

Innovative Liposomal Vitamin C by West Bengal Chemical Industries Ltd., Kolkata, India: Enhancing Nutraceutical Effectiveness

Poulami Gupta Banerjee¹, Atanuka Paul², Argha Chakraborty³, Subrata Kundu⁴

From, ¹Scientific Editor, ²Scientist, ³Manager, ⁴Senior Executive, Department of Scientific Research, West Bengal Chemical Industries Ltd. Kolkata, India

ABSTRACT

Objective: To evaluate the physicochemical properties, stability, and absorption behavior of a liposomal Vitamin C formulation developed by West Bengal Chemical Industries Ltd. (WBCIL) using non-genetically modified sunflower-derived phosphatidylcholine, with a focus on its potential as a topical alternative to conventional Vitamin C supplements. **Methods:** The liposomal formulation was synthesized and subjected to comprehensive characterization using advanced analytical techniques. High-Performance Liquid Chromatography (HPLC) was used to determine encapsulation efficiency. Fourier Transform Infrared Spectroscopy (FTIR) was employed to identify functional groups, while X-Ray Photoelectron Spectroscopy (XPS) confirmed elemental composition. Dynamic Light Scattering (DLS) was used to measure hydrodynamic particle size, zeta potential, and polydispersity index. Stability and sustained release of Vitamin C were evaluated under physiological and thermal conditions. **Results:** The formulation demonstrated a high encapsulation efficiency of 91.98%, with an average particle size of 184 nm. The zeta potential was -33.7 mV, indicating excellent electrostatic stability, while the polydispersity index (PDI) of 0.334 reflected uniform particle distribution. FTIR confirmed the presence of functional groups associated with Vitamin C and phosphatidylcholine, and XPS analysis detected the target elements on the surface of the liposomal Vitamin C. The formulation exhibited sustained release properties and maintained structural integrity under various conditions, suggesting good thermal and physiological stability. **Conclusion:** WBCIL's liposomal Vitamin C formulation offers high encapsulation efficiency, nanoscale size, and excellent stability, making it a promising and bioavailable alternative to traditional Vitamin C supplements. Its potential for topical application enhances its value for skin health, particularly in promoting collagen synthesis and photoprotection.

Key words: Liposomal Vitamin C, Bioavailability, Phosphatidylcholine, Nanocarrier Delivery System, Nutraceutical Encapsulation

Vitamin C, or ascorbic acid, is a water-soluble essential nutrient that plays a critical role in numerous physiological functions, including immune defence, collagen synthesis, antioxidant protection, iron absorption, and the maintenance of cartilage, bones, and teeth [1]. As a potent antioxidant, it scavenges free radicals and helps reduce oxidative stress, thereby contributing to the prevention and management of chronic diseases such as cardiovascular disorders, certain cancers, and neurodegenerative conditions [2]. It is also crucial for the biosynthesis of neurotransmitters and L-carnitine, making it indispensable for energy metabolism and brain health [3]. The absorption of Vitamin C in the human body primarily occurs in the small intestine through a saturable, active transport mechanism mediated by sodium-dependent vitamin C transporters (SVCTs), particularly SVCT1 and SVCT2.

SVCT1, expressed on the apical membrane of intestinal epithelial cells, facilitates the uptake of the reduced form of ascorbic acid from the intestinal lumen into the enterocytes. Once inside the cells, Vitamin C is transported into the bloodstream via the basolateral membrane and distributed to various tissues.

However, due to the saturation of SVCT1 at moderate doses, increasing oral intake beyond a certain threshold (approximately 200–400 mg/day) does not proportionally increase plasma concentrations, as excess Vitamin C is rapidly excreted via the kidneys [4]. In contrast, liposomal Vitamin C employs a different and more efficient absorption pathway. Liposomes are nano-sized phospholipid bilayer vesicles that encapsulate hydrophilic compounds like Vitamin C within their aqueous core. When ingested, liposomal Vitamin C is absorbed primarily through passive diffusion or endocytosis

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Correspondence to: Dr. Poulami Gupta Banerjee, Department of Scientific Research, West Bengal Chemical Industries Ltd. Kolkata, India.

Email: banerjee.pg@wbCIL.co.in

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across the intestinal mucosa. The phospholipid composition of liposomes mimics biological membranes, allowing them to fuse with cellular membranes and facilitate direct intracellular delivery of Vitamin C, bypassing the saturable SVCT transporters. This mechanism significantly enhances systemic bioavailability, allowing for higher and more sustained plasma levels of ascorbic acid [5]. Moreover, liposomes protect Vitamin C from degradation in the gastrointestinal tract, preserving its potency until cellular uptake. By circumventing traditional absorption limitations and offering a more stable, targeted delivery system, liposomal formulations present a promising advancement in Vitamin C supplementation, both for systemic health and localized dermatological applications.

To address these pharmacokinetic challenges, liposomal encapsulation technology has emerged as a transformative strategy for enhancing the delivery and efficacy of water-

soluble micronutrients like Vitamin C. Liposomes are microscopic vesicles composed of phospholipid bilayers that can encapsulate both hydrophilic and lipophilic substances, protecting them from degradation and facilitating improved absorption through the gastrointestinal tract [6]. This delivery method not only shields Vitamin C from oxidative damage and enzymatic breakdown but also promotes cellular uptake via endocytosis and membrane fusion mechanisms, resulting in higher bioavailability and prolonged circulation in the body [7].

Table 1 below presents a comparative overview of different formulations, dosages, and modes of administration of Vitamin C, highlighting the distinctions in absorption efficiency and clinical relevance across various studies and trials.

Table 1: Summary of Liposomal Vitamin C Absorption Studies from the literature

| Dose | Mode of Administration | Absorption | Formulation | Reference |
|---------------|------------------------|--|---|--|
| 1000 mg/day | Oral | 70% absorption in 4 hours (Caco-2 cell line) | Liposomal formulation with phosphatidylcholine | Padayatty et al., 2004 |
| 10% w/v | Topical | 2.9× higher skin absorption | Lipo-oil-some (a lipid-based vesicle encapsulating oil-soluble compounds or oil-loaded liposomal structure) with sodium deoxycholate & camellia oil | Lee et al., 2023 |
| 1 g IV | Injectable | 100% bioavailability | Standard IV solution (non-liposomal) | Padayatty et al., 2004 |
| 500 mg | Oral | Plasma saturation beyond 400 mg; low systemic benefit | Non-liposomal ascorbic acid | Levine et al., 1996 |
| 1 g | Oral | Higher plasma and leukocyte vitamin C levels compared to standard formulation | Liposomal vitamin C powder (Double Nutri™) | National Library of Medicine (U.S.), 2025 [8] |
| 1 g | Oral | 3.91× higher plasma vitamin C levels over 48 hours compared to standard formulation | Liposomal vitamin C (Zooki) | National Library of Medicine (U.S.), 2025 [9] |
| 500 mg | Oral | Significantly increased plasma and leukocyte vitamin C levels compared to standard and placebo | Liposomal vitamin C (LipoVantage®) | National Library of Medicine (U.S.), 2025 [10] |
| 1 g | Oral | Enhanced absorption and metabolism compared to standard formulation | Liposomal vitamin C | National Library of Medicine (U.S.), 2025 [11] |
| Not specified | Oral | Improved physical performance and reduced fatigue in older adults | Liposomal vitamin C with L-arginine | National Library of Medicine (U.S.), 2025 |
| Not specified | Oral | Improved fatigue severity and physical performance in COPD patients | Liposomal vitamin C with L-arginine | National Library of Medicine (U.S.), 2025 |
| Not specified | Oral | 1.77× more bioavailable than non-liposomal vitamin C | Liposomal vitamin C | Purpura et al. (2024) |
| Not specified | Oral | Increased serum vitamin C levels by 55% more than ascorbic acid after two hours | Liposomal vitamin C | Kim et al. (2024) |

The physicochemical characteristics of liposomal Vitamin C formulations play a crucial role in determining their effectiveness in various applications, as outlined in Table 2.

Table 2: An outline of particle size, zeta potential and encapsulation efficiency of liposomal Vitamin C reported in the literature

| Particle Size (nm) | Zeta Potential (mV) | Encapsulation Efficiency (%) | Reference |
|--------------------|---------------------|------------------------------|-------------------------|
| 110 ± 5 | -20 ± 2 | 85–90 | Danaei et al., 2018 |
| 133.9 | -31.87 | 85–90 | Akbarzadeh et al., 2013 |
| ~100 | -33.7 | Not specified | Mozafari, 2005 |
| <200 | -25 to -35 | Up to 90 | Lee et al., 2023 |

In summary, smaller particle sizes facilitate better absorption and skin penetration, while negative zeta potentials ensure stability and reduce the likelihood of aggregation. High encapsulation efficiency ensures the likelihood of the active ingredient being effectively delivered to the target site.

West Bengal Chemical Industries Ltd. (WBCIL), Kolkata, India, has developed a proprietary Liposomal Vitamin C formulation using non-GMO sunflower-derived phosphatidylcholine and nano-encapsulation technology. This formulation aims to provide a potent, stable, and highly bioavailable form of Vitamin C, addressing the limitations of

traditional supplements and offering an alternative to invasive, costly intravenous (IV) administration. With the growing demand for immune-boosting, anti-aging, and performance-enhancing nutraceuticals—especially amid global health challenges and increasing preventive healthcare awareness—liposomal formulations are gaining attention in both clinical and consumer health sectors. This article critically analyzes the formulation, efficacy, and advantages of WBCIL's Liposomal Vitamin C based on scientific data and current literature. A study using Caco-2 cell monolayers demonstrated that the liposomal formulation significantly enhanced absorption compared to free Vitamin C [8].

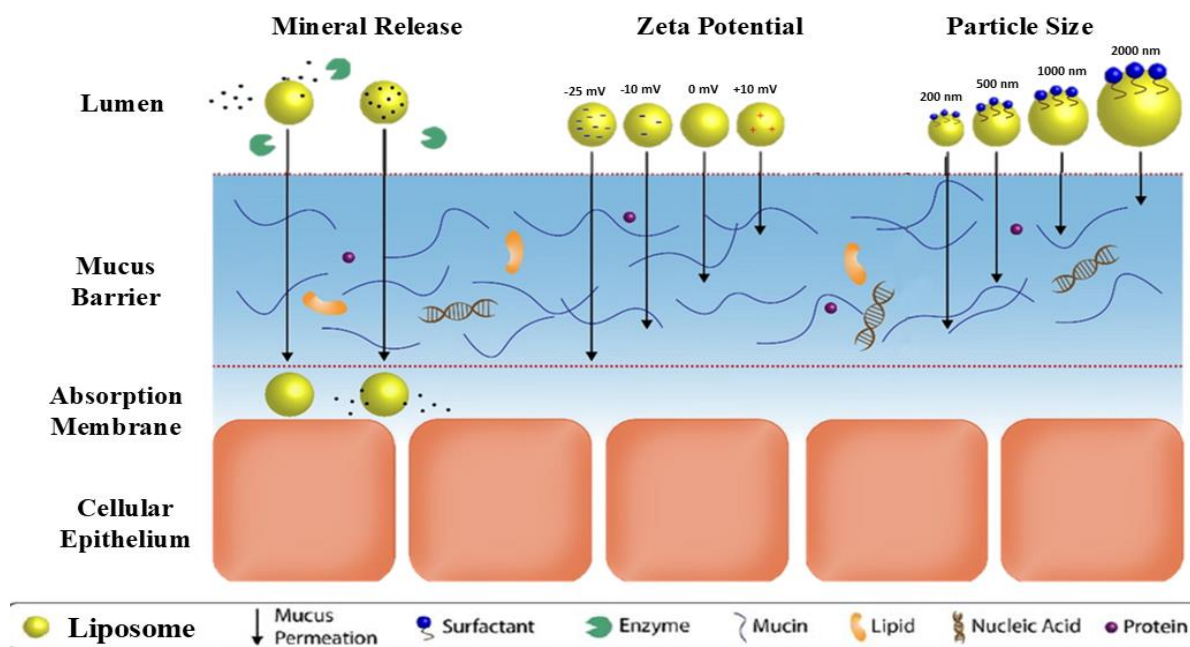


Figure 1: Pictorial Representation of Epidermal Vitamin C Absorption [8]

The bilayer structure facilitated fusion with intestinal membranes, supporting direct nutrient transfer. This substantiates the rationale for liposomal delivery in oral Vitamin C supplementation [9].

Superior features of Liposomal Vitamin C over conventional Vitamin C

Conventional Vitamin C supplementation is limited by low oral bioavailability and rapid renal clearance, as only a small

amount of ingested ascorbic acid is absorbed in the intestines, with absorption tightly regulated by active transport mechanisms [10]. To address this, liposomal delivery systems offer a solution. Liposomes, composed of phospholipid bilayers surrounding an aqueous core, encapsulate hydrophilic compounds like Vitamin C, enhancing absorption through passive diffusion, endocytosis, and fusion with cellular membranes, thus improving bioavailability and cellular uptake [11] [7]. Lecithin, a natural emulsifier and source of phospholipids, is a

key component in liposomal formulations. Sunflower-derived lecithin, known for its non-GMO status, solvent-free processing, and low allergenicity, is preferred for pharmaceutical and nutraceutical use [12]. Its high phosphatidylcholine (PC) content improves liposomal stability and performance by contributing to membrane fluidity, permeability, and stability [13].

Process Flow of 75% Sunflower Lecithin for Liposomal Encapsulation used by WBCIL

Sunflower lecithin, comprising Phosphatidylcholine (PC): 20–30%, Phosphatidylethanolamine (PE): 15–25%, Phosphatidylinositol (PI): 10–20%, Phosphatidic acid (PA): 5–10%, and trace amounts of triglycerides and glycolipids, is used in liposomal Vitamin C formulations due to its structural and functional properties that ensure high encapsulation efficiency and stability [14]. The preparation process involves several stages: Extraction (using mechanical cold pressing and aqueous enzymatic extraction), Purification (centrifugation and filtration), Degumming (to increase phospholipid concentration), and Fractionation (to concentrate Phosphatidylcholine to 75%).

The lecithin is then dried under low heat to produce a stable powder, followed by hydration with an aqueous Vitamin C solution for liposome formation. Size reduction and homogenization under high pressure or sonication yield nanosized liposomes (100–200 nm), enhancing bioavailability. Finally, antioxidants like Vitamin E are added to prevent lipid peroxidation, and the formulation is packaged in opaque, airtight containers. This process, used by West Bengal Chemical Industries Ltd. (WBCIL), ensures potent, stable liposomal Vitamin C with effective gastrointestinal uptake. The use of sunflower lecithin supports clean-label and allergen-free preferences while meeting pharmaceutical-grade standards.

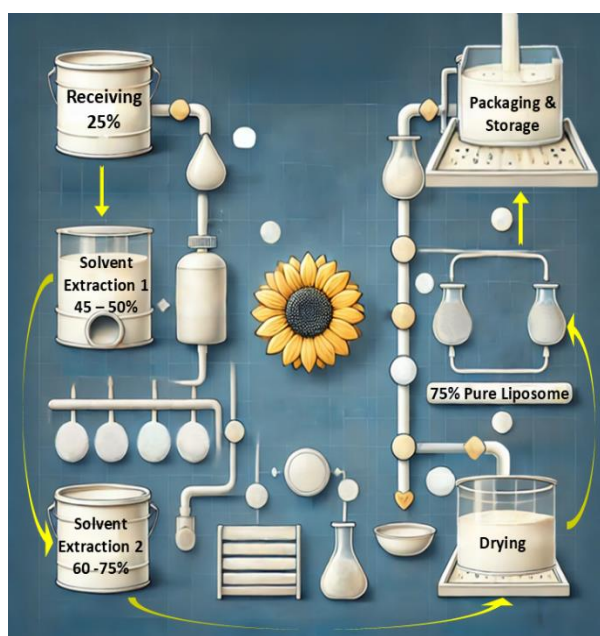


Figure 2: Purification process of Sunflower Lecithin at WBCIL

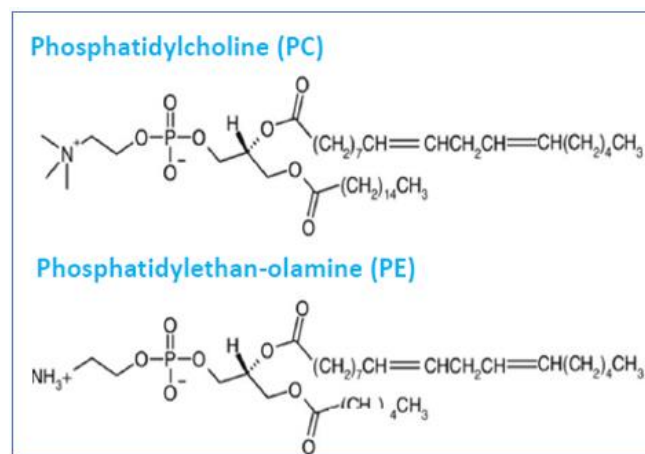


Figure 3: Chemical structures of phospholipids that make up lecithin

Efficacy of Liposomal Vitamin C Absorption in the Body and Skin

Liposomal vitamin C is recognized for its enhanced absorption, both systemically and through the skin, due to its encapsulation in lipid vesicles that improve stability and bioavailability. This delivery system protects vitamin C from degradation in the digestive tract and aids in its transport across cellular membranes, ensuring higher concentrations reach the bloodstream to support immune function and collagen synthesis. A 2023 study introduced a novel "lipo-oil-some" (LOS) system, incorporating neutral oils and edge activators to enhance skin delivery of vitamin C. The study found that using sodium deoxycholate as an edge activator increased skin absorption by 2.9 times compared to cholesterol-based formulations. The type of neutral oil used, such as camellia oil, tricaprilyn, and grapeseed oil, influenced the absorption, emphasizing the importance of oil selection in liposomal design. These optimized formulations could improve topical vitamin C delivery, enhancing skin health and promoting collagen production [20].

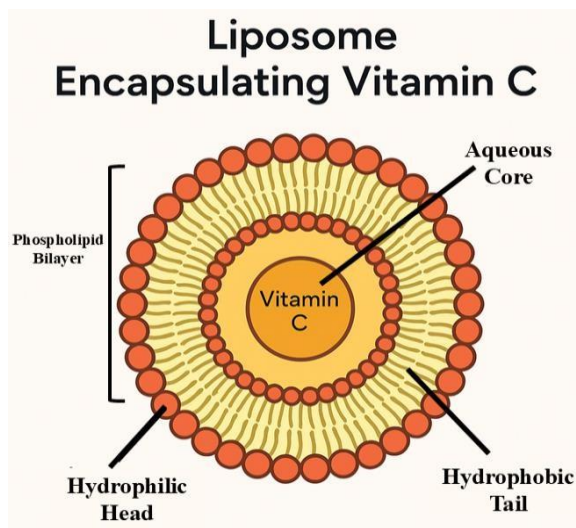


Figure 4: Cross-sectional view of a liposome encapsulating Vitamin C, highlighting the aqueous core and phospholipid bilayer components

MATERIALS AND METHODS

Liposome Characterisation

The characterization of liposomes is vital to assess their efficiency, stability, and functionality as a delivery system. At West Bengal Chemical Industries Ltd. (WBCIL), Kolkata, we employed a comprehensive approach to characterize liposomes for Liposomal Vitamin C using chemical and morphological analyses. High Performance Liquid Chromatography (HPLC) with a C18 column, methanol-water mobile phase, and 254 nm detection confirmed Vitamin C encapsulation, efficiency, and stability. Fourier Transform Infrared (FTIR) Spectroscopy (4000–500 cm^{-1} , ATR method) analyzed interactions between phospholipids, focusing on hydroxyl, phosphate, and lipid ester groups. X-ray Photoelectron Spectroscopy (XPS) was conducted using PHI 5000 VERSA PROBE III (ULVAC-PHI, USA) at the Central Research Facility, IIT-Kharagpur. Dynamic Light Scattering (DLS) was conducted at IACS, Jadavpur using Malvern Zetasizer ZEN 3600 to assess particle size, distribution, and Polydispersity Index (PDI) to evaluate homogeneity, while zeta potential measurements confirmed electrostatic stability, preventing agglomeration.

Encapsulation Efficiency of Liposomal Vitamin C

Encapsulation efficiency (EE) is quantified by the formula and represents the proportion of liposomal vitamin C successfully entrapped within liposomes.

$$\text{Encapsulation Efficiency (EE\%)} = \frac{\text{Encapsulated Vitamin C}}{\text{Total Vitamin C added}} \times 100$$

To determine EE, liposomal vitamin C was incorporated into a phosphate buffer solution with a pH of 7.4. The mixture was then centrifuged to separate the encapsulated and unencapsulated fractions, allowing for the calculation of encapsulation efficiency based on the amount of vitamin C retained within the liposomes compared to the total amount initially added.

Dynamic Light Scattering (DLS) Analysis of Liposomal Vitamin C

Dynamic Light Scattering (DLS) was performed at IACS, Jadavpur using Malvern Zetasizer ZEN 3600. DLS provides crucial insight into the nanometric scale and monodispersity of the formulation. This method is widely used in nanoparticle research and was conducted following protocols described by Danaei *et al.* (2018) and Wagner & Vorauer-Uhl (2011).

FTIR Spectra of API, Liposome, and Liposomal Vitamin C

FTIR spectroscopy was employed to characterize the molecular interactions and functional group integrity of the active pharmaceutical ingredient (Vitamin C), pure lecithin liposomes, and the liposomal Vitamin C formulation. This

technique helps confirm whether Vitamin C is physically encapsulated within the liposomal matrix without undergoing structural degradation or chemical interaction [15].

For sample preparation, the formulations were analyzed directly using attenuated total reflectance (ATR). A small amount of the sample was placed on the ATR crystal, and uniform contact was ensured to obtain high-quality spectra. The spectra were recorded using a FTIR (Agilent, USA) instrument in the range of 4000–400 cm^{-1} with a resolution of 4 cm^{-1} , and 32 scans per sample were collected to enhance the signal-to-noise ratio. Background spectra were recorded using a clean ATR crystal under the same conditions [16]. Each spectrum was carefully analyzed to detect any shifts in peak position, broadening, or intensity change, which would suggest potential interactions between molecule the and the lipid bilayer. Special attention was paid to peaks corresponding to O–H, C=O, and P=O functional groups, as these are critical markers for Vitamin C and phospholipid structures.

Differential Scanning Calorimetry (DSC) Analysis

The thermal characteristics of the liposomal Vitamin C sample were evaluated using Differential Scanning Calorimetry (DSC) at Sapala Organics, located in Secunderabad, India [17]. For the analysis, the sample was sealed in a standard aluminum pan, while an empty aluminum pan served as the reference. The temperature program initiated at 40°C and increased at a steady rate of 10°C per minute until reaching 500°C. The resulting DSC thermogram was used to identify melting points, phase transitions, and to assess the thermal stability of the formulation.

Morphological Characterization Using Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) was utilized to assess the surface morphology and physical integrity of the liposomes containing Vitamin C. This technique provides high-resolution images, allowing detailed observation of the vesicular structure and surface topography of the liposomes. Samples were analysed using Field Emission Gun-Scanning Electron Microscope (Merlin, Gemini II, Zeiss, Germany) at Central Research Facility, IIT Kharagpur.

Assessment of Vitamin C Leakage from Liposomes

To evaluate the retention capacity of the liposomal bilayer and its effectiveness in securely encapsulating Vitamin C, a leakage study was conducted. This study involved monitoring the release of Vitamin C from liposomes over time under controlled conditions, simulating potential storage or physiological environments [18].

Liposomal suspension was stored over a period of 6 months at 40°C \pm 2 °C and at a relative humidity of 75% \pm 5%. The liposomes were separated from the medium using ultracentrifugation. The supernatant, which contained any

Vitamin C that may have leaked from the liposomes was analyzed using High-Performance Liquid Chromatography (HPLC) [19]. The amount of free Vitamin C was quantified and compared to the initial encapsulated amount to calculate the percentage of leakage.

Thermal Stability Testing of Liposomal Vitamin C

Thermal stability studies were conducted on the liposomal Vitamin C suspension to assess its resilience under stress conditions, focusing on encapsulation and chemical integrity at elevated temperatures. Aliquots were stored at room temperature and exposed to 105°C for 4 hours. The samples were evaluated for physical changes (e.g., phase separation, color changes), encapsulation efficiency (via centrifugation and HPLC), and chemical stability (Vitamin C retention). This test provides key insights into the robustness of the liposomal bilayer and shelf stability, particularly under tropical or room-temperature storage conditions [20] [21].

RESULTS AND DISCUSSION

Liposome Characterisation

High-Performance Liquid Chromatography (HPLC) analysis

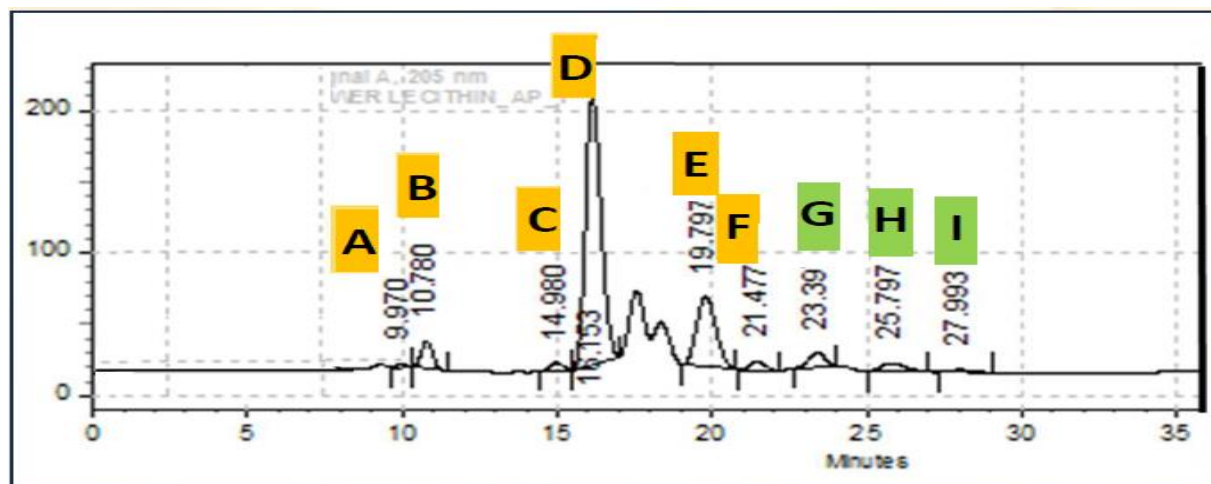


Figure 5: Chromatogram of Liposome with peaks of PC (A-F) and PE (G-I)

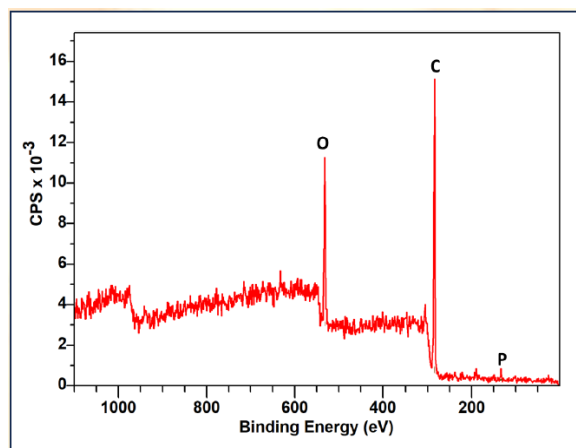


Figure 6: XPS spectrum representing different elements present in the Liposome

revealed that the liposomes contained 82.05% Phosphatidylcholine (PC) and 10.82% Phosphatidylethanolamine (PE), indicating a high total phospholipid content of 93%, essential for forming a stable bilayer structure conducive to encapsulating hydrophilic molecules like Vitamin C (Figure - 4) [22]. FTIR spectroscopy further confirmed the structural integrity of the liposomes, with characteristic peaks such as C=O stretching at $\sim 1738\text{ cm}^{-1}$, CH₂ vibrations at ~ 2853 and $\sim 2920\text{ cm}^{-1}$, and broad -OH stretching around $3138\text{--}3320\text{ cm}^{-1}$, signifying both hydrophilic and hydrophobic interactions necessary for stable aqueous dispersion (Figure - 9) [23]. X-Ray Photoelectron Spectroscopy (XPS) validated the surface composition, showing the presence of Carbon (81.14%), Oxygen (17.61%), and Phosphorus (1.25%), confirming the phospholipid-based structure and biocompatibility of the liposomes (Figure - 5) [24]. Dynamic Light Scattering (DLS) analysis showed an average particle size of 133.9 nm with a Polydispersity Index (PDI) of 0.294, and a zeta potential of -31.87 mV, indicating a moderately uniform and electrostatically stable liposomal formulation suitable for pharmaceutical applications [25].

Liposomal Vitamin C Characterisation

Encapsulation Efficiency of Liposomal Vitamin C

The results showed that the liposomal formulations has an encapsulation efficiency of 91.98%, indicating high entrapment of Vitamin C within the liposomes (Figure-6). The formulation with the highest lipid-to-non-lipid ratio demonstrated the greatest encapsulation efficiency, suggesting that the lipid concentration plays a significant role in improving the entrapment of the non-liposomal Vitamin C. A high EE is crucial because it ensures that a greater amount of Vitamin C is protected within the liposomes, enhancing its stability against degradation from environmental factors like light, heat, or oxidation. This high EE of 85% to 90% is beneficial as it maximizes the delivery of active Vitamin C to

target sites in the body, improving bioavailability and therapeutic efficacy for applications such as antioxidant therapy and immune support, while minimizing waste of the active compound.

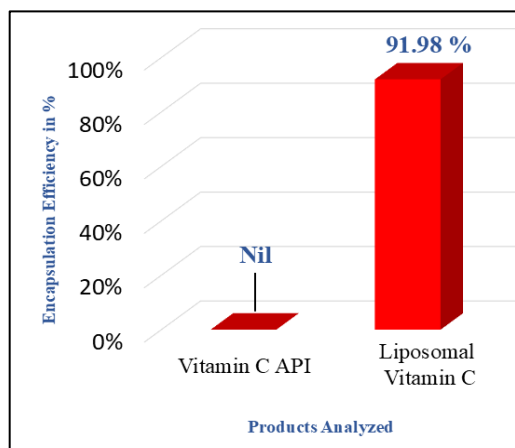


Figure 7: Encapsulation Efficiency measured by validated HPLC analytical data

Thus, the lipid-to-non-lipid ratio was a significant determinant of EE, with higher lipid content yielding better encapsulation. This high efficiency underlines the suitability of liposomes as carriers for hydrophilic molecules like Vitamin C, especially given their ability to shield the active ingredient from external degradation until release [26].

Dynamic Light Scattering (DLS) Analysis of Liposomal Vitamin C

Dynamic Light Scattering (DLS) was employed to determine the size distribution and zeta potential of the liposomal Vitamin C formulations. The average size of the liposomes was found to be approximately 184 nm, with a narrow size distribution (PDI = 0.334), indicating uniformity in the formulation (Figure-8). This result suggests that the liposomal Vitamin C formulations are consistently sized, enhancing their stability and potential efficacy for targeted delivery.

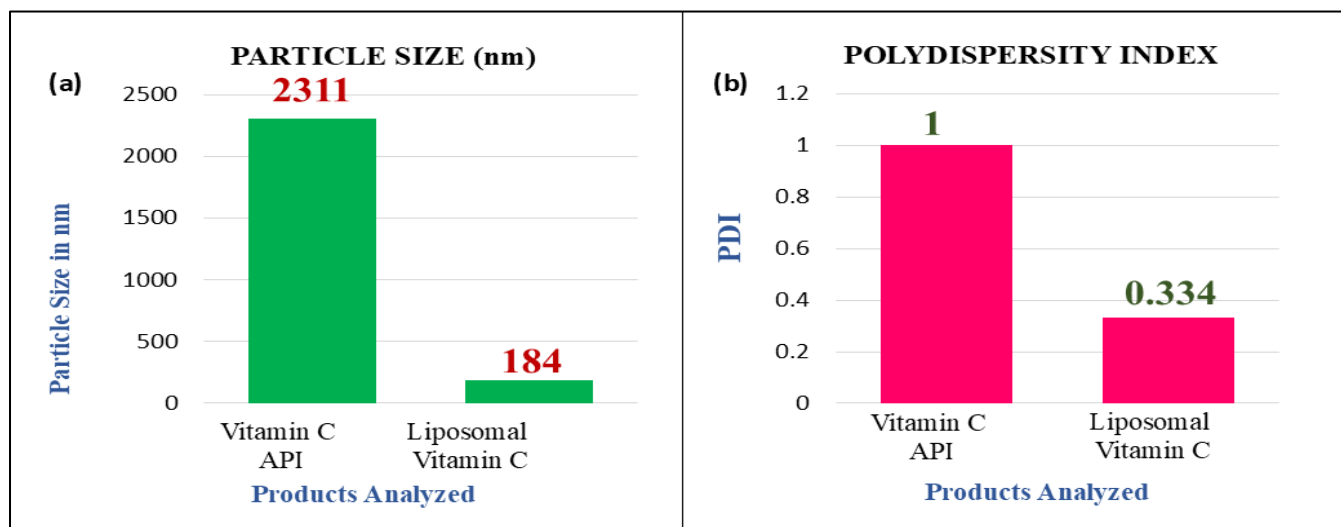


Figure 8: Chart comparing (a) Particle size and (b) PDI between Vitamin C API and liposomal vitamin C

Hence, these characteristics are favourable for oral delivery systems, allowing liposomes to traverse biological membranes efficiently while remaining stable in aqueous formulations [25].

Surface Charge Properties as Determined by Zeta Potential

These results indicate that liposomal Vitamin C remains stable under physiological conditions, with slight sensitivity to acidic environments. Its zeta potential, measured at -33.7 mV compared to -15.41 mV for unencapsulated Vitamin C, demonstrates superior electrostatic stability, preventing aggregation and enhancing absorption (Figure-9). The strongly negative charge supports stable dispersion, re-dispersibility, and protection against degradation, contributing to improved bioavailability. Overall, the formulation shows

good colloidal stability and shelf-life under ambient conditions [27].

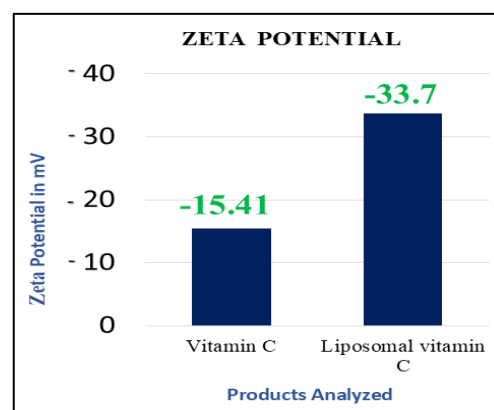


Figure 9: Chart comparing Zeta Potential between non-liposomal vitamin C and liposomal vitamin C

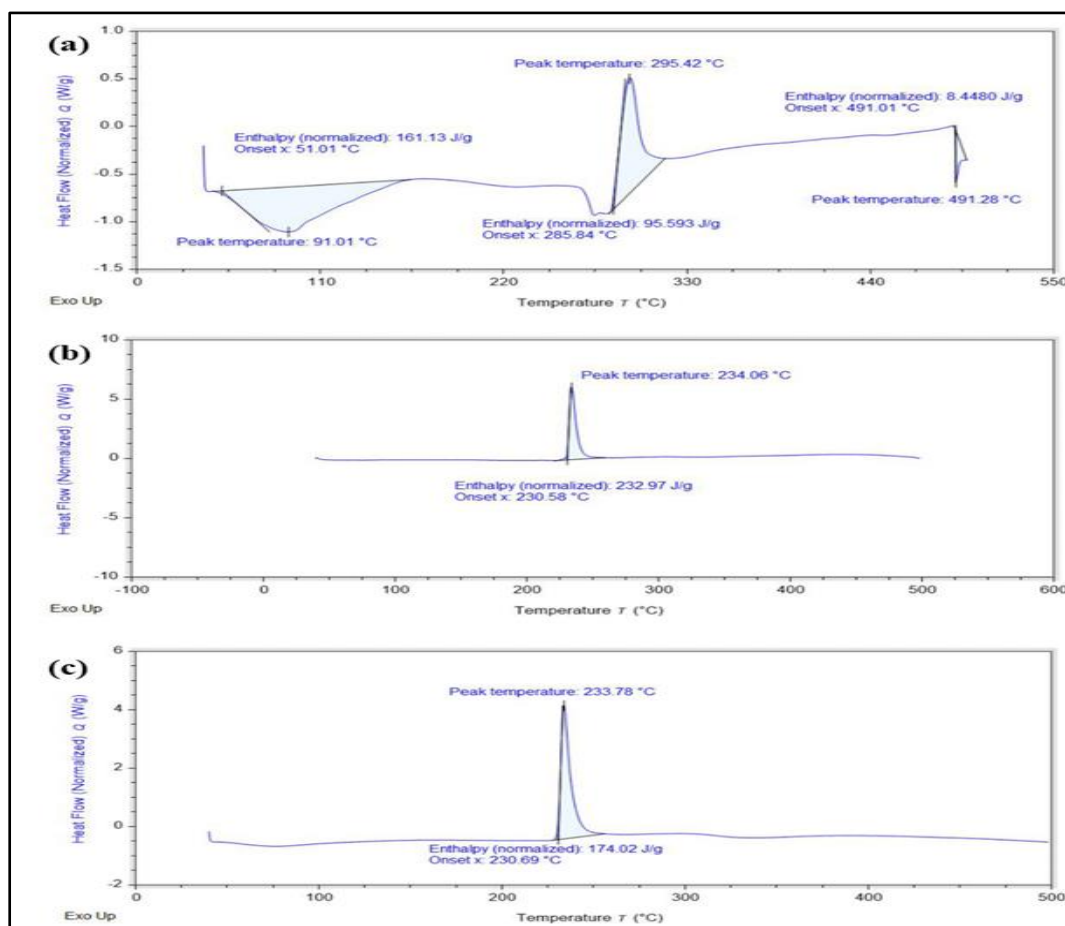
Table 3: Allotment of FTIR peaks in empty liposome, Non-Liposomal Vitamin C, and Liposomal Vitamin C

| Parameter | Empty Liposome | Non-Liposomal Vitamin C | Liposomal Vitamin C |
|----------------------------|---|--|---|
| Confirmation of OH Groups | Broad peak near 3370–3340 cm^{-1} (due to lipid hydration) [28] | Broad OH stretch near 3500–3200 cm^{-1} | Retention of broad OH stretch near 3500–3300 cm^{-1} confirming encapsulation with hydration stability |
| Confirmation of C=O Groups | Peak near 1730 cm^{-1} (ester carbonyl from phospholipids) [27] | Strong peak near 1700 cm^{-1} indicating C=O from API | Peak shift near 1725–1740 cm^{-1} indicating interaction of API with lipid bilayer |
| Hydrophobic Interactions | CH_2 symmetric and asymmetric stretch at 2920–2850 cm^{-1} [29] | Less prominent hydrophobic CH stretches | Distinct CH_2 and CH_3 stretches at 2920–2850 cm^{-1} confirming lipid tail organization around API |
| Hydrophilic Interactions | PO_4^- stretching at 1024 cm^{-1} , confirming phospholipid head groups. [30] | Presence of C=O stretching (~1650 cm^{-1}) from carboxyl groups of Vitamin C | Both C=O (Liposomal Vitamin C) and PO_4^- (Liposome) peaks retained but slightly shifted. Confirms strong interaction between hydrophilic Vitamin C groups and liposome polar heads. |
| Encapsulation Stability | Stable peaks for CH_2 , CH_3 , and phosphate groups [31] | Strong Vitamin C peaks ~1688 cm^{-1} , 1402 cm^{-1} . | Retention of both lipid and API peaks, confirming successful encapsulation |
| Lipid Tail Organization | Ordered lipid bilayer peaks (e.g., CH_2 at 2850–2920 cm^{-1}) [32] | Not applicable | Similar CH_2 peaks but reduced sharpness, indicating lipid rearrangement post-encapsulation |

These subtle modifications indicate successful encapsulation and potential stabilization of Vitamin C without compromising its chemical identity [28].

Differential Scanning Calorimetry analysis

The Differential Scanning Calorimetry (DSC) thermograms of (a) liposome, (b) Vitamin C API, and (c) liposomal Vitamin C demonstrate distinct thermal behaviors reflecting their structural and compositional characteristics (Figure 10).

**Figure 11: DSC Thermograms of (a) Liposome, (b) Vitamin C API, and (c) Liposomal Vitamin C**

In the thermogram of the liposome (Figure 11a), multiple thermal events were observed. An initial endothermic peak at 91.01 °C (onset at 51.01 °C) with an enthalpy of 161.13 J/g indicates the melting transition of the liposomal bilayer, corresponding to the gel-to-liquid crystalline phase transition of phospholipids. A sharp endothermic peak at 295.42 °C (onset at 285.84 °C) with an enthalpy of 95.593 J/g suggests the degradation of the liposomal structure. Additionally, a minor peak at 491.28 °C with an enthalpy of 8.448 J/g is associated with further decomposition or oxidation processes. In contrast, the DSC profile of pure Vitamin C API (Figure 10b) exhibited a single sharp endothermic peak at 234.06 °C (onset at 230.58 °C) with a high enthalpy of 232.97 J/g, corresponding to its melting point followed by rapid degradation. The thermogram of liposomal Vitamin C (Figure 10c) shows a similar melting event at 233.78 °C (onset at 230.69 °C), but with a lower enthalpy of 174.02 J/g compared to pure Vitamin C. This reduction in enthalpy suggests successful encapsulation of Vitamin C within the liposomal matrix, leading to altered thermal stability. The slight shift in peak temperature and change in thermal behaviour confirm the interaction between Vitamin C and the liposomal components, indicating the formation of a stable liposomal delivery system.

Morphological Characterization Using Scanning Electron Microscopy (SEM)

This morphological examination was intended to confirm the formation of spherical, smooth, and distinct vesicles, as well as to assess any possible aggregation or deformation resulting from the manufacturing process. SEM images of the liposomal Vitamin C showed well-defined, spherical liposomes with smooth surfaces. The average diameter observed was consistent with the size distribution obtained from DLS. The liposomes appeared uniform in shape, confirming the effectiveness of the lipid hydration method used for preparation. No significant aggregation of liposomes was observed, indicating the stability of the formulation under the experimental conditions.

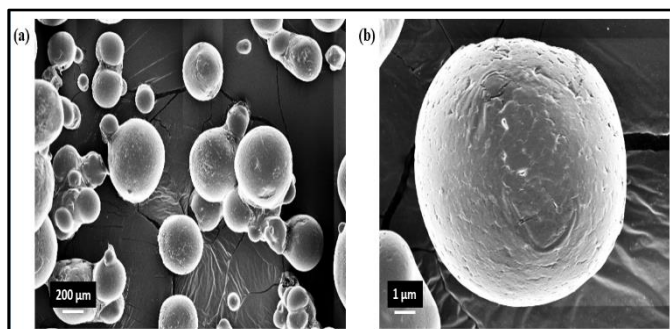


Figure 12: SEM image showing (a) several Vitamin C Liposomes scattered within the field of view under observation and (b) SEM image of a Liposomal Vitamin C

SEM analysis of liposomal Vitamin C revealed uniform, spherical vesicles in agreement with DLS data (Figure 12).

The liposomes showed no signs of fusion or collapse, confirming their structural resilience. The smooth surface morphology also implies minimal surface defects, which is important for reproducibility in release and stability. These visual confirmations solidify the reliability of the manufacturing process and the stability of the final formulation [27].

Assessment of Vitamin C Leakage from Liposomes

The mineral leakage assay conducted over six months demonstrated consistently high encapsulation efficiency of Vitamin C, ranging from 90% to 92%, with an initial value of 91.98% and a slight increase to 92% by six months, indicating excellent stability of the encapsulation method. Assay values, reflecting Vitamin C availability, showed moderate fluctuation—from 49.22% initially to 46% at two months, then rising to 49.10% at six months—likely due to minor oxidative or handling losses. Overall, the results confirm effective retention and minimal leakage of Vitamin C, supporting the formulation's suitability for long-term use in functional foods or nutraceutical applications where nutrient stability is essential.

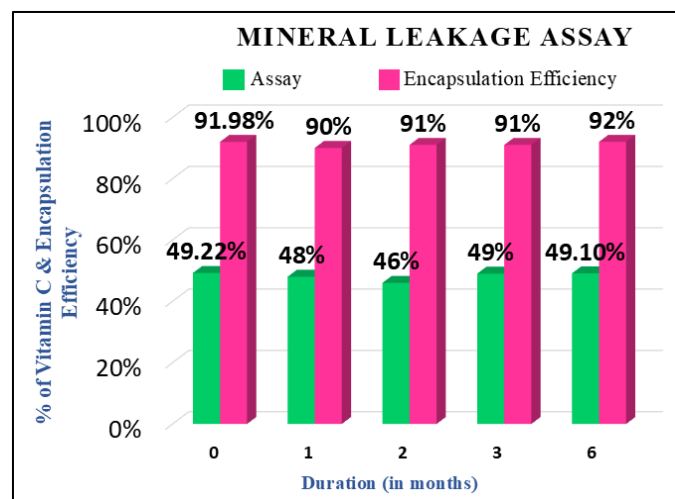


Figure 13: Chart comparing the stability of liposomal vitamin C stored over a period of 6 months at 40 °C ± 2 °C and a relative humidity of 75% ± 5%

This sustained release profile indicates that the liposomal bilayer effectively retains the encapsulated Vitamin C, reducing premature release. Such characteristics are advantageous for applications requiring prolonged nutrient availability and stability during storage [28].

Thermal Stability Testing of Liposomal Vitamin C

Figure 14 compares the stability of non-liposomal and liposomal Vitamin C under room temperature and 105 °C for 4 hours. Assay values remained stable, with a slight increase from 48.22% to 48.58%, indicating minimal impact of heat on core Vitamin C stability. Encapsulation efficiency also rose slightly from 91.98% to 93%, suggesting the liposomal system retained structural integrity under thermal stress. These

findings confirm the formulation's thermal stability and effectiveness in protecting Vitamin C, supporting its suitability for heat-involved applications in food, pharmaceutical, and nutraceutical products requiring extended shelf life and temperature resilience.

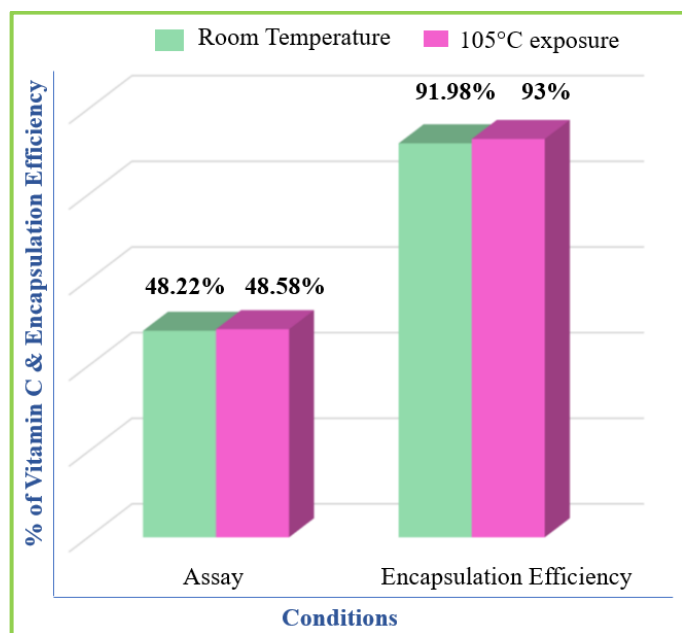


Figure 14: Chart comparing the stability of liposomal Vitamin C both at room temperature (RT) and at 105°C exposure for 4 hours

CONCLUSION

This study underscores the successful development and comprehensive characterization of Liposomal Vitamin C by West Bengal Chemical Industries Ltd. (WBCIL), utilizing sunflower-derived phosphatidylcholine to enhance bioavailability, stability, and controlled release. The formulation showed high encapsulation efficiency (91.98%), nanoscale particle size (184 nm), excellent electrostatic stability (zeta potential -33.7 mV), and uniformity (PDI 0.334). Advanced techniques (HPLC, FTIR, XPS, SEM, DLS) confirmed structural integrity and functional performance. Sustained release, thermal stability, and enhanced in vitro absorption demonstrated superiority over traditional oral supplements.

Future research could explore alternative lipids, ligand-functionalized liposomes for targeted delivery, and integration into foods, beverages, or dermal products. Combining Vitamin C with other bioactives (e.g., zinc, glutathione, polyphenols) may offer synergistic benefits. Scaling up manufacturing while preserving quality and cost-efficiency is essential for broader commercial and clinical adoption.

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