



# Summary of Characterizations Performed on Liposomal Vitamin C



Identification and quantification of lipid species



Quantification of the encapsulated, un-encapsulated, and total API

Critical quality attributes (CQAs) of Liposomal Vitamin



- Morphology and structure (lamellarity)
- Mean particle size and size distribution
- Surface charge (zeta potential)



- Physical stability (fusion and aggregation)
- Chemical stability (degradation of lipids and API)

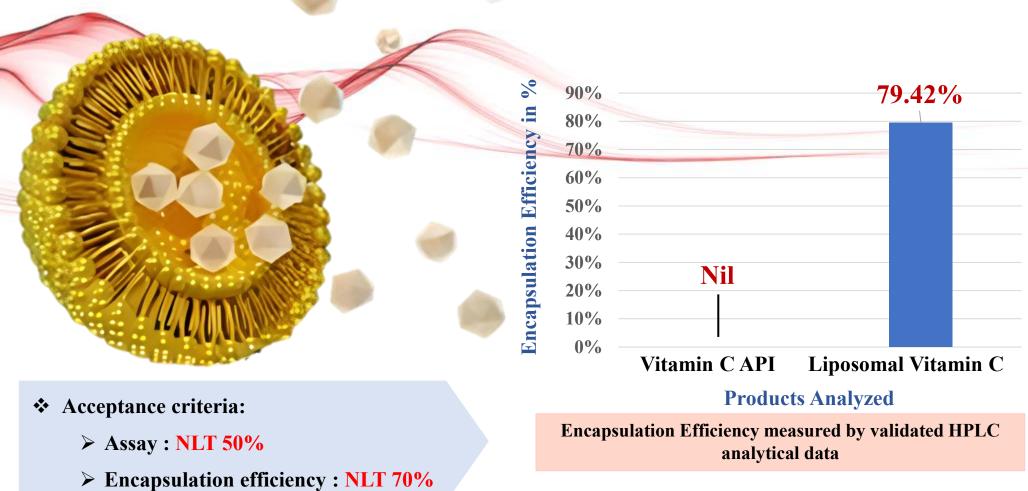


In vitro release kinetics of the encapsulated API

- 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



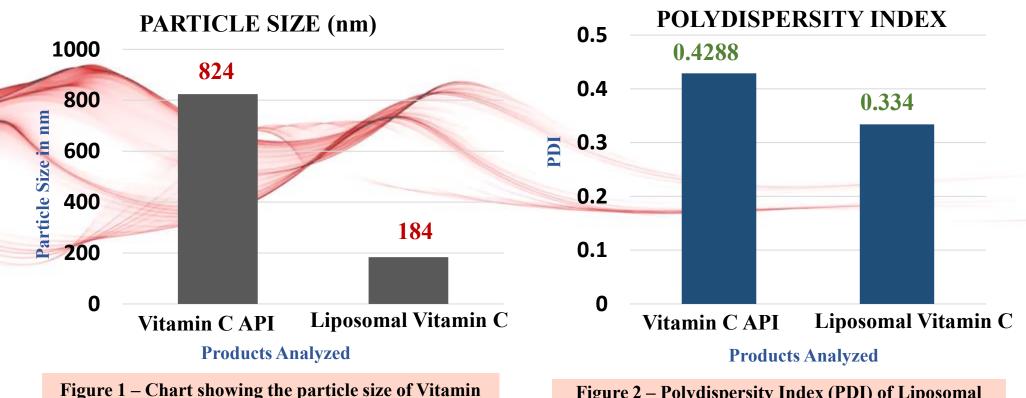
## 1. Encapsulation Efficiency of 55.76% Liposomal Vitamin C



- ➤ Liposomal encapsulation ensures **79.42% efficiency.**
- 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



➤ Nanosized, uniform particles offer greater colloidal stability and improved shelf life.

C API with Liposomal Vitamin C

- > 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.

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

#### \* Acceptance criteria:

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



## 3a. Behavior of Liposomal Vitamin C

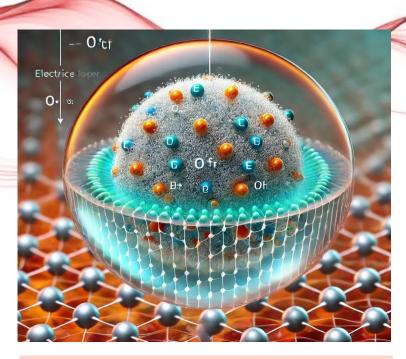


Figure 1 – Zeta potential visualization showing a negatively charged particle suspended in a liquid solution, surrounded by a well-defined electric double layer of positively charged ions.

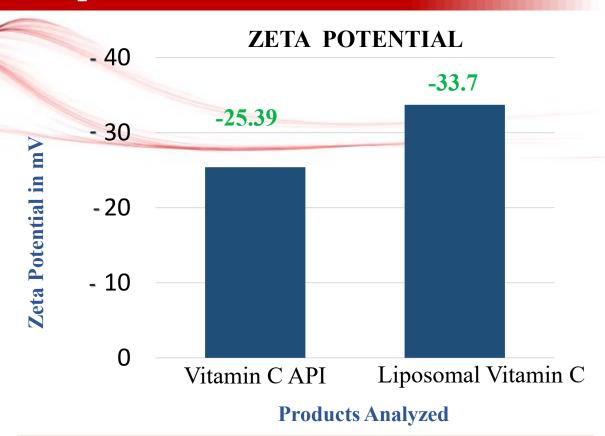


Figure 2 – Chart comparing the Zeta potential of Vitamin C API and Liposomal Vitamin C showing Vitamin C in Liposomal form is stable and less prone to agglomerate in solution.

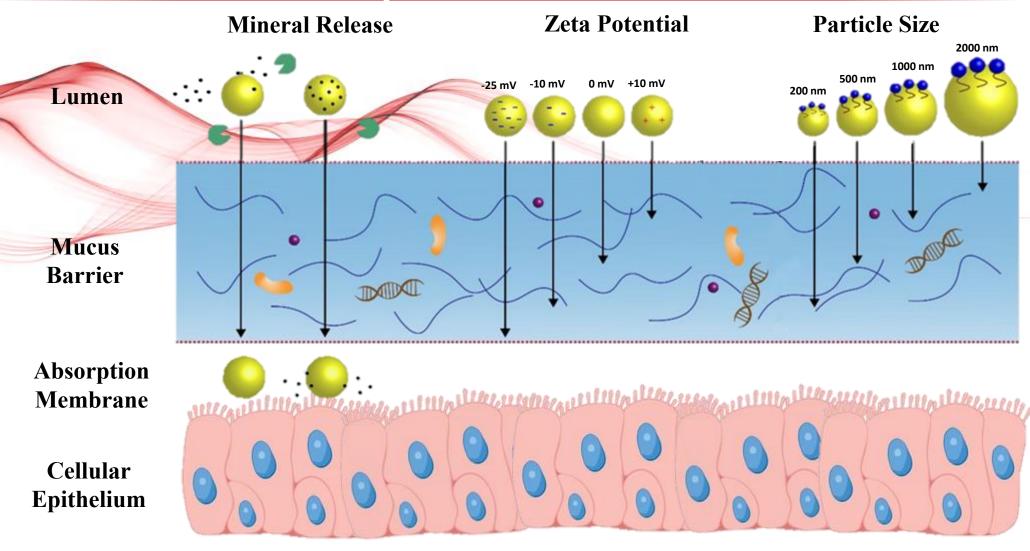
- ➤ Liposomal Vitamin C shows **high zeta potential (-33.7 mV)** → excellent colloidal stability.
- $\triangleright$  Prevents particle aggregation  $\rightarrow$  ensures uniform suspension.
- > Enhances product shelf life and bioavailability in liquid form.

#### \* Acceptance criteria:

> Zeta Potential : < -30 mV



# 3b. Absorption of Liposomal Vitamin C Represented Schematically on a Cellular Cross-Section



Enzyme

Mucus

Permeation

Liposome

Surfactant

Mucin

Lipid

Protein

Nucleic Acid



### 4a. FTIR Spectra of API, Liposome & Liposomal Vitamin C

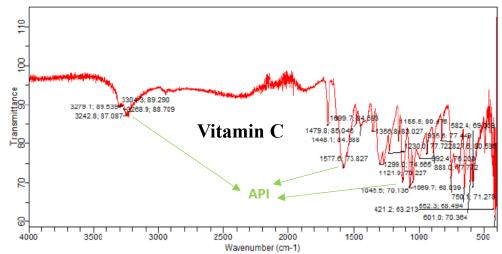


Figure 1: IR Transmission spectrum showing bands at different wavelengths of Vitamin C API

Figure 2: Hydrophobic and Hydrophilic interactions within Empty Liposome



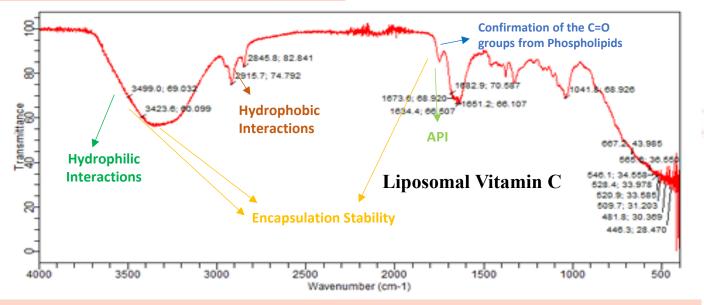


Figure 3: IR Transmission spectrum of Liposomal Vitamin C is shown



# 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<sup>-1</sup>, confirming the presence of phospholipids
- 2. Hydrophobic Interactions C-H stretch around 2915 cm<sup>-1</sup> and 2845 cm<sup>-1</sup>, alkyl chains of phospholipids, hydrophobic interactions) (C-H bending at 1450 cm<sup>-1</sup>, further confirming hydrophobic interactions
- 3. Hydrophilic Interactions O-H stretching around 3499 cm<sup>-1</sup> and C=O stretch at 1073 cm<sup>-1</sup> and 1041 cm<sup>-1</sup> suggesting water and polar group interactions.
- 4. API O-H stretching peak at 3423.6 cm<sup>-1</sup> and C=O stretching at 1634.4 cm<sup>-1</sup> confirm the presence of ascorbic acid.
- 5. Encapsulation Stability C=O stretch at 1734 cm<sup>-1</sup> and the O-H stretching at 3499 cm<sup>-1</sup> and 3423 cm<sup>-1</sup> confirm the encapsulation of Vitamin C within the Liposomal bilayer.



# 5. Elemental Analysis of Liposomal Vitamin C

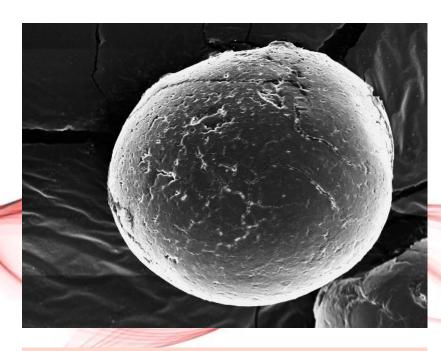
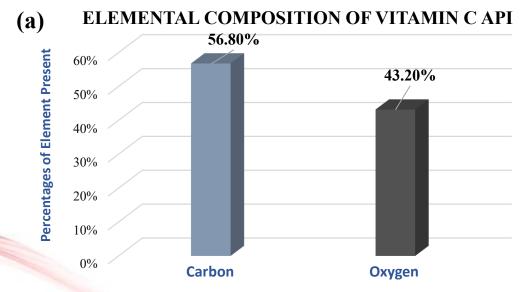


Figure 1 – SEM image of Liposomal Vitamin C showing the area scanned using Energy Dispersive X-Ray Spectroscopy (EDAX)

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



#### (b) ELEMENTAL COMPOSITION OF LIPOSOMAL VITAMIN C

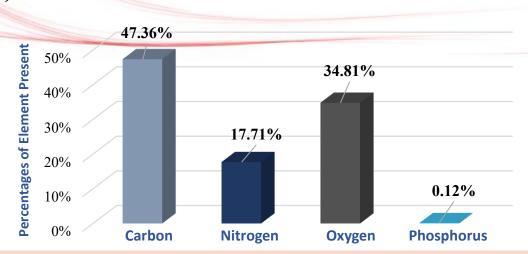
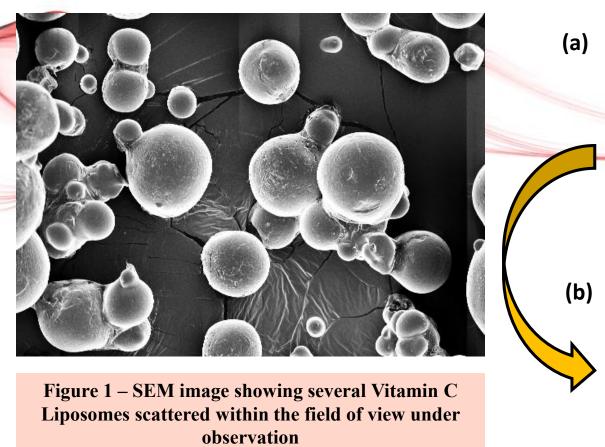


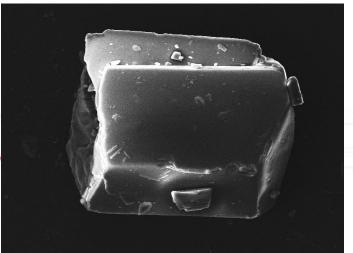
Figure 2 – A graphical representation of the percentages of elements composing (a) Vitamin C API and (b) Liposomal Vitamin C



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



- Spherical morphology observed in liposomal Vitamin C 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.



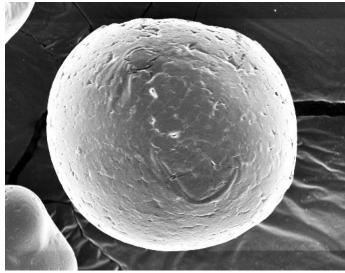


Figure 2 – SEM panels showing transformation from (a) Vit C API to (b) Liposomal Vit C after encapsulation.



## 7. Leakage of Liposomal Vitamin C



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

- Encapsulation efficiency remains high (~79%) throughout 3 years of storage, indicating stable liposome structure.
- Assay values for free Vitamin C remain low (~55%), showing minimal leakage over time.
- The formulation shows **excellent retention of Vitamin** C, confirming its suitability for longterm shelf storage.

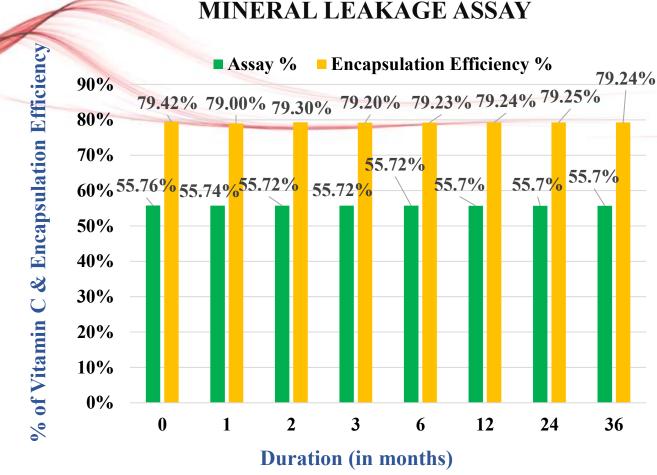


Figure 2 – Chart comparing the stability of Liposomal Vitamin C stored over a period of 3 years at  $40^{\circ}$ C  $\pm 2^{\circ}$ C and a relative humidity of  $75\% \pm 5\%$ .



# 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 (≈78%) even after exposure to 105°C for 4 hours.
- Assay values (~55.76% at RT vs. ~55.73% 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.

#### TEMPERATURE EXPOSURE STUDY

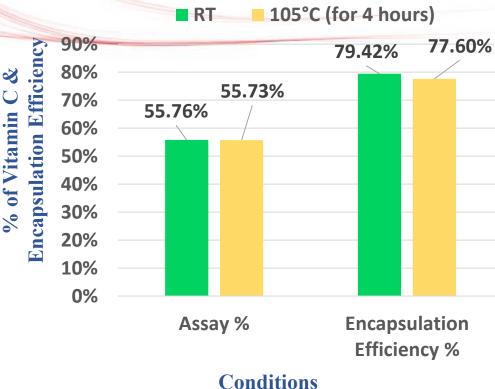


Figure 2 – Chart comparing the stability of Liposomal Vitamin C both at room temperature (RT) and at 105°C exposure for 4 hours.











