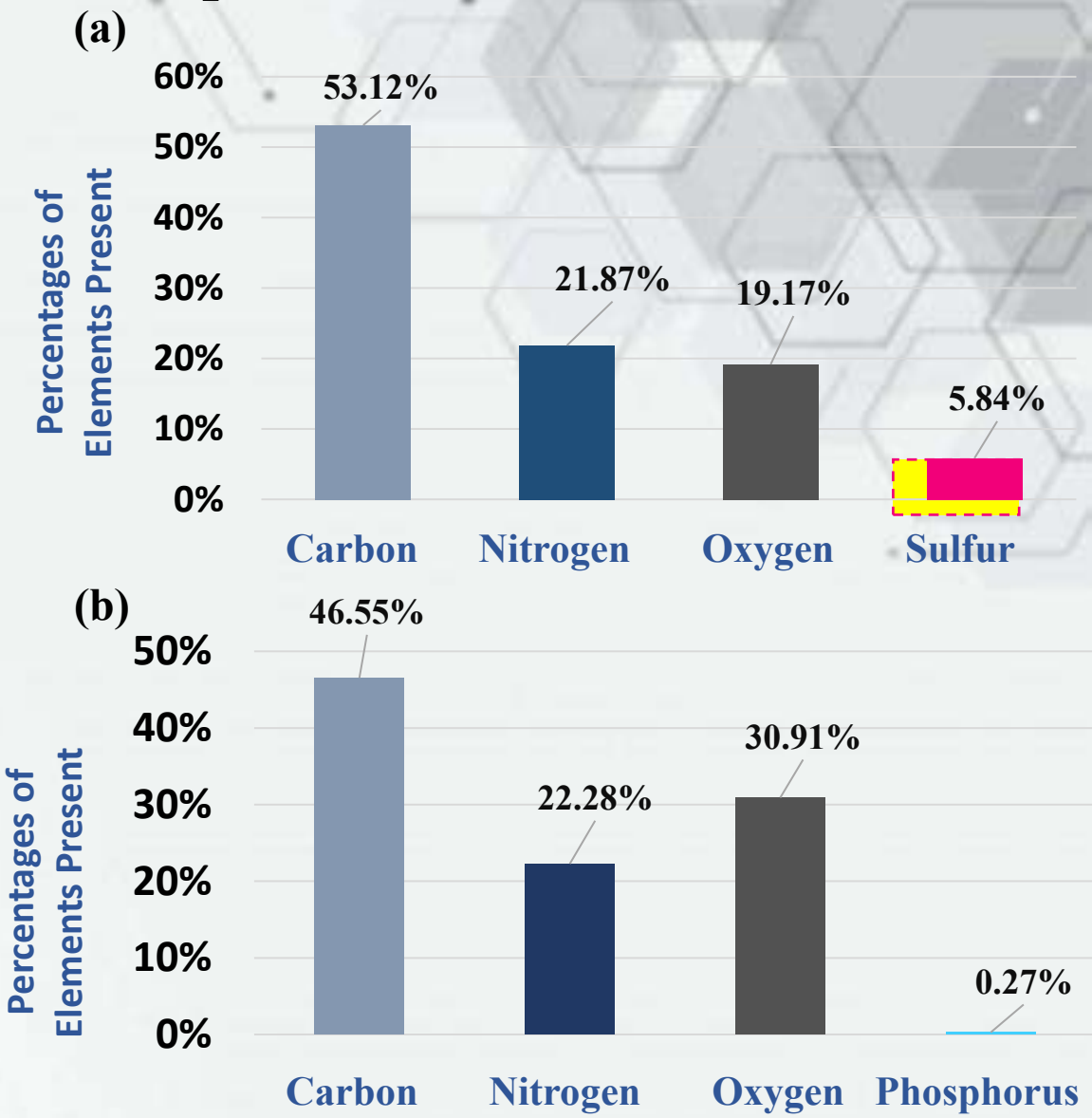


Figure 2 – A graphical representation of the percentages of elements composing (a) L-Glutathione API and (b) Liposomal L-Glutathione

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

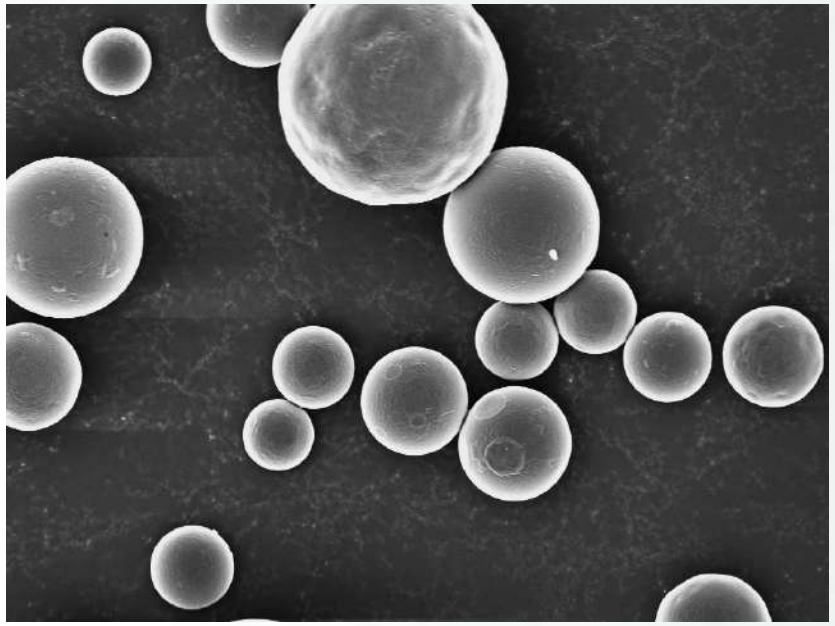


Figure 1 – SEM image showing several L-Glutathione Liposomes scattered within the field of view under observation

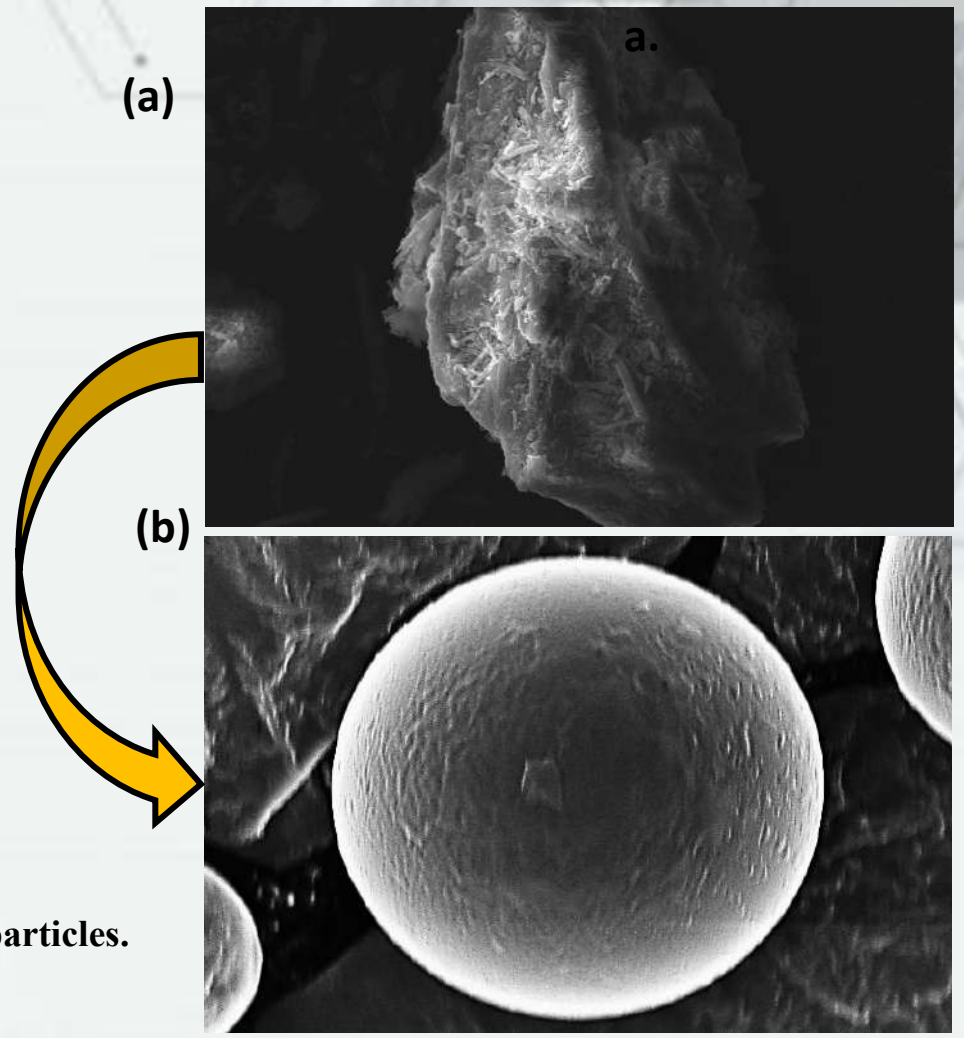


Figure 2 – SEM panels showing transformation from (a) L- Glutathione API, to (b) Liposomal L-Glutathione after encapsulation.

- **Spherical morphology observed in liposomal L-Glutathione 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.**

7. Leakage of Liposomal L-Glutathione

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

- **Encapsulation efficiency remains high (~80%)** throughout 3 years of storage, indicating stable liposome structure.
- **Assay values for L-Glutathione remain within the same range (~92%)**, showing minimal leakage over time.
- The formulation shows **excellent retention of L-Glutathione**, confirming its suitability for long-term shelf storage.

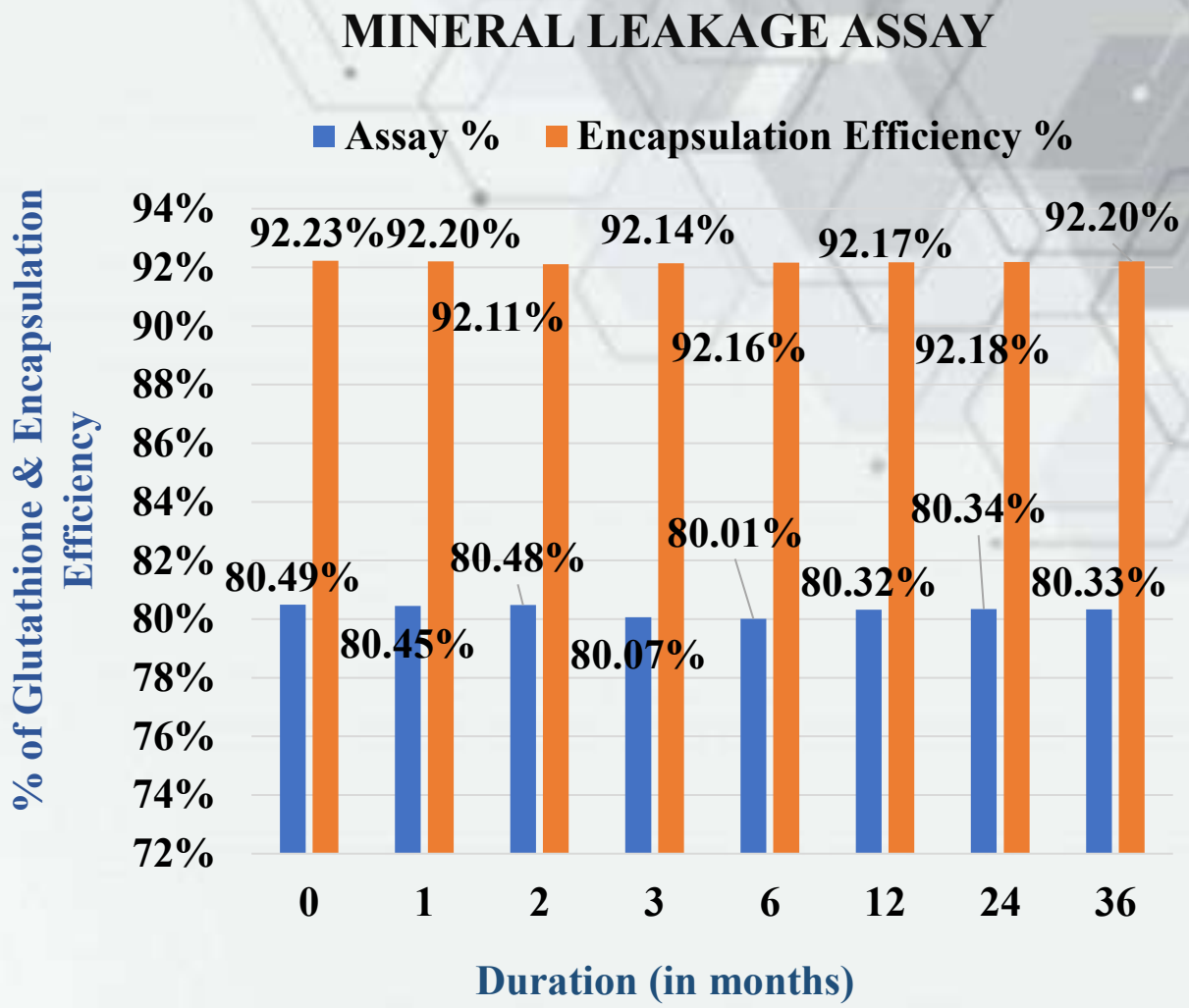


Figure 2 – Chart comparing the stability of Liposomal L-Glutathione stored over a period of 3 years at 40°C ± 2 °C and a relative humidity of 75% ± 5%.

8. Stability of Liposomal L-Glutathione at Elevated Temperatures

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

- **Encapsulation efficiency remains high (≈80%)** even after exposure to 105°C for 4 hours.
- **Assay values (92.23% at RT vs. 91.88% at 105°C)** show minimal variation, indicating **negligible L-Glutathione leakage**.
- Demonstrates **thermal robustness**, making the formulation suitable for transport and storage in hot climates.

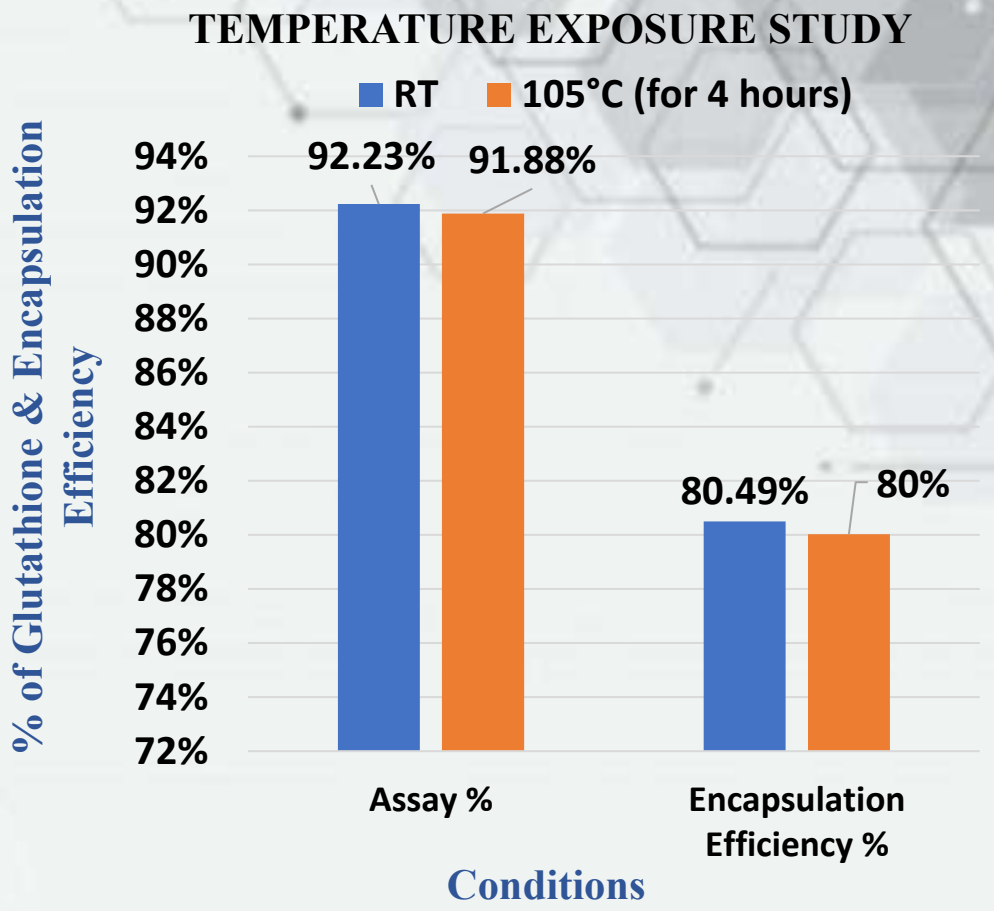


Figure 2 – Chart comparing the stability of L-Glutathione API and the encapsulation efficiency of liposomal L-Glutathione both at room temperature (RT) and at 105°C exposure for 4 hours.

Thank You!!!

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