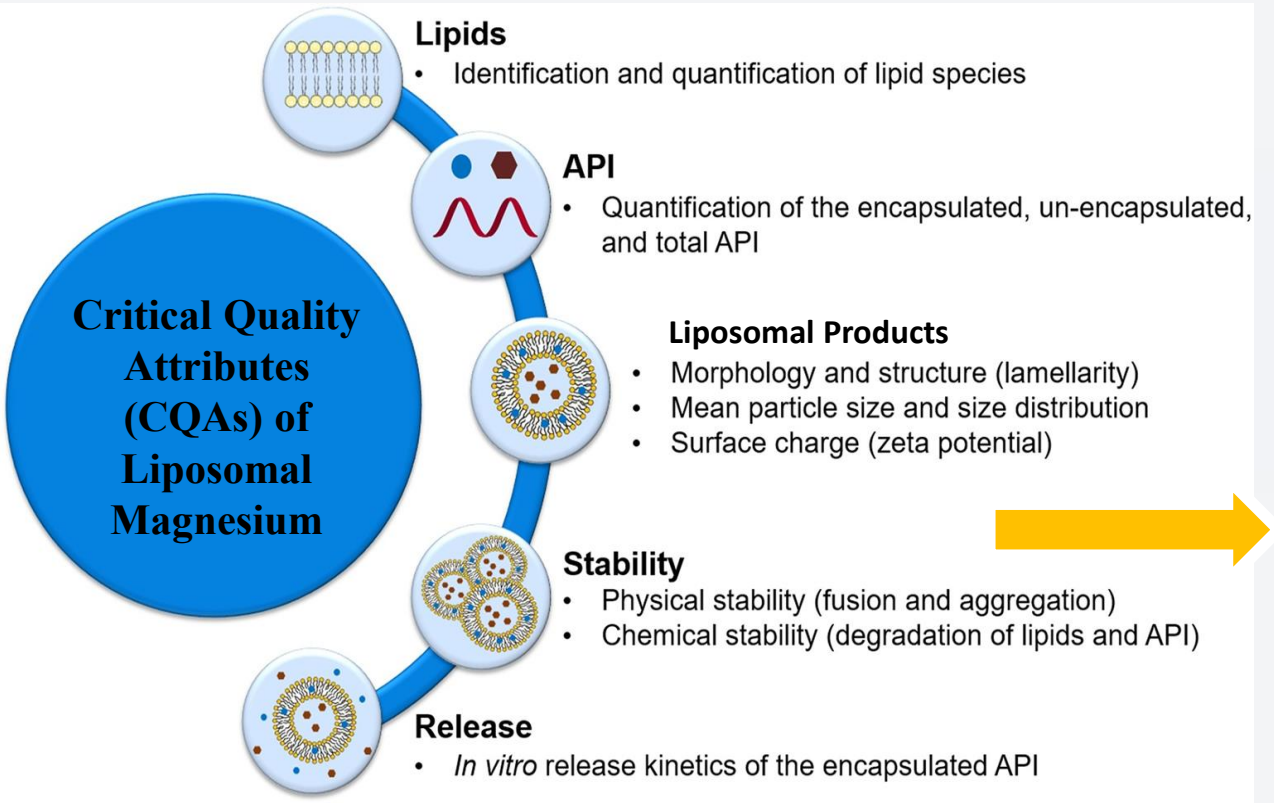
West Bengal Chemical Industries Limited

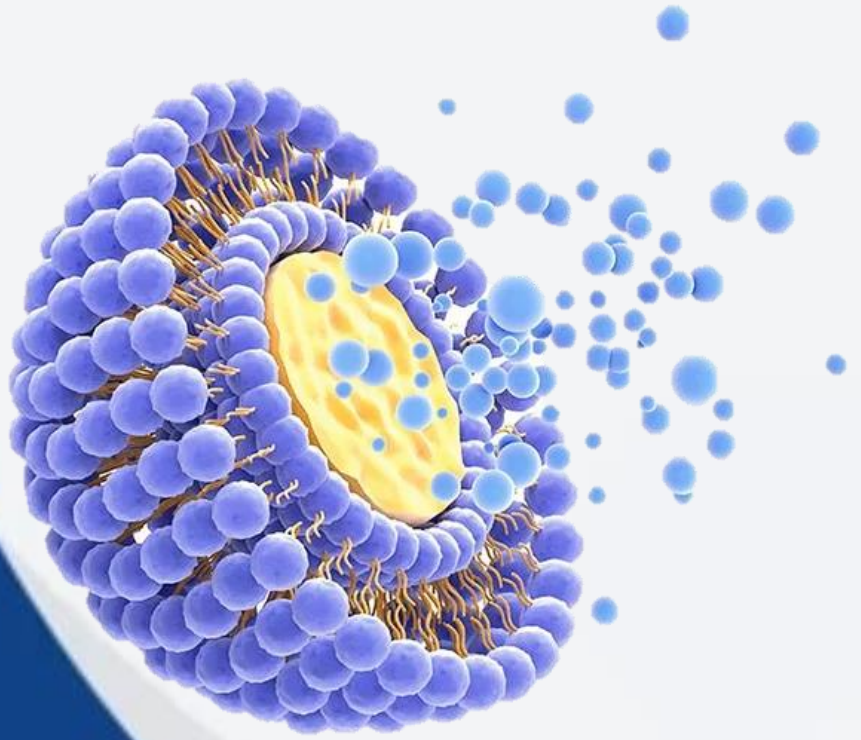
Summary of Characterizations Performed on Liposomal Magnesium

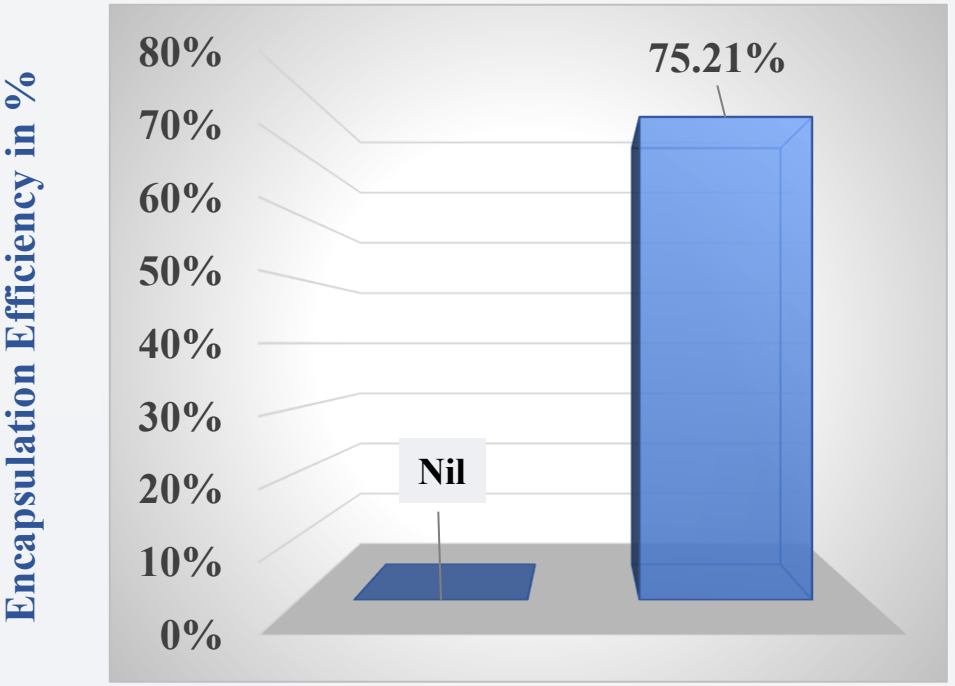


1. ***Encapsulation efficiency of Liposomal Magnesium***
2. ***Analysis of particle size and uniformity of Liposomal Magnesium using DLS***
3. ***Behavior of Liposomal Magnesium particles in liquid medium using DLS Zeta-sizer***
4. ***FTIR analysis of Liposomal Magnesium composition***
5. ***Elemental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

https://www.sciencedirect.com/science/article/pii/S0731708520315284

1. Encapsulation Efficiency of 22.25% Liposomal Magnesium





Products Analyzed

- **Acceptance criteria:**
  - **Assay : 22% - 23%**
  - **Encapsulation efficiency : NLT 70%**

Encapsulation Efficiency measured by validated titrimetric analytical data

- Liposomal encapsulation ensures **75.21% efficiency.**
- Efficient encapsulation minimizes mineral loss, improving bioavailability and therapeutic efficacy.
- Offers protection against oxidation and gastrointestinal irritation, common with conventional Magnesium forms.

2. Dynamic Light Scattering Analysis of Liposomal Magnesium

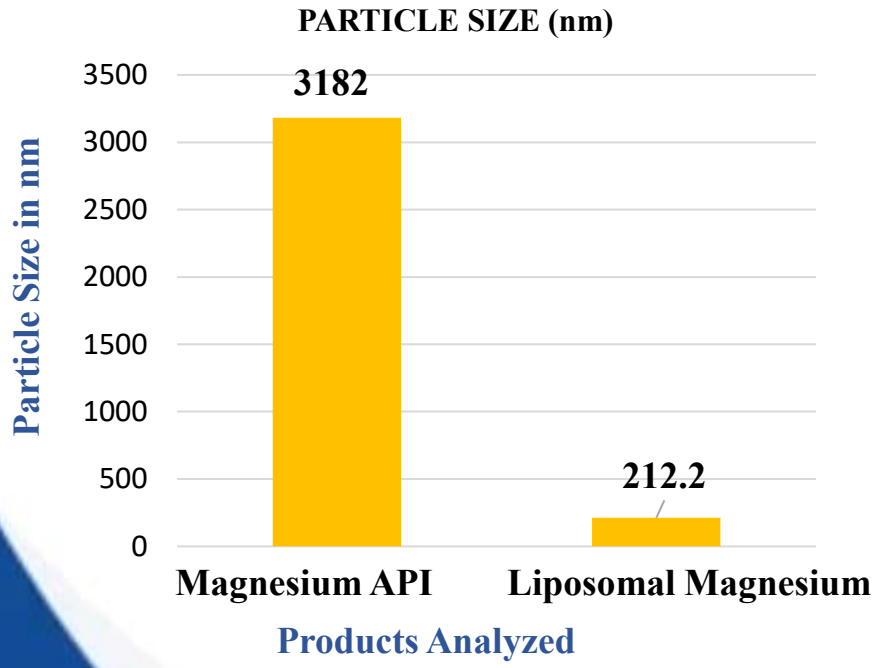


Figure 1 – Chart showing the particle size of Magnesium API with Liposomal Magnesium

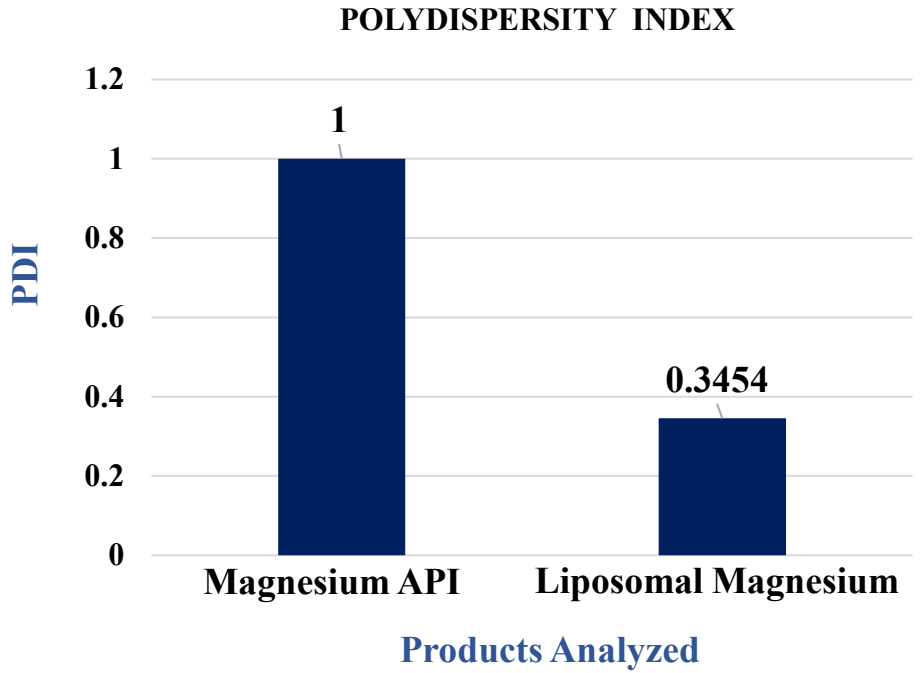


Figure 2 – Chart comparing the Polydispersity Index of Magnesium API and Liposomal Magnesium in solution

- Nanosized, uniform particles offer greater colloidal stability and improved shelf life.
- Smaller particles (**Particle size: 212.2** and **PDI 0.3454**) support increased mucosal permeability and cellular uptake.
- DLS characterization confirms high formulation control and batch-to-batch reproducibility.

Acceptance criteria:

- **Particle Size : < 200 nm**
- **Polydispersity Index : <1**

3a. Behavior of Liposomal Magnesium

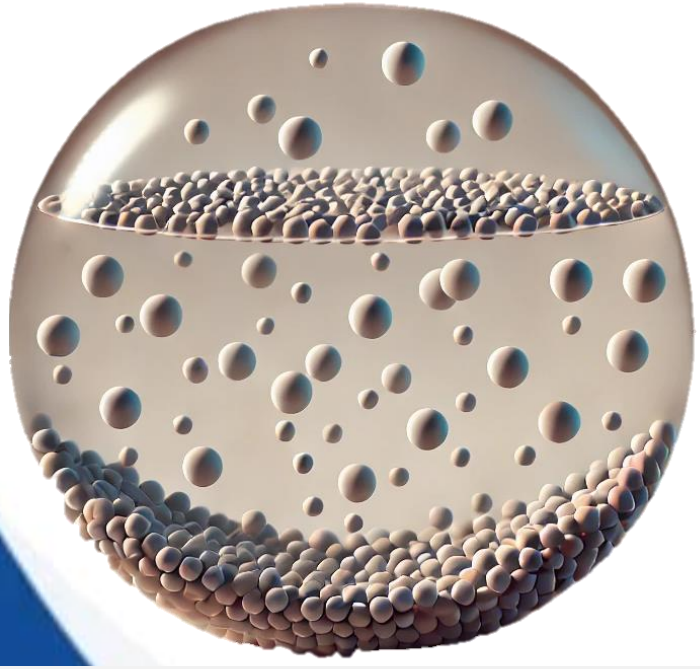


Figure 1 – Illustration of zeta potential, showing the electrostatic interactions of particles in suspension

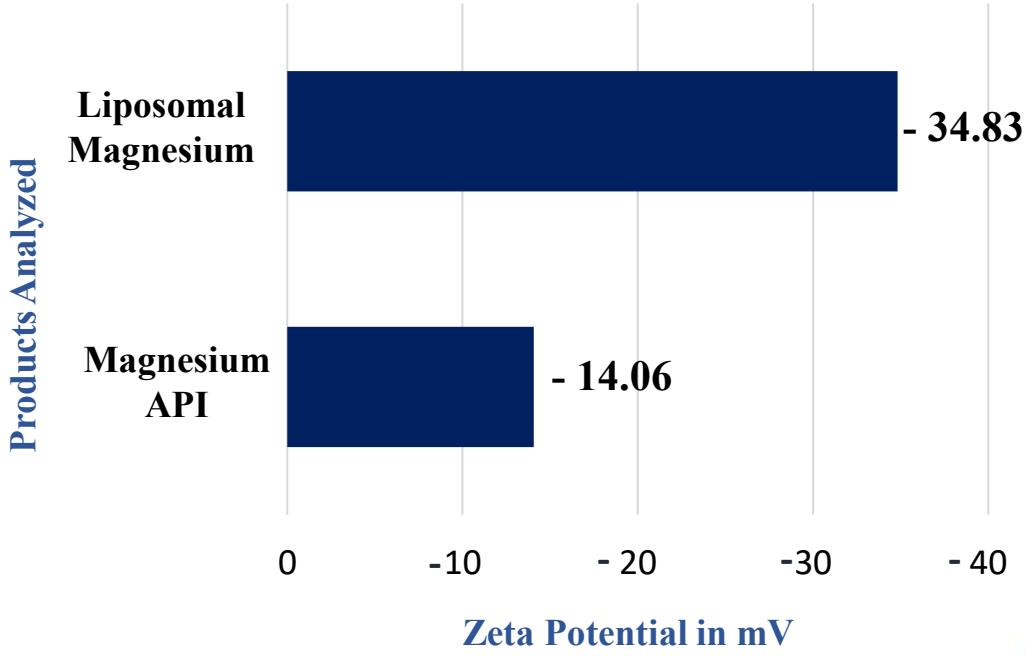


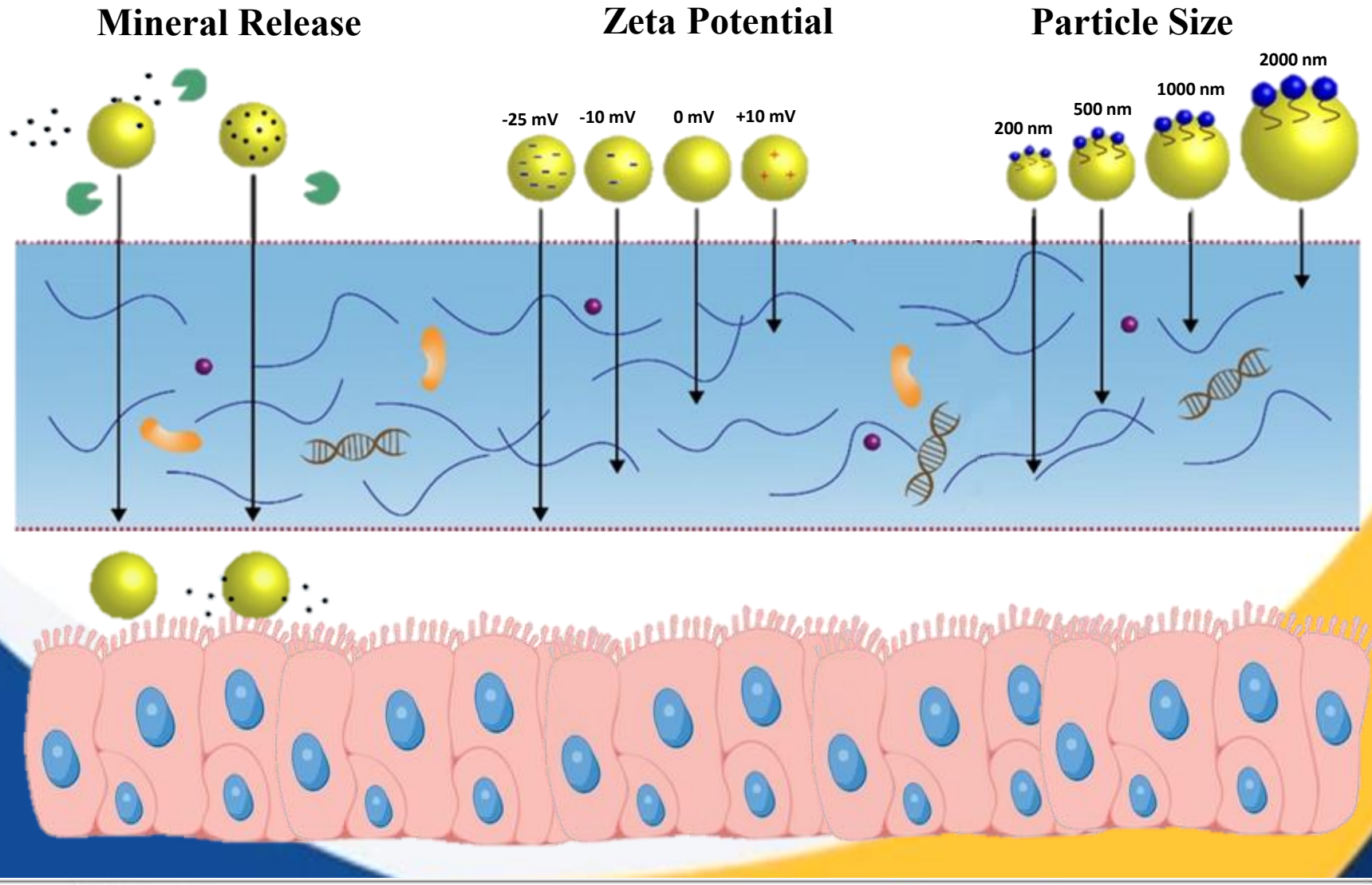
Figure 2 – Chart comparing the zeta potential of Magnesium API and Liposomal Magnesium showing Magnesium in Liposomal form is stable and unlikely to agglomerate in solution.

- Liposomal Magnesium shows **high zeta potential (-34.83 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 Magnesium Represented Schematically on a Cellular Cross-Section



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

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

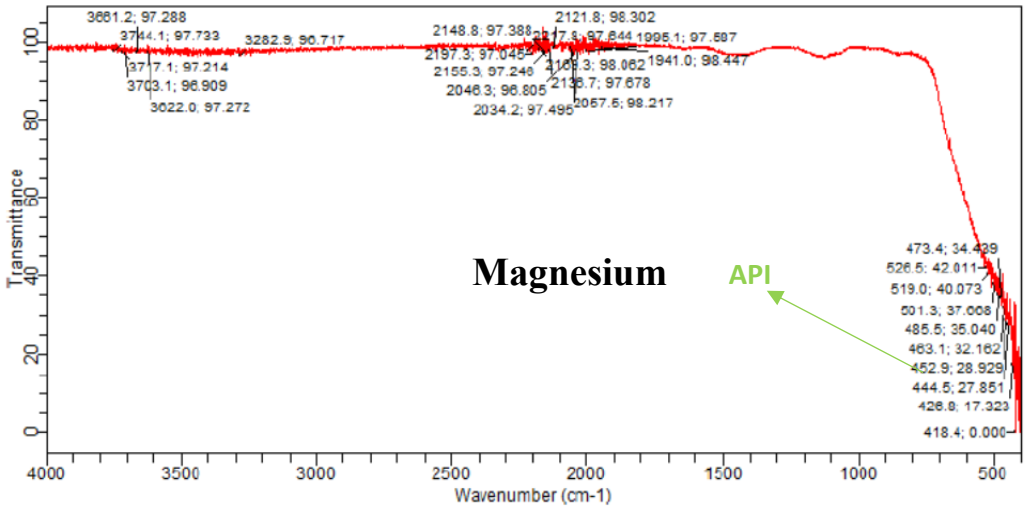


Figure 1: IR Transmission spectrum showing bands at different wavelengths of Magnesium Oxide API

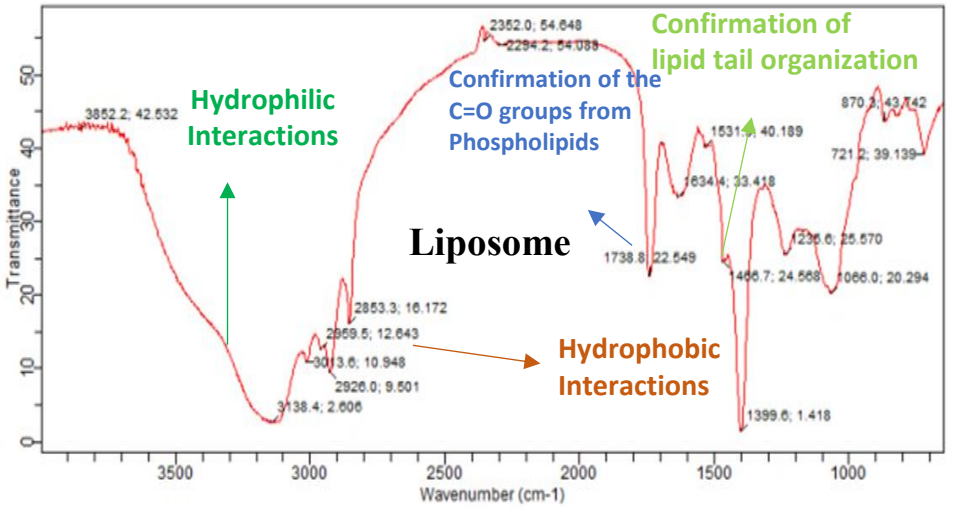


Figure 2: Hydrophobic and Hydrophilic interactions within Empty Liposome

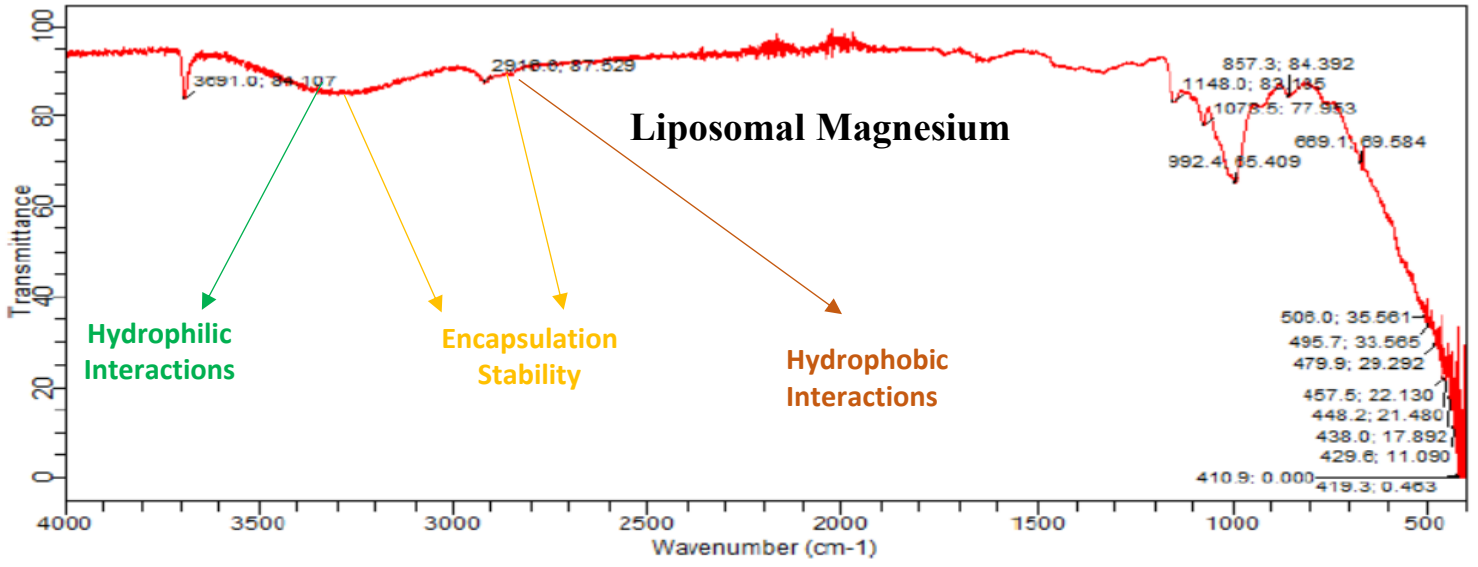


Figure 3: IR Transmission spectrum of Liposomal Magnesium is shown

4b. Summary of FTIR Analysis of Liposomal Magnesium

1. **Confirmation of the C=O and O-H Groups**: The broad O-H stretching peak at ~3391 cm^{-1} confirms the presence of hydroxyl groups, while the C=O stretching peak at ~1695 cm^{-1} confirms the presence of a carbonyl group.

2. **Hydrophobic Interactions** - C-H stretching at **2918 cm^{-1}** and **2845 cm^{-1}** and C-H bending at **1450 cm^{-1}**, suggesting water and polar group interactions.

3. **Hydrophilic Interactions -** O-H stretching around **3410 cm^{-1}** suggesting water and polar group interactions.

4. **Encapsulation Stability -** C-H Stretching at **2918 cm^{-1}** and **2845 cm^{-1}** indicates the presence of lipid alkyl chains, confirming the hydrophobic part of the Liposome structure.

5. Elemental Analysis of Liposomal Magnesium

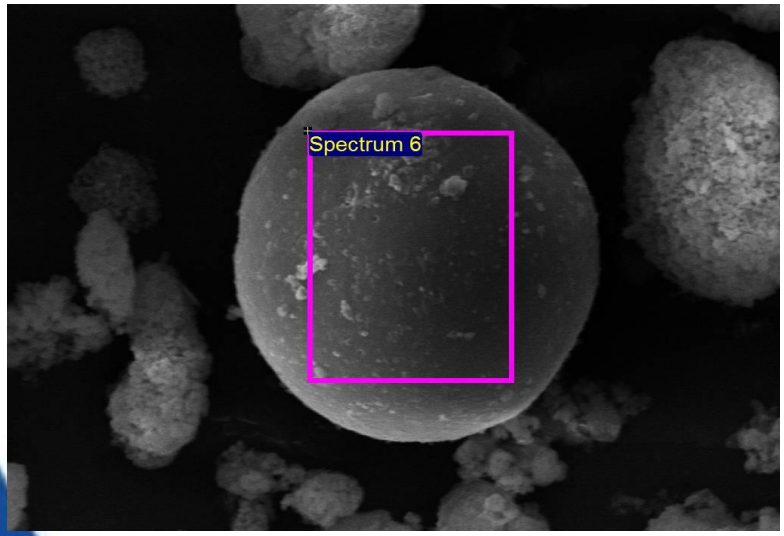


Figure 1 – SEM image of Liposomal Magnesium showing the area scanned using Energy Dispersive X-Ray Spectroscopy (EDAX)

- Magnesium API contains 53.72% magnesium and 46.28% oxygen.
- Liposomal magnesium shows 56.58% carbon, 25.85% oxygen, 17.33% nitrogen, and 0.24% phosphorus.
- The absence of magnesium on the surface suggests it is encapsulated or localized within the liposome.

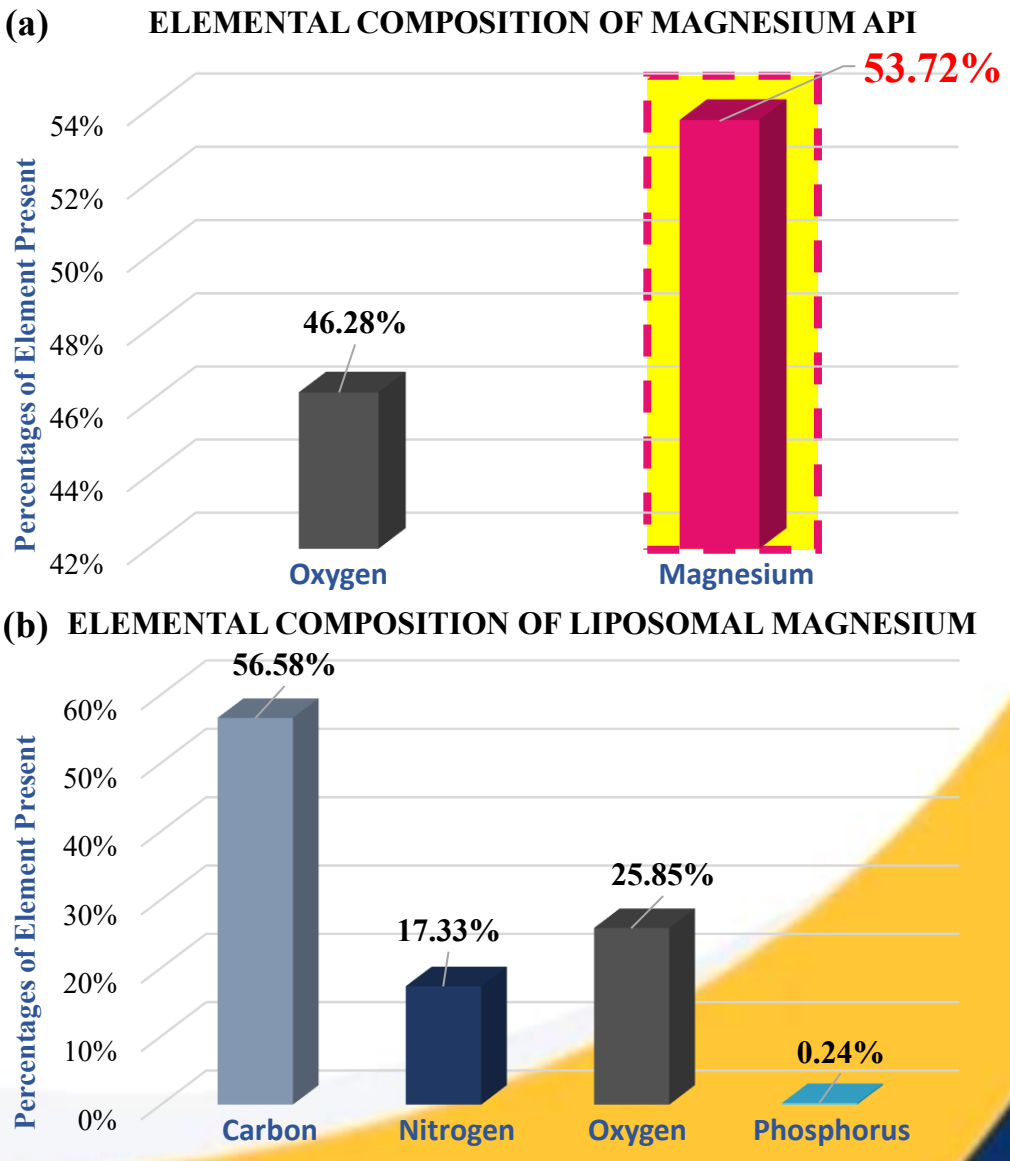


Figure 2 – A graphical representation of the percentages of elements composing (a) Magnesium API and (b) Liposomal Magnesium

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

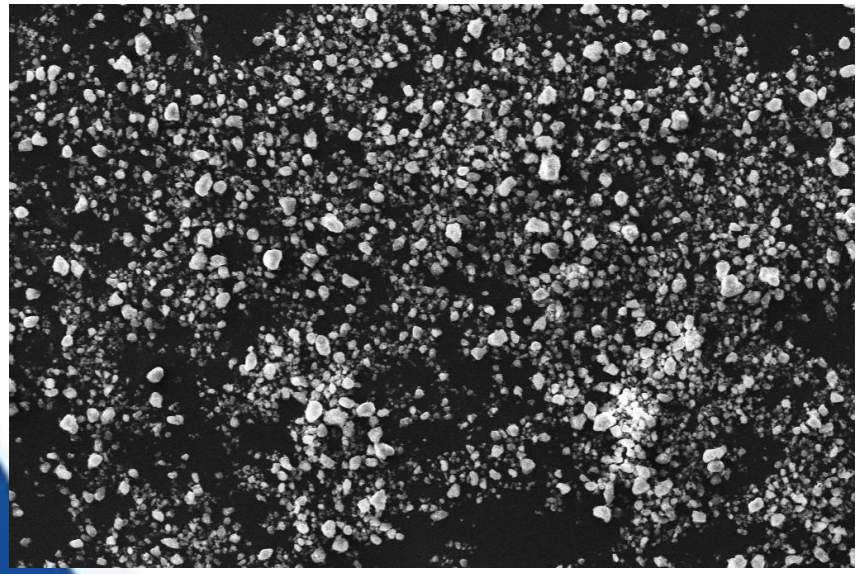


Figure 1 – SEM image of few Magnesium Liposomes scattered within the field of view under observation

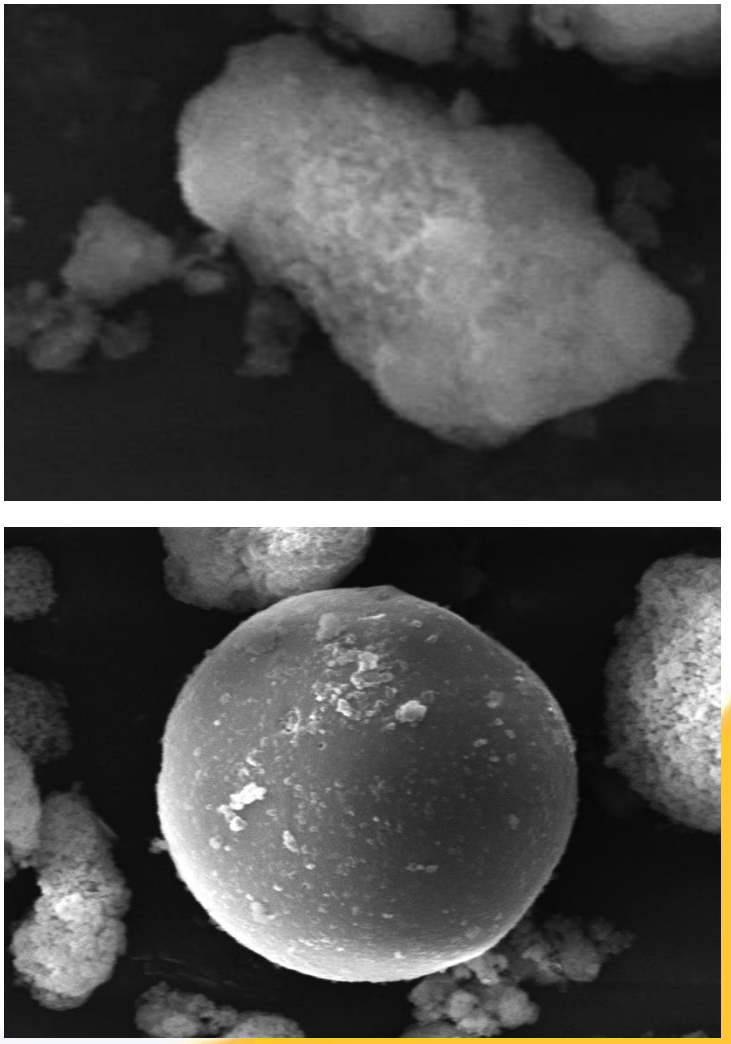


Figure 2 – SEM panels showing transformation from (a) Magnesium API to (b) Liposomal Magnesium after encapsulation.

- Spherical morphology observed in liposomal Magnesium 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 Magnesium from Liposomes

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

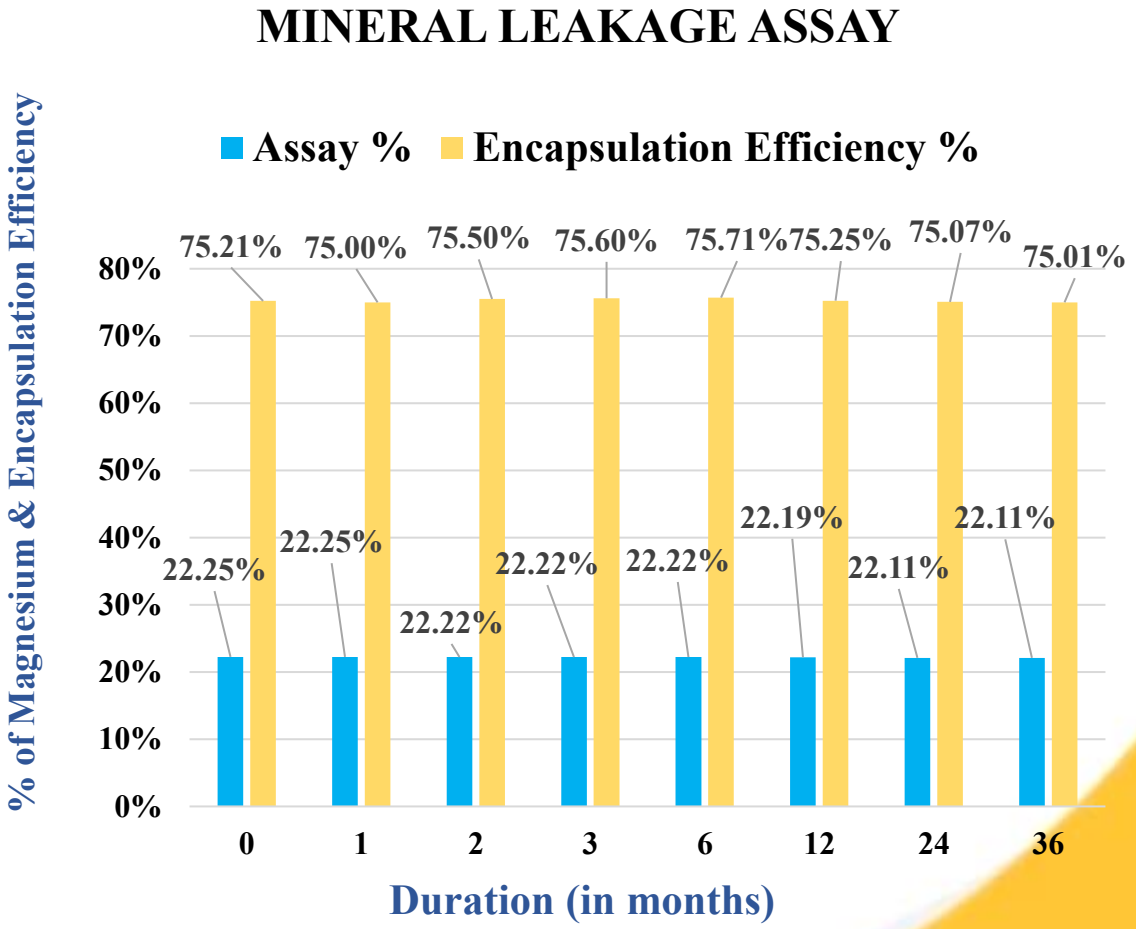


Figure 2 – Chart comparing the stability of Liposomal Magnesium stored over a period of 3 years at 40°C ± 2 °C and a relative humidity of 75% ± 5%.

- **Encapsulation efficiency remains consistent (~75%)** throughout 3 years of storage, indicating stable liposome structure.
- **Assay values for free Magnesium remain low (~22%)**, showing minimal leakage over time.
- The formulation shows **excellent retention of Magnesium**, confirming its suitability for long-term shelf storage.

8. Stability of Magnesium Liposomes at Elevated Temperatures

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

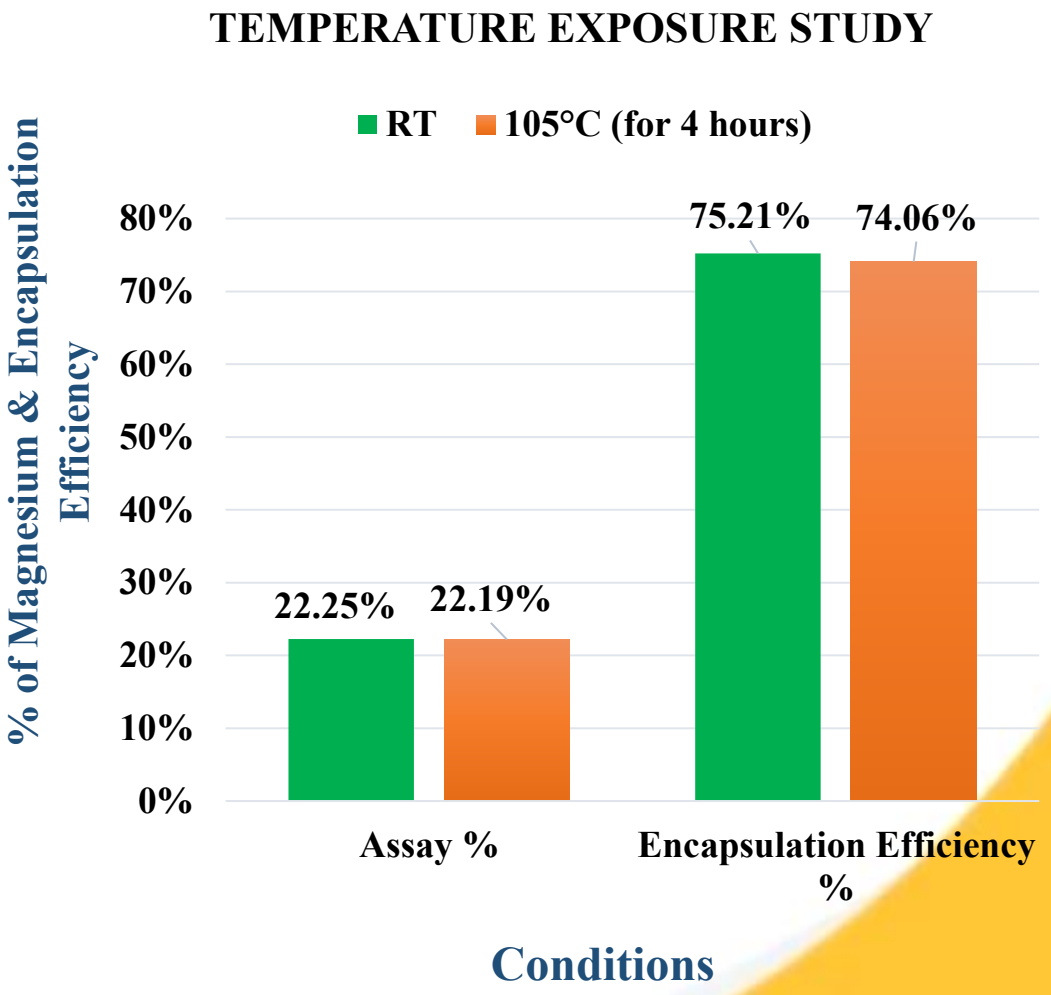


Figure 2 – Chart comparing the stability of Liposomal Magnesium both at room temperature (RT) and at 105°C for 4 hours of exposure.

- **Encapsulation efficiency remains high (≈74%)** even after exposure to 105°C for 4 hours.
- **Assay values (approximately 22% at RT and at 105°C)** show minimal variation, indicating **negligible Magnesium leakage**.
- Demonstrates **thermal robustness**, making the formulation suitable for transport and storage in hot climates.

Thank You !

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