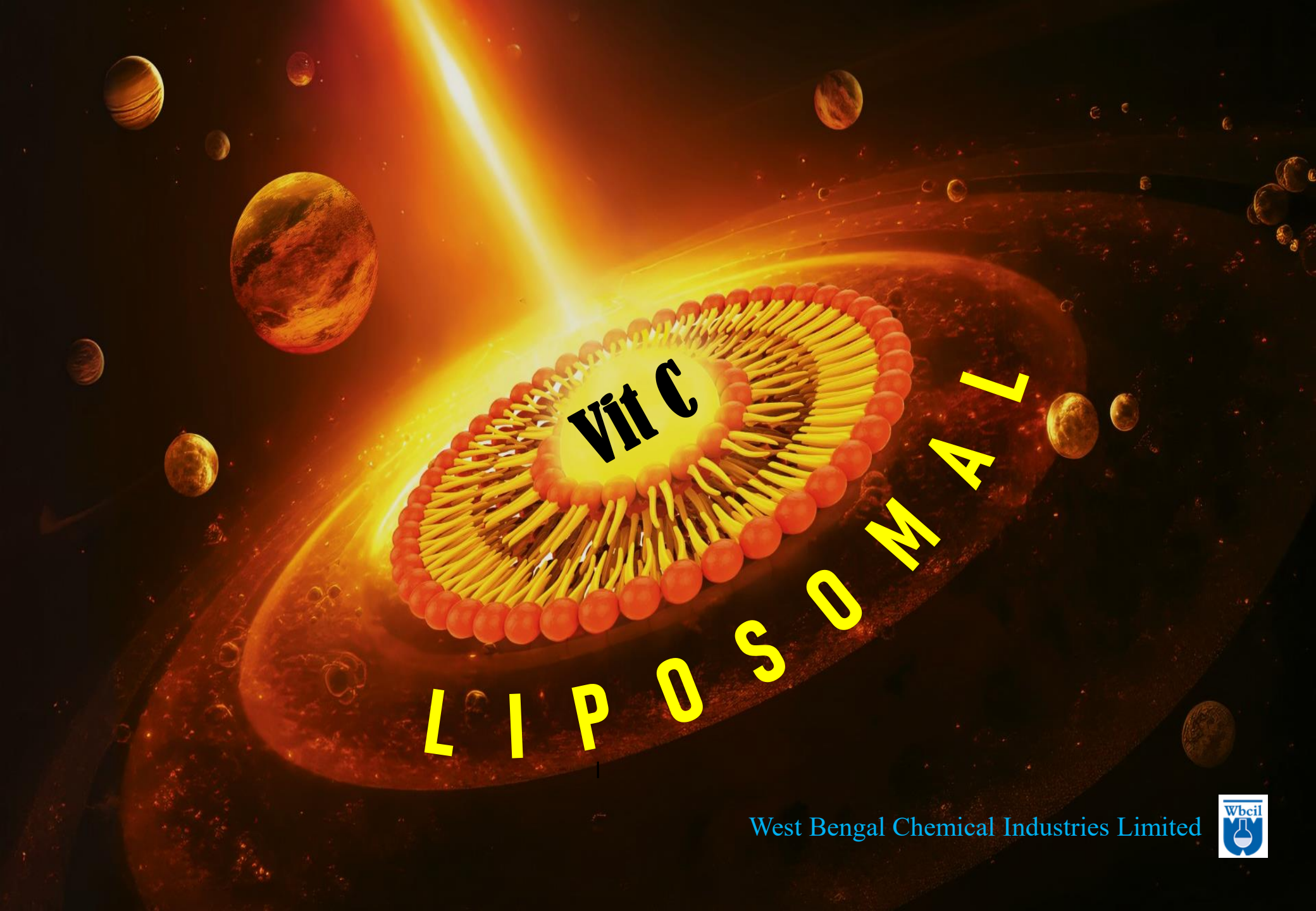
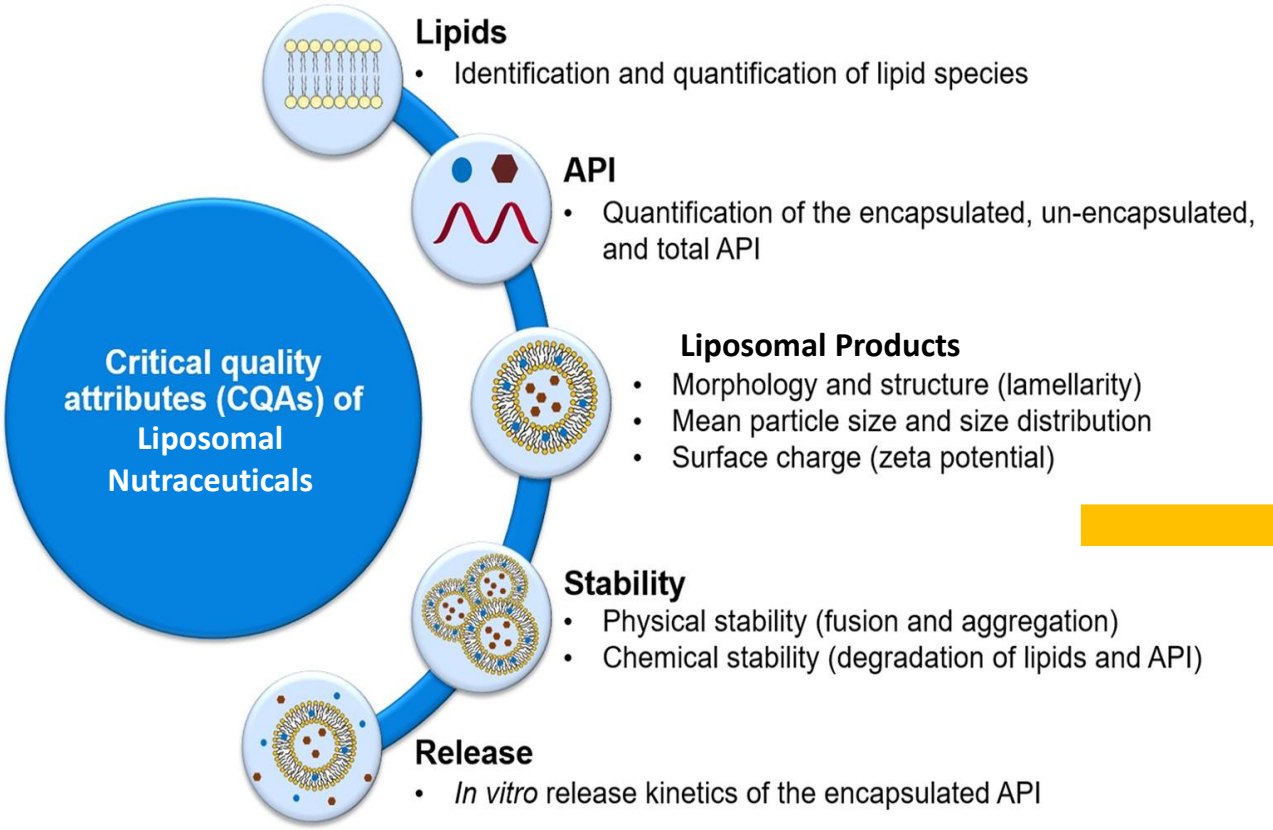


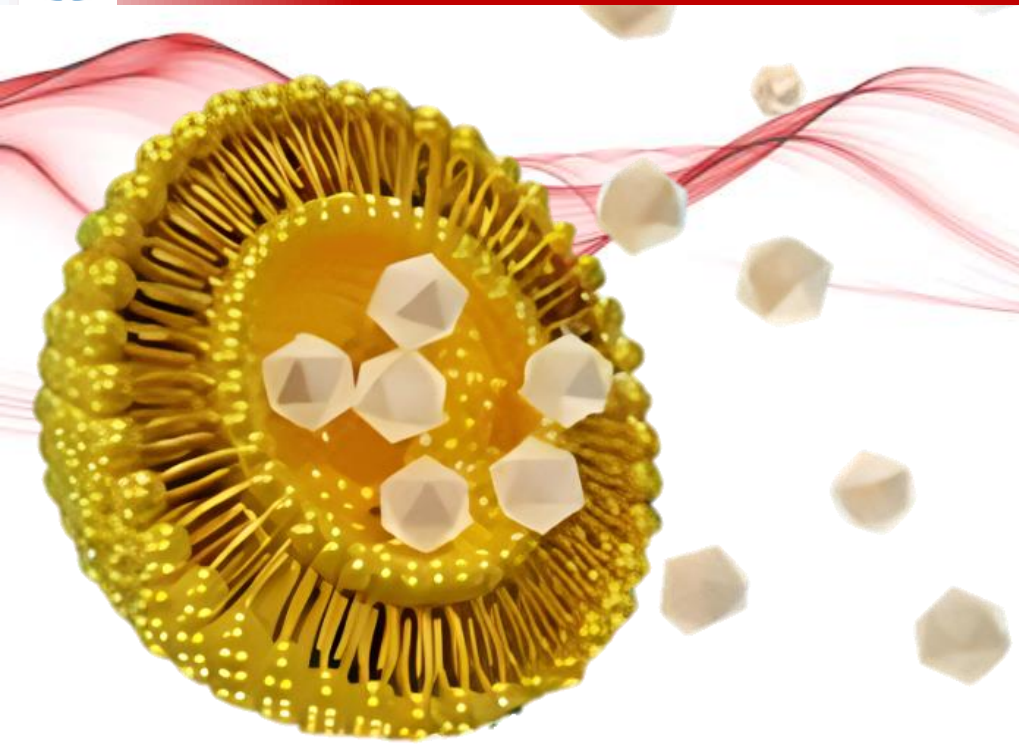
West Bengal Chemical Industries Limited

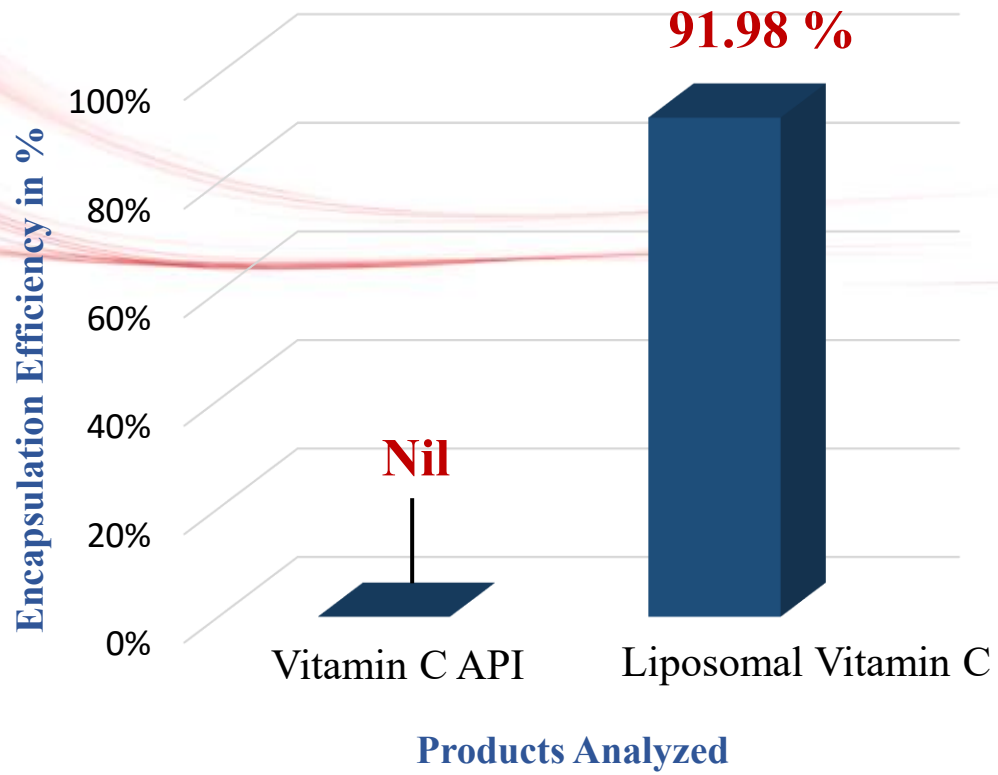
Summary of Characterizations Performed on Liposomal Vitamin C



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

1. Encapsulation Efficiency of 55.76% Liposomal Vitamin C





- **Acceptance criteria:**
  - **Assay : NLT 50%**
  - **Encapsulation efficiency : NLT 70%**

Encapsulation Efficiency measured by validated HPLC analytical data

- Liposomal encapsulation ensures **91.98% 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 vitamin C forms.

2. Dynamic Light Scattering Analysis of Liposomal Vitamin C

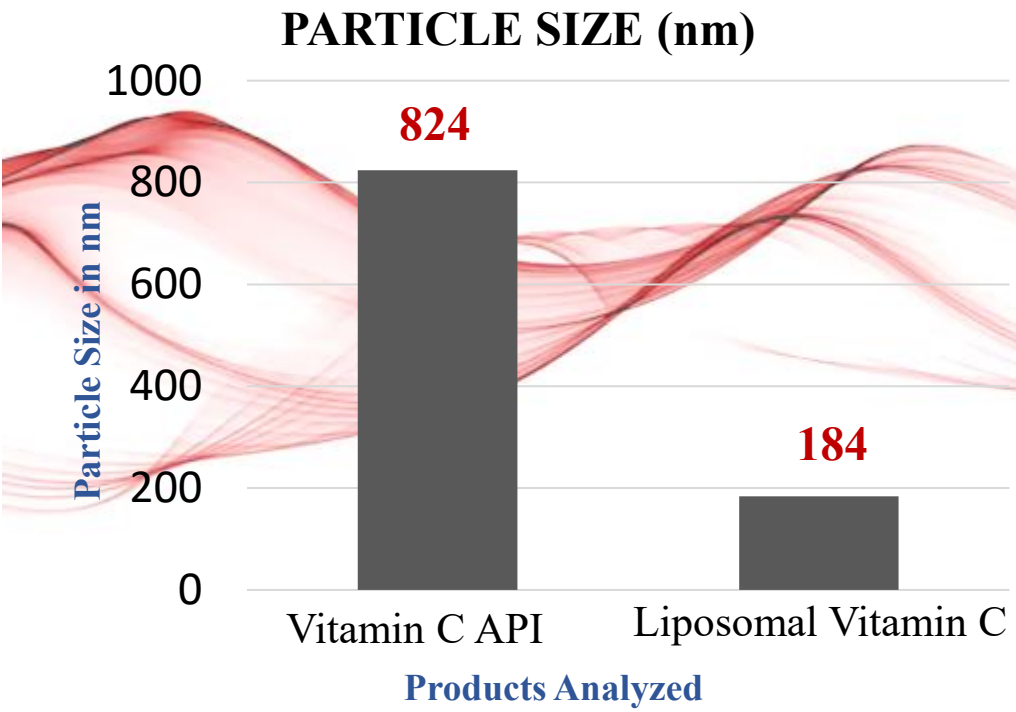


Figure 1 – Chart showing the particle size of Vitamin C API with Liposomal Vitamin C

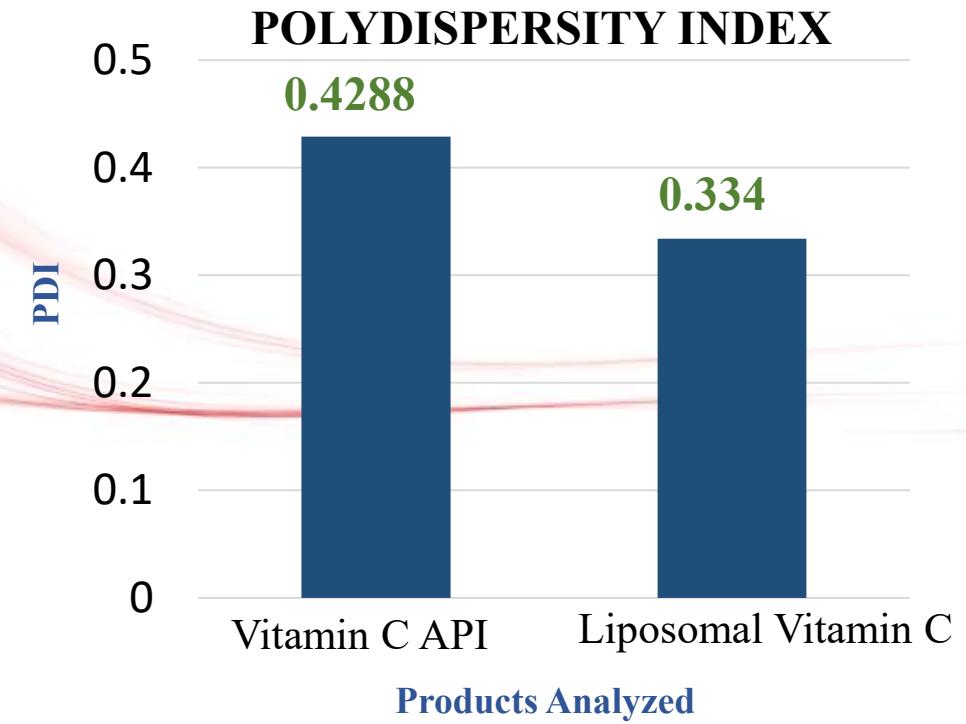


Figure 2 – Polydispersity Index (PDI) of Liposomal Vitamin C in solution

- Nanosized, uniform particles offer greater colloidal stability and improved shelf life.
- Smaller particles (particle size: 184 and PDI 0.334 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 Vitamin C

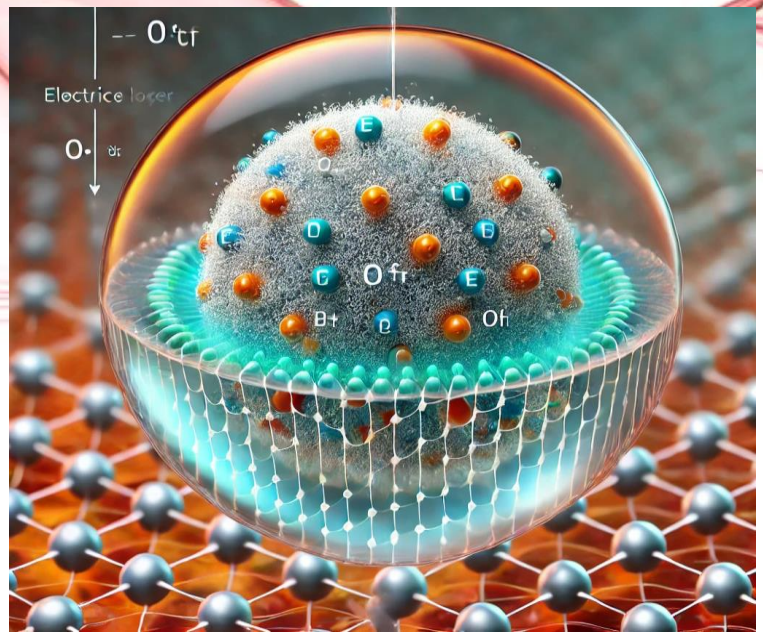


Figure 1 – Zeta potential visualization showing a negatively charged particle suspended in a liquid solution, surrounded by a well-defined electric double layer of positively charged ions.

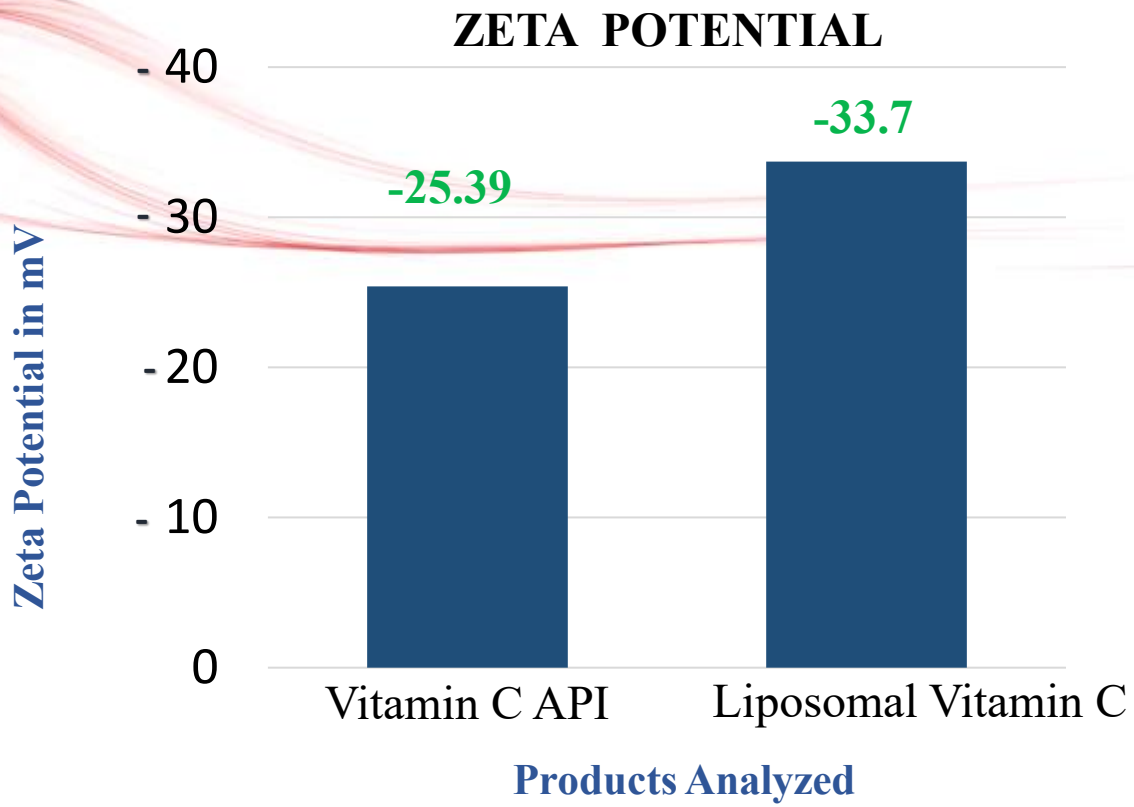


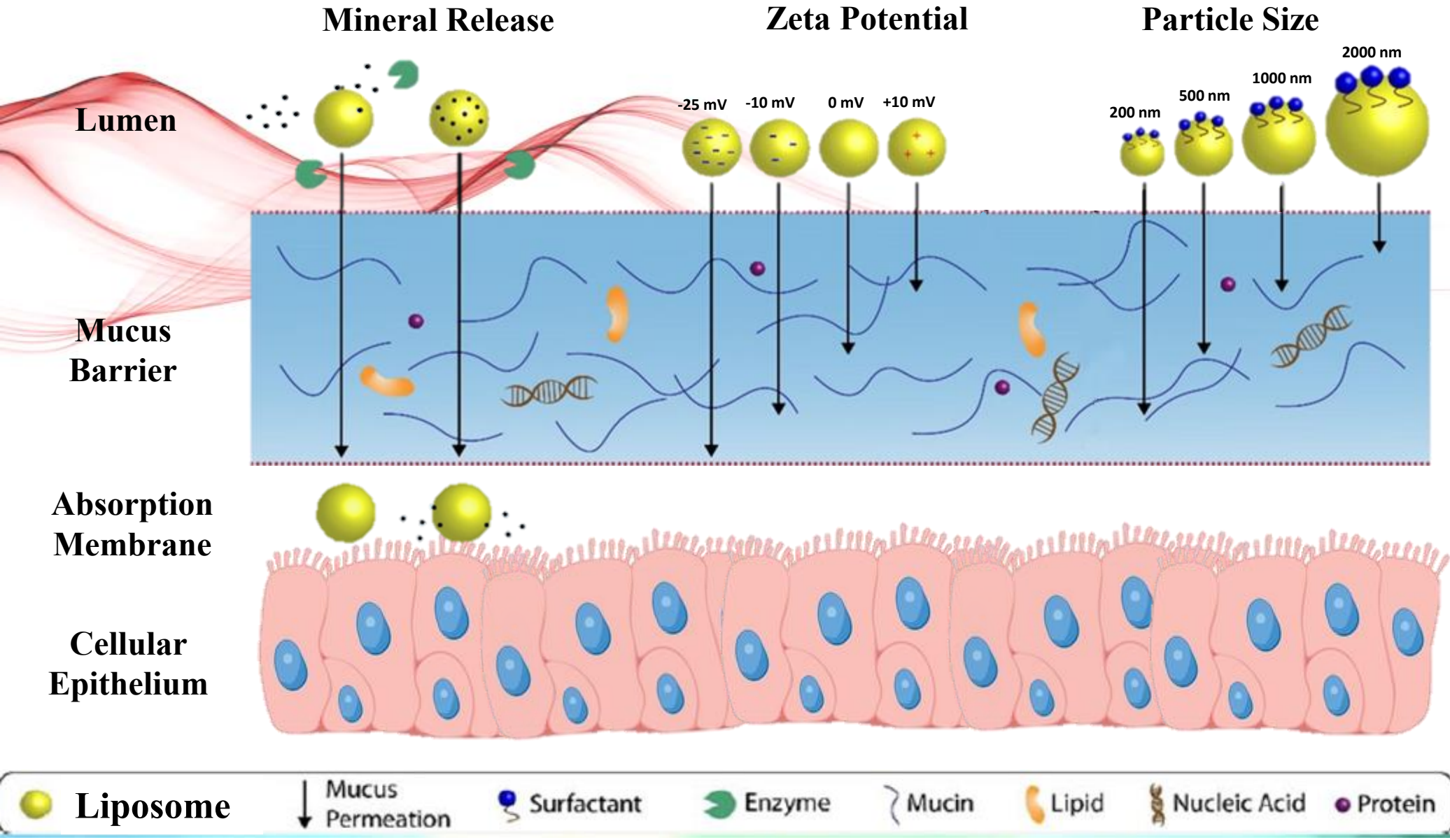
Figure 2 – Chart comparing the Zeta potential of Vitamin C API and Liposomal Vitamin C showing Vitamin C in Liposomal form is stable and less prone to agglomerate in solution.

- Liposomal Vitamin C shows **high zeta potential (-33.7 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 Vitamin C 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 Vitamin C

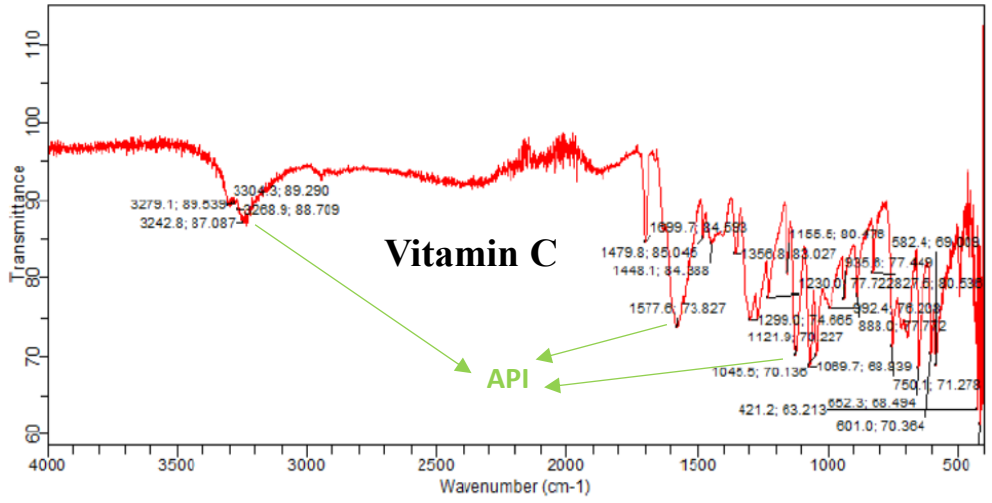


Figure 1: IR Transmission spectrum showing bands at different wavelengths of Vitamin C API

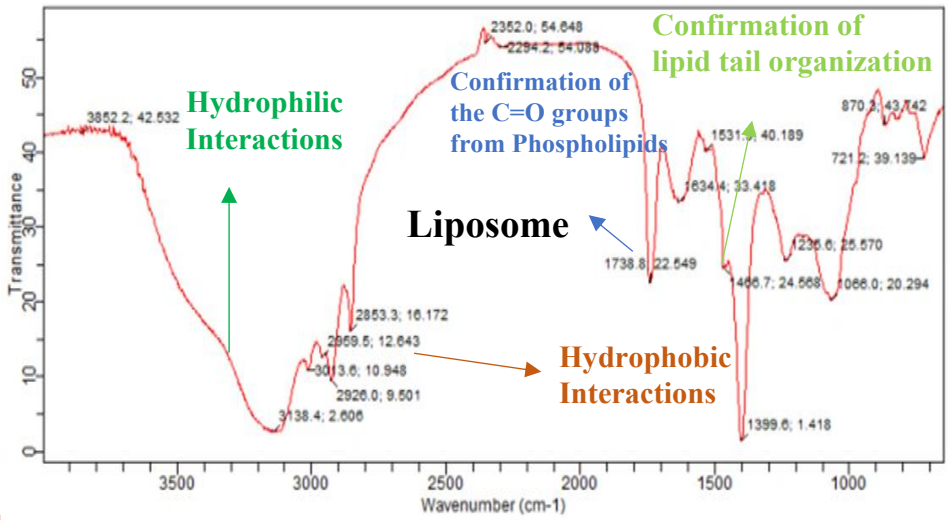


Figure 2: Hydrophobic and Hydrophilic interactions within Empty Liposome

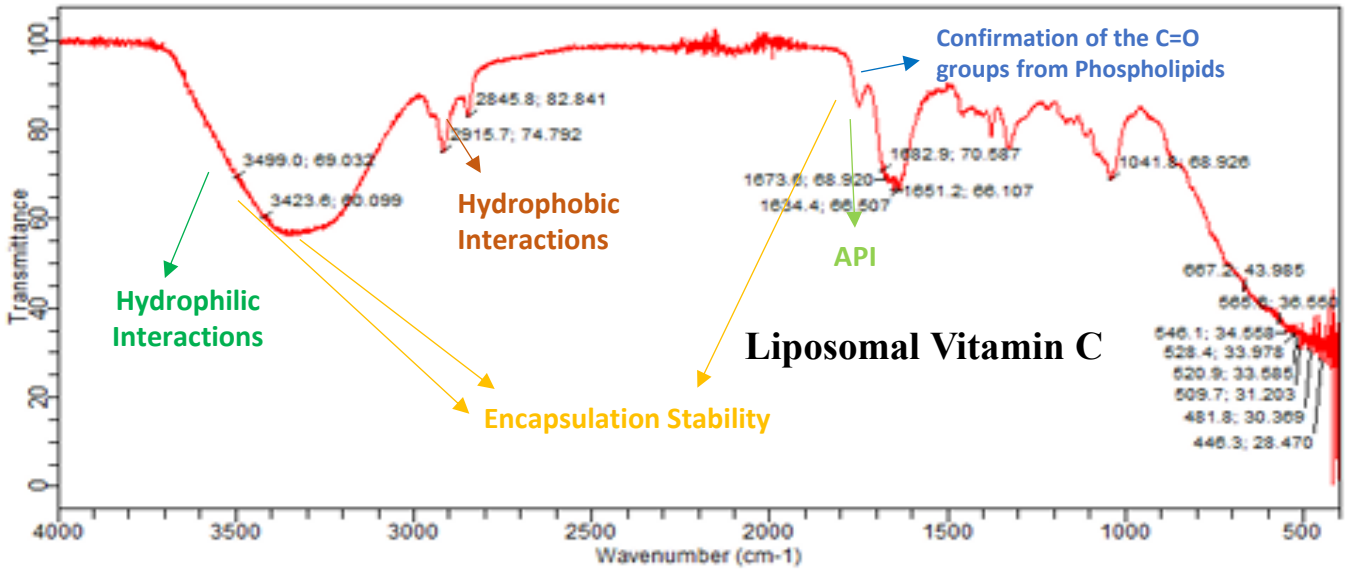


Figure 3: IR Transmission spectrum of Liposomal Vitamin C is shown

4b. Summary of FTIR Analysis of Liposomal Vitamin C

1. **Confirmation of the C=O and OH groups –** C=O stretch from ester group of phospholipids at **1734 cm^{-1}** , confirming the presence of phospholipids

2. **Hydrophobic Interactions -** C-H stretch around **2915 cm^{-1}** and **2845 cm^{-1}** , alkyl chains of phospholipids, hydrophobic interactions) (C-H bending at **1450 cm^{-1}**, further confirming hydrophobic interactions

3. **Hydrophilic Interactions -** O-H stretching around **3499 cm^{-1}** and C=O stretch at **1073 cm^{-1}** and **1041 cm^{-1}** suggesting water and polar group interactions.

4. **API -** O–H stretching peak at **3423.6 cm^{-1}** and C=O stretching at **1634.4 cm^{-1}** confirm the presence of ascorbic acid.

5. **Encapsulation Stability -** C=O stretch at **1734 cm^{-1}** and the O-H stretching at **3499 cm^{-1}** and **3423 cm^{-1}** confirm the encapsulation of Vitamin C within the Liposomal bilayer.

5. Elemental Analysis of Liposomal Vitamin C

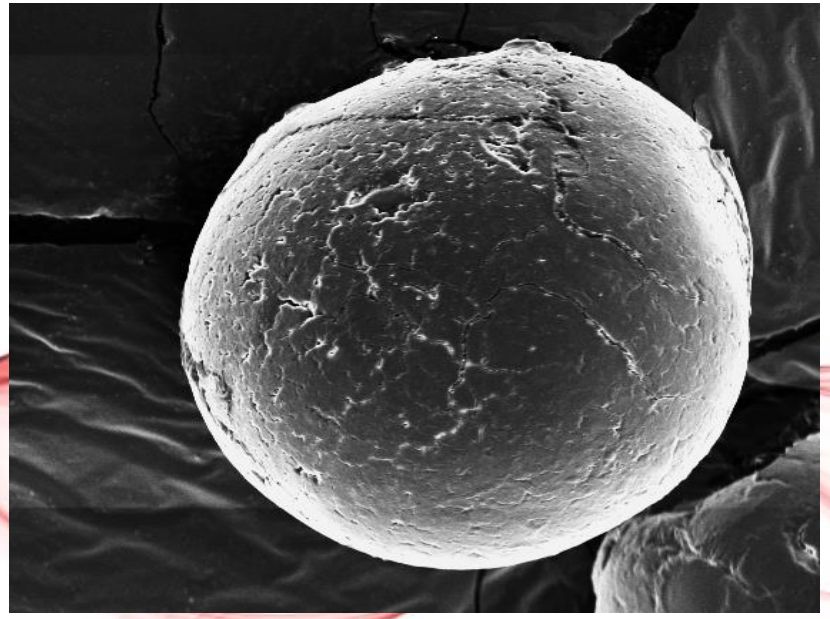


Figure 1 – SEM image of Liposomal Vitamin C showing the area scanned using Energy Dispersive X-Ray Spectroscopy (EDAX)

- **EDAX scan** shows that only the liposomal shell elements are detected, proving that the Vitamin C core is completely encapsulated within the liposome.

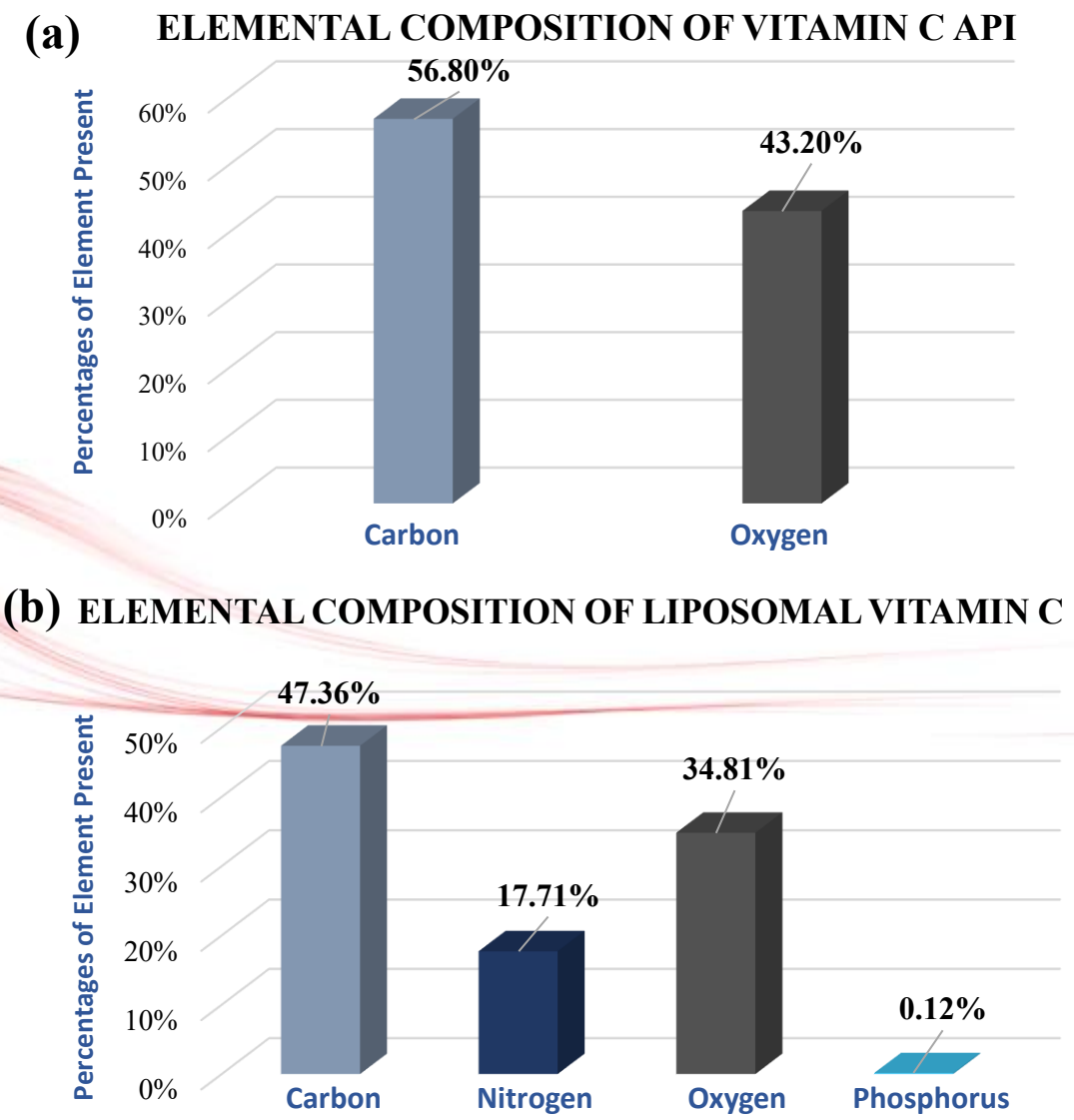


Figure 2 – A graphical representation of the percentages of elements composing (a) Vitamin C API and (b) Liposomal Vitamin C

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

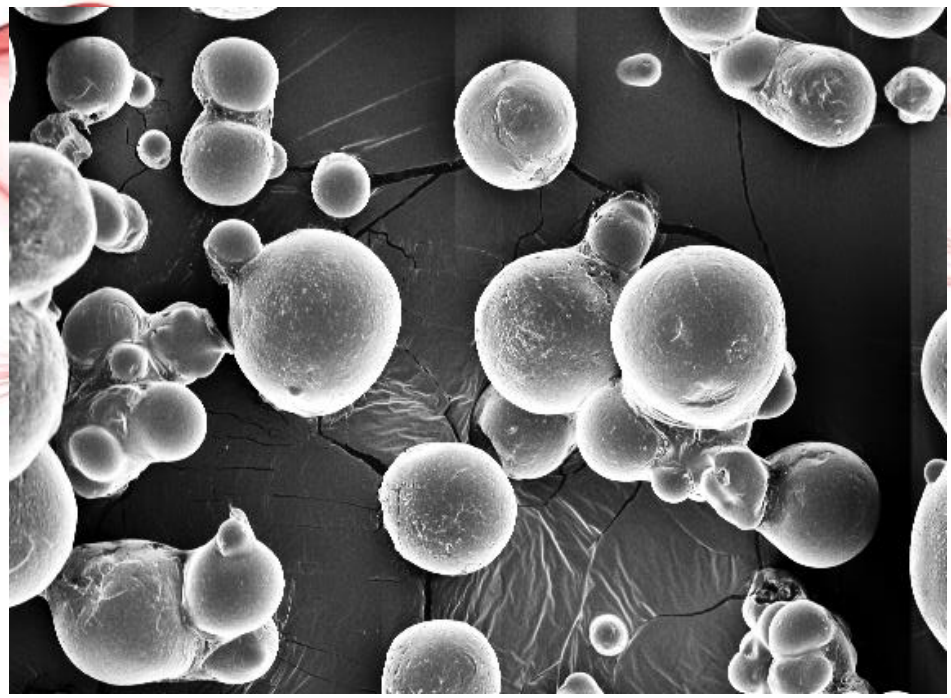


Figure 1 – SEM image showing several Vitamin C Liposomes scattered within the field of view under observation

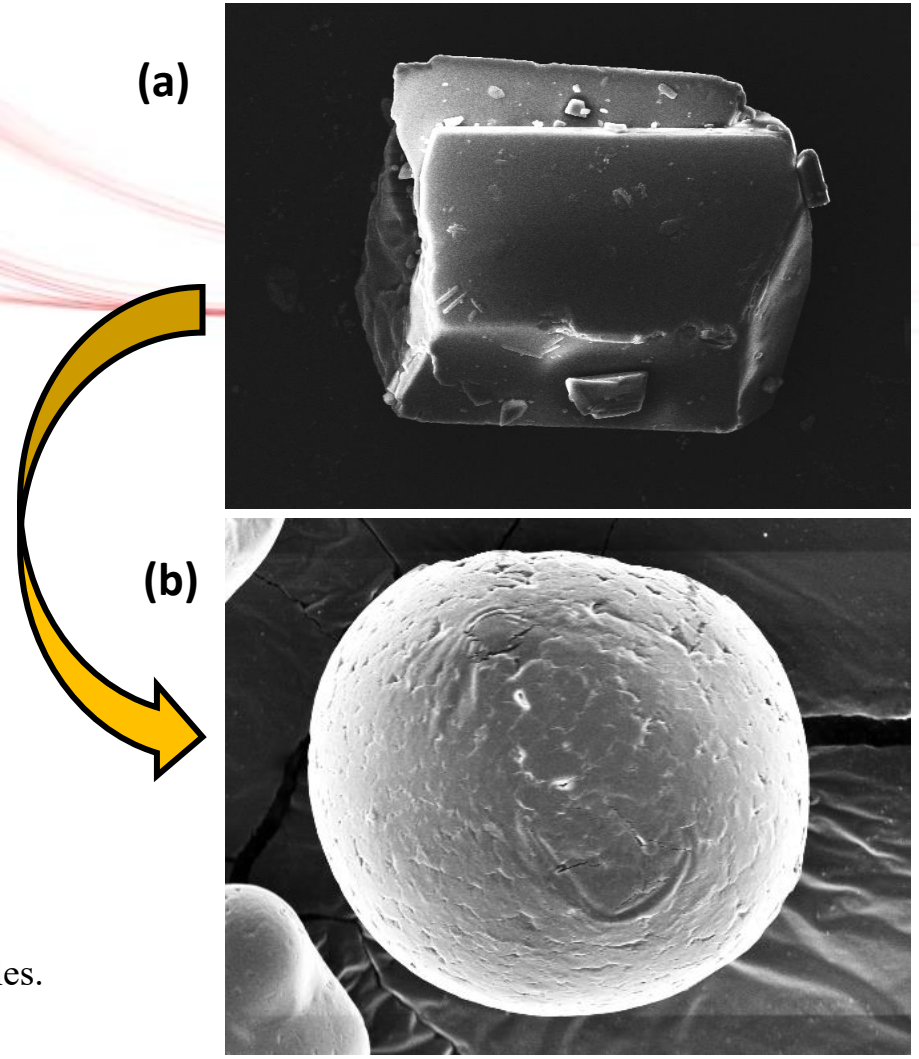


Figure 2 – SEM panels showing transformation from (a) Vit C API to (b) Liposomal Vit C after encapsulation.

- Spherical morphology observed in liposomal Vitamin C 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 Vitamin C

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

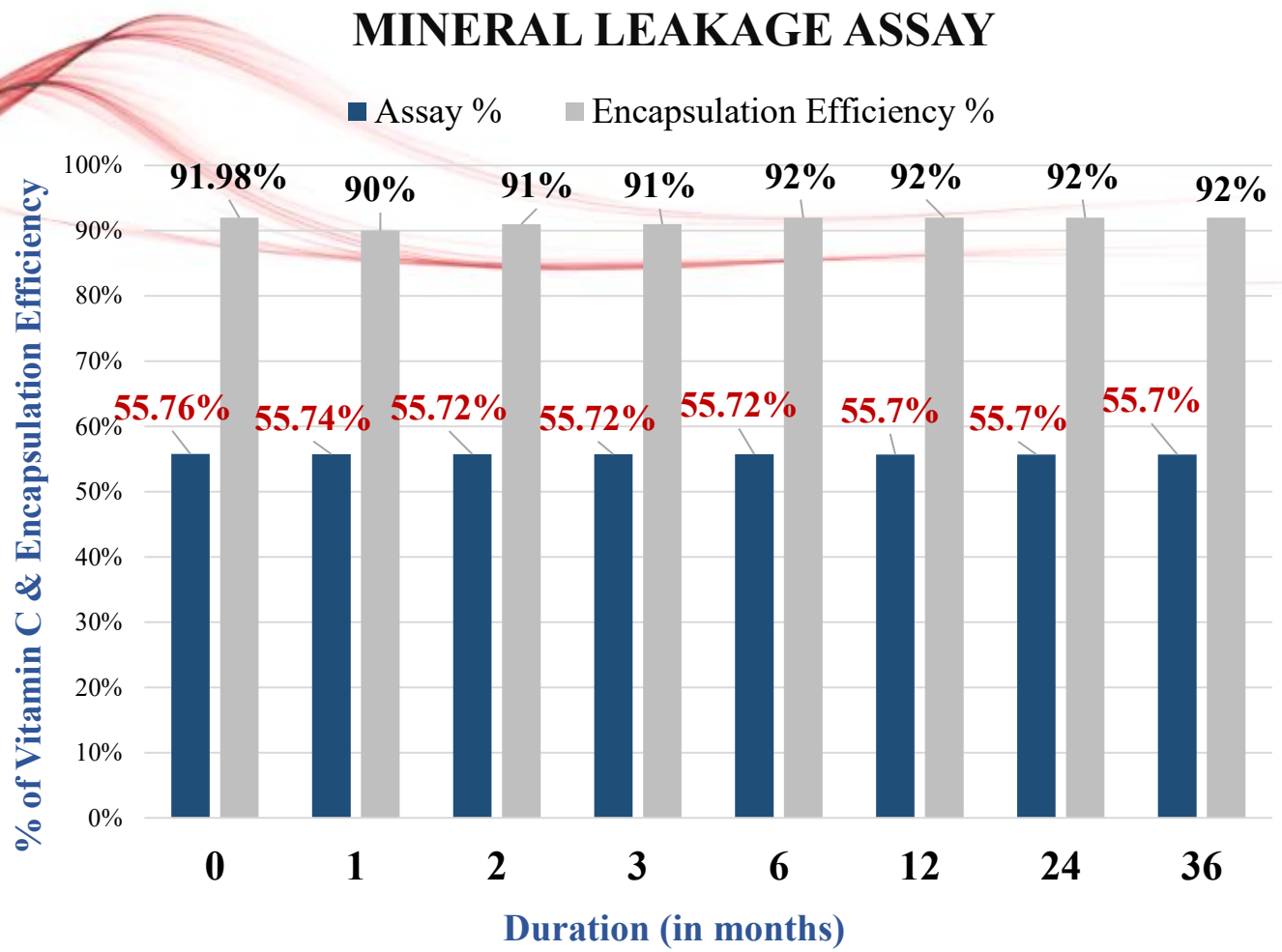


Figure 2 – Chart comparing the stability of Liposomal Vitamin C stored over a period of 3 years at 40°C ± 2 °C and a relative humidity of 75% ± 5%.

- **Encapsulation efficiency remains high (91–92%)** throughout 3 years of storage, indicating stable liposome structure.
- **Assay values for free Vitamin C remain low (~50%)**, showing minimal leakage over time.
- The formulation shows **excellent retention of Vitamin C**, confirming its suitability for long-term shelf storage.

8. Stability of Liposomal Vitamin C at Elevated Temperatures

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

- **Encapsulation efficiency remains high (≈93%)** even after exposure to 105°C for 4 hours.
- **Assay values (50% at RT vs. 33.61% at 105°C)** show minimal variation, indicating **negligible Vitamin C leakage**.
- Demonstrates **thermal robustness**, making the formulation suitable for transport and storage in hot climates.

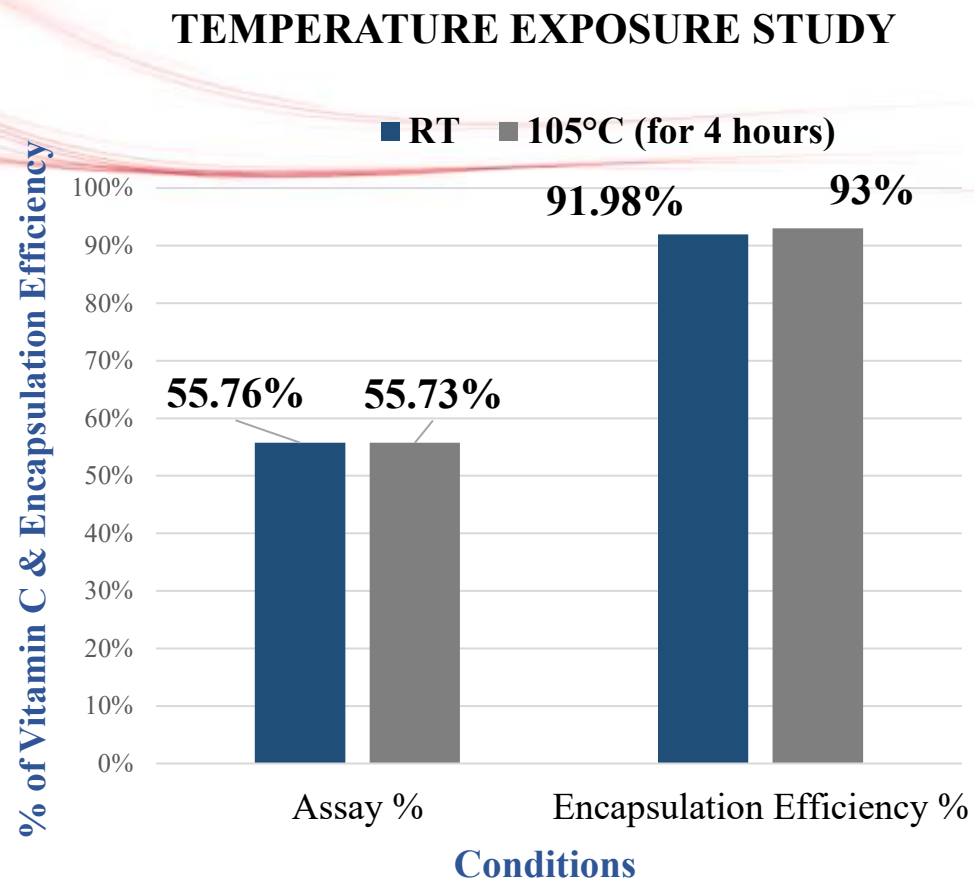


Figure 2 – Chart comparing the stability of Liposomal Vitamin C both at room temperature (RT) and at 105°C exposure for 4 hours.

9a. Differential Scanning Calorimetry Analysis

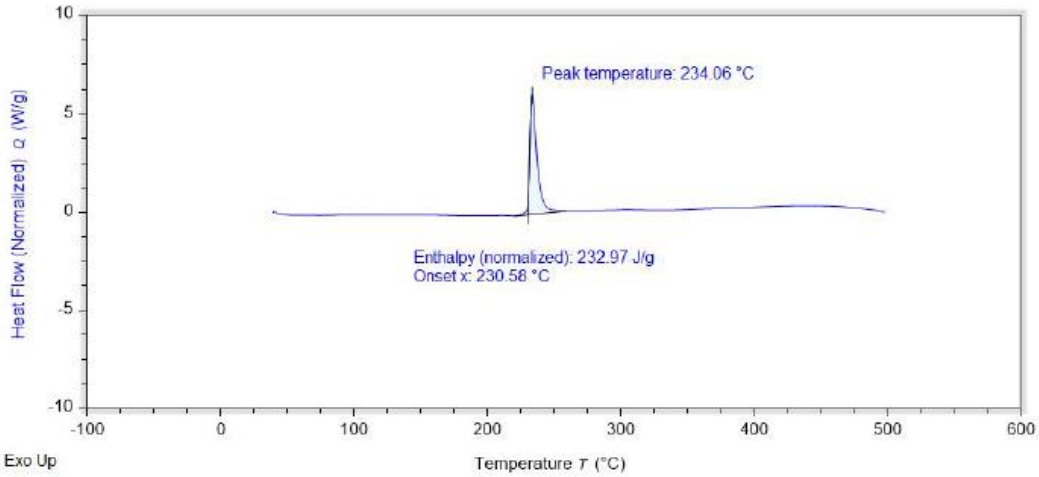


Figure 1: DSC Thermogram of Vitamin C API

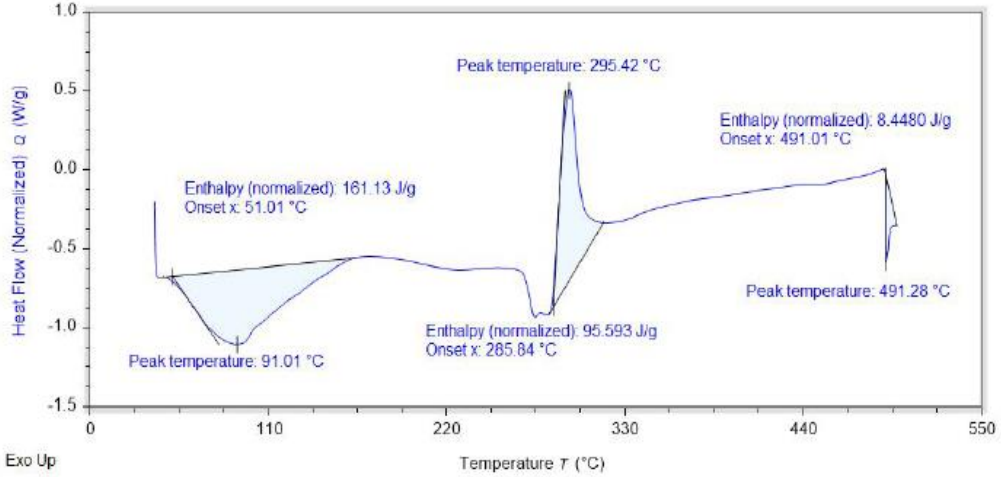


Figure 2: DSC Thermogram of Empty Liposome

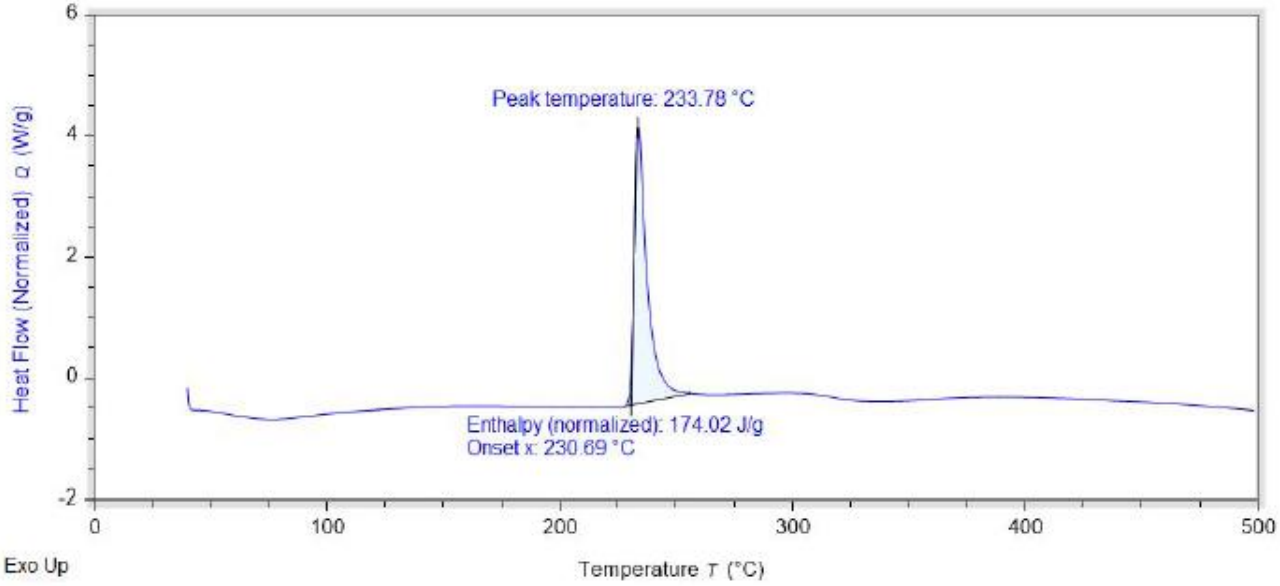
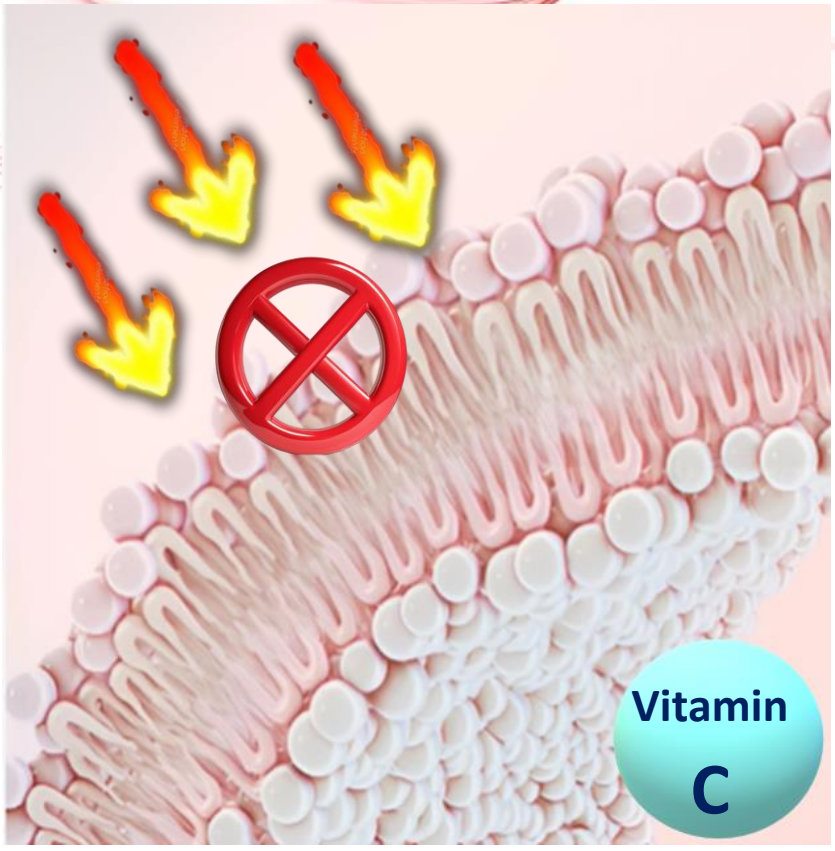


Figure 3: DSC Thermogram of Liposomal Vitamin C

9b. Endothermic Study of Liposomal Vitamin C Using Differential Scanning Calorimetry Analysis

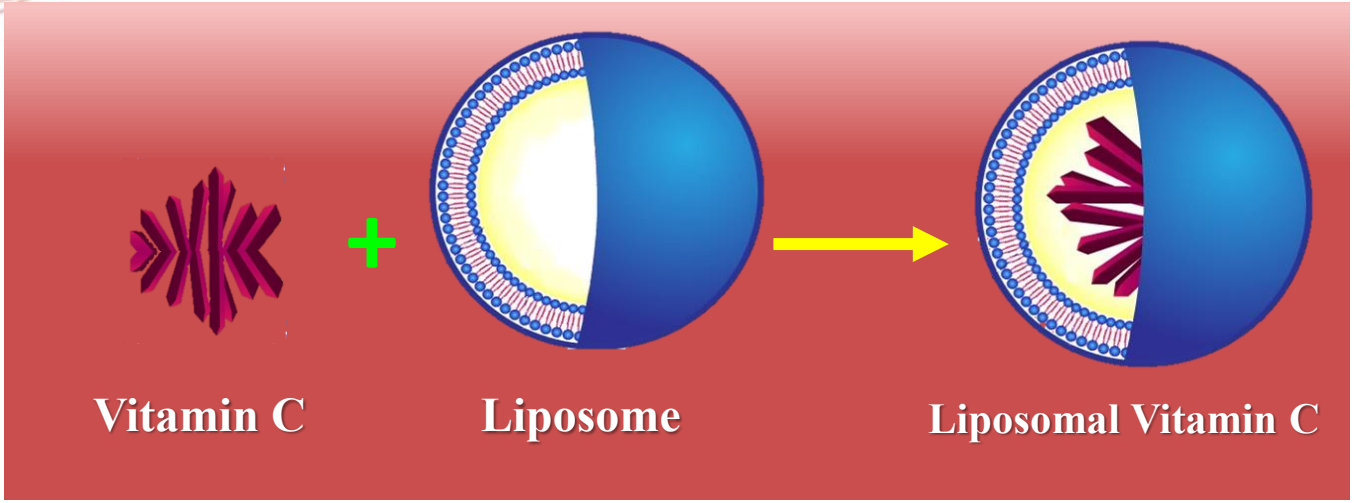


An illustration showing how the phospholipid bilayer is hindering the heat from reaching Vitamin C API which improves thermal stability of Liposomal Vitamin C.

Sample	Thermal Events (°C)	Enthalpy (J/g)	Inference
Vitamin C API	234.06 °C	232.97	Sharp peak suggests significant thermal event, likely melting or decomposition.
Empty Liposome	91.01 °C, 295.42 °C	161.13, 95.593, 8.4480	Peaks are for thermal events corresponding to lipid melting or phase transition in the liposome.
Liposomal Vitamin C	233.78 °C	174.02	The lower enthalpy of Liposomal Vitamin C indicates that the liposomal encapsulation is protecting the Vitamin C API from the direct effects of heat.

**Thermograms available for reference*

10. Mineral Loading Capacity



Formulation of Vitamin C in Liposomes

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

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

Thank You!!!

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