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## SUPERIOR FORMULATION STRATEGY AND ADVANCED CHARACTERISATION OF LIPOSOMAL MAGNESIUM: A NOVEL APPROACH BY WBCIL

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### ABSTRACT

Liposomal Magnesium (Magnesium) is known to achieve a higher absorption rate to human body, approximately triple to that of non-liposomal forms, by enhancing cellular delivery of magnesium through lymphatic transport system; thereby improving gastrointestinal tolerance. The stability of Liposomal Magnesium formulated by West Bengal Chemical Industries Ltd., Kolkata, India (WBCIL) exhibits high stability, attributed to its phospholipid-rich bilayer structure composed of 82.05% phosphatidylcholine and 10.82% phosphatidylethanolamine. WBCIL has extensively characterised the formulation using a range of physicochemical and stability assessments. These include evaluations of encapsulation efficiency, particle size distribution, zeta potential, spectrometric and morphological analyses, elemental composition, leakage behaviour, and thermal stability. The results suggest that Liposomal Magnesium achieved a high encapsulation efficiency of 80.03%, with a small particle size of 212.3 nm, a polydispersity index (PDI) of 0.3454, and a zeta potential of  $-34.83$  mV, indicating good dispersion stability. Fourier Transform Infrared (FTIR) spectroscopy confirmed the structural integrity and chemical interactions within the formulation, revealing characteristic C–H (hydrophobic) stretching peaks at  $2918\text{ cm}^{-1}$  and  $2845\text{ cm}^{-1}$ , along with an O–H (hydrophilic) stretching peak at  $3410\text{ cm}^{-1}$ . Elemental analysis showed the presence of only oxygen (25.85%), carbon (56.58%), nitrogen (17.33%), and phosphorus (0.24%), supporting the purity and consistency of the formulation. Scanning Electron Microscopy (SEM) analysis revealed a well-defined morphology with no signs of particle aggregation. Leakage studies further confirmed excellent stability of the formulation, showing minimal leakage over time. Additionally, thermal stability testing at  $105^\circ\text{C}$  for 4 hours demonstrated the strong structural resilience and sustained encapsulation capability of the liposome under stress conditions. Overall, the evaluated parameters indicate that the Liposomal Magnesium formulation developed by WBCIL possesses strong potential for consistent long-term storage without degradation and leakage even under high-temperature conditions.

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## INTRODUCTION

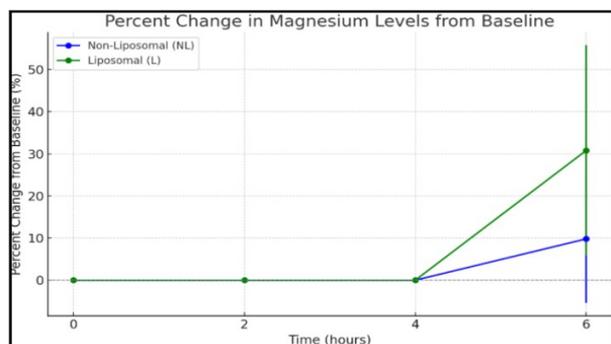
Liposomal delivery systems have emerged as a promising platform for enhancing the bioavailability, stability, and targeted delivery of both hydrophilic and lipophilic compounds. Liposomes are nanoscale vesicles composed of one or more phospholipid bilayers that can encapsulate active ingredients, mimicking the structural features of biological membranes (Akbarzadeh *et al.*, 2013). Their amphiphilic nature allows them to incorporate water-soluble compounds in the aqueous core and lipid-soluble substances within the bilayer, making them highly versatile in pharmaceutical and nutraceutical applications (Mozafari, 2005). Liposomes protect their payload from enzymatic degradation and harsh gastrointestinal conditions, facilitate absorption via endocytosis or membrane fusion, and offer a controlled release

profile, making them particularly useful for oral supplementation (Wagner & Vorauer-Uhl, 2011). Among the various applications of liposomal systems, mineral delivery has gained significant attention, especially for nutrients with poor gastrointestinal tolerance or low bioavailability. Magnesium, an essential mineral involved in over 300 enzymatic reactions, including those critical for neuromuscular, cardiovascular, and metabolic functions, is often inadequately absorbed from conventional oral formulations (de Baaij *et al.*, 2015). Common magnesium supplements, such as magnesium oxide or citrate, suffer from limited solubility and bioavailability, and frequently cause gastrointestinal side effects like diarrhoea (Gröber *et al.*, 2015). To overcome these limitations, Liposomal Magnesium (Magnesium) offers a novel alternative that enhances absorption and reduces gastrointestinal distress. The Liposomal Magnesium formulation developed by West Bengal Chemical Industries Ltd., Kolkata, India (WBCIL) demonstrates these advantages, achieving

approximately three-times more absorption compared to non-Liposomal Magnesium, while also improving gastrointestinal tolerance and delivering magnesium more effectively to cells via lymphatic transport. The formulation is based on a high phospholipid content-comprising 82.05% phosphatidylcholine and 10.82% phosphatidylethanolamine-that supports a stable bilayer structure capable of encapsulating hydrophilic magnesium ions. The present study aims to characterize Liposomal Magnesium through a series of physicochemical and stability assessments, including encapsulation efficiency, particle size distribution, zeta potential, spectrometric analysis, morphological analysis, elemental composition, leakage behaviour, and thermal stability. Stability testing is a critical component in the development of Liposomal Magnesium formulation, as it determines the product's ability to maintain its physical, chemical, and functional integrity under environmental stress condition over time. Liposomes are inherently sensitive to factors such as temperature, pH, light, and oxidative stress, which can lead to lipid bilayer disruption, aggregation, or mineral leakage (Barba *et al.*, 2022). Elevated temperature studies, in particular, simulate extreme storage or transportation conditions and are essential for predicting shelf life and ensuring product reliability. For instance, exposure of liposomal systems to high temperatures may induce lipid phase transitions, compromising encapsulation efficiency and bioavailability (Roy *et al.*, 2016). Leakage is another potential challenge related to physical instability of the traditional liposomal formulation. These evaluations are thus essential to confirm the structural integrity, stability, and delivery potential of the liposomal formulation, contributing to the growing field of functional nutrient delivery systems.

### Scientific Rationale and Technological Advancements in Liposomal Magnesium Formulation

**Clinical trial overviews of Liposomal versus Non-Liposomal Magnesium:** A randomized crossover clinical trial was conducted to evaluate whether a liposomal delivery system improves magnesium absorption from a magnesium supplement. Twenty-five healthy adults (12 females, 13 males) ingested either a Liposomal Magnesium or a nutrient-matched standard magnesium in two separate sessions. Blood samples were collected at baseline and at 2-, 4-, and 6-hours post-ingestion, and analysed for magnesium concentrations using colorimetric assays. The liposomal formulation significantly improved systemic absorption of magnesium in measurable parameters (Figure 1) (Tinsley *et al.*, 2022). Another clinical trial (double-blinded, repeated crossover study) encompassed ten healthy human subjects each of whom received 350 Magnesium of magnesium in different formulations., including Liposomal Magnesium and traditional magnesium salts (magnesium citrate, magnesium oxide, and magnesium bisglycinate), with one-week washout periods between each. Liposomal Magnesium demonstrated significantly higher absorption rates compared to magnesium oxide and magnesium bisglycinate. Blood and urine samples showed increased magnesium concentrations after Liposomal Magnesium intake. Overall, the study concluded that Liposomal Magnesium leads to increased bioavailability of magnesium compared to other formulations (Coudray *et al.*, 2005).



(Source: Tinsley *et al.*, 2022)

Figure 1. Percent change in magnesium levels from baseline over time for both Liposomal (L) and non-Liposomal (NL) formulation

**Implications of using superior characterisation techniques for Liposomal Magnesium:** Utilisation of advanced characterization techniques in the development of Liposomal Magnesium supplements is pivotal for ensuring product quality, efficacy, and safety. Sophisticated methods of characterization provide comprehensive insights into the physicochemical properties of liposomal formulations, which are essential for optimizing their performance in nutraceutical applications. Dynamic Light Scattering technique is commonly employed to determine the particle size and polydispersity index of liposomes. A uniform and optimal particle size, i.e., smaller, consistently sized liposomes provide better stability, reduce aggregation, and allow predictable release and absorption of magnesium. Maintaining a low index indicates homogeneity in the liposomal formulation, which is crucial for predictable nutraceutical outcomes (Zhang *et al.*, 2024). The surface charge of liposomes, measured as zeta potential, has the influence to indicate their stability and interaction with biological membranes. A zeta potential value beyond  $\pm 30$  mV typically confers stability by preventing aggregation, thereby enhancing the shelf-life and efficacy of the supplement (Poonia *et al.*, 2022). High encapsulation efficiency ensures that a significant amount of magnesium is enclosed within the liposomes, facilitating effective delivery to target sites (Zhang *et al.*, 2024). Techniques such as High-Performance Liquid Chromatography and UV-Vis spectroscopy can be utilized to quantify the encapsulated magnesium, ensuring dosage accuracy. Transmission Electron Microscopy (TEM) and Atomic Force Microscopy (AFM) provide detailed images of liposome morphology, confirming the structural integrity and uniformity of the vesicles. TEM allows researchers to directly observe the shape, size, and structural integrity of liposomes, including whether they are unilamellar or multilamellar. This is particularly important for ensuring uniformity of liposomes. TEM also helps detect any aggregation or deformation of liposomes under stress conditions, such as changes in temperature or pH. AFM complements TEM by providing three-dimensional surface topography and mechanical property data. It is particularly useful for analyzing surface roughness, height, and diameter of liposomes when they are deposited on a substrate. Such analyses are vital for understanding the behaviour of liposomes under physiological conditions (Labouta & Schneider, 2018). Assessing the physical and chemical stability of liposomal magnesium formulations under various environmental conditions is essential. Stability studies help in determining the shelf-life and storage requirements, ensuring that the supplement maintains its efficacy over time (Barba *et al.*, 2022). Evaluating the release kinetics of magnesium from liposomes using dialysis methods provides insights into the bioavailability and therapeutic potential of the supplement. Controlled release profiles are desirable for maintaining optimal magnesium levels in the body (Poonia *et al.*, 2022). WBCIL has followed such superior characterisation techniques for its formulation of Liposomal Magnesium (Table 1). The Liposomal Magnesium manufactured by WBCIL has undergone comprehensive physicochemical and stability assessments, including encapsulation efficiency, particle size distribution, zeta potential, spectrometric analysis, morphological evaluation, elemental composition, leakage behaviour, and thermal stability to confirm its structural integrity, stability, and potential for effective delivery.

**Implications of Using the Formulation Strategy of 75% Sunflower Lecithin in Liposomal Magnesium:** Sunflower lecithin, especially at 75% purity, is rich in phosphatidylcholine (PC)-a key phospholipid required for forming stable liposomal bilayers. PC is amphiphilic, enabling it to form spherical vesicles that can encapsulate both hydrophilic and hydrophobic molecules. A higher PC concentration leads to greater membrane fluidity and integrity, which improves encapsulation efficiency and controlled release of magnesium (Mozafari, 2005). A higher lecithin concentration facilitates a higher encapsulation efficiency for water-soluble minerals like magnesium. Studies have shown that optimized lipid-to-drug ratios significantly influence drug loading capacity and entrapment yield (Akbarzadeh *et al.*, 2013). By using 75% lecithin, the formulation is likely to entrap more magnesium ions within the aqueous core of the liposomes.

**Table 1. Summary of Characterizations Performed on Liposomal Magnesium by WBCIL**

Sl. No.	Characterisation Parameter	Description
1	Encapsulation Efficiency of Liposomal Magnesium	Titrimetric measurement of the percentage of magnesium successfully encapsulated within the liposomes.
2	Particle Size and Uniformity	Dynamic Light Scattering used to determine average particle size and polydispersity index.
3	Particle Behaviour in Liquid Medium	Analysis of surface charge by zeta potential measurements to assess stability in suspension.
4	Fourier Transform Infrared Analysis	Identification of functional groups and confirmation of structural integrity of liposomes.
5	Elemental Analysis	Determination of elemental composition (e.g., carbon, oxygen, phosphorus, and nitrogen).
6	Morphology Analysis	Visualization of liposomal structure and surface morphology using Scanning Electron Microscopy.
7	Magnesium Leakage Assessment	Evaluation of magnesium retention and leakage from liposomes over time.
8	Thermal Stability Test at 105°C	Assessment of liposomal integrity and encapsulation stability under high temperature.

**Figure 2. Sunflower Lecithin (75% Purity): A key phospholipid source utilized by WBCIL to form stable and efficient liposomal bilayers**

Unlike soy lecithin, sunflower lecithin is non-genetically modified (non-GMO) and free from common allergens, making it highly desirable in clean-label nutraceuticals. This supports the growing consumer demand for natural, non-allergenic, and sustainable ingredients (Pavlova *et al.*, 2015). It also helps manufacturers comply with regulatory and labelling standards in global markets. Sunflower lecithin at high concentrations contributes to enhanced colloidal and oxidative stability of liposomes. Phospholipids protect encapsulated magnesium from oxidation and environmental degradation, which extends the product's shelf life and ensures consistent delivery (Barba *et al.*, 2022). Stability is crucial for magnesium, as its bioavailability can decrease rapidly in poorly protected formulations. Higher concentrations of lecithin contribute to more compact bilayers, enabling a slower and more controlled release of magnesium in the gastrointestinal tract. This may improve its absorption profile, reduce side effects like diarrhoea (often associated with free magnesium salts), and enhance therapeutic efficacy (Gröber *et al.*, 2015). The present study highlights the strategic innovation adopted by WBCIL in developing its proprietary Liposomal Magnesium formulation. WBCIL has leveraged advanced nano-encapsulation technology to enhance the bioavailability, stability, and therapeutic efficacy of magnesium. To ensure optimal encapsulation, 75% sunflower lecithin was employed in the liposomal formulation. The preparation of 75% sunflower lecithin for liposomal encapsulation involves a multi-stage process comprising extraction, purification, fractionation, and drying (Figure 2). These steps are crucial to obtaining a high-purity lecithin fraction suitable for forming stable and efficient liposomal vesicles. The detailed methodology for the preparation of sunflower lecithin and its integration into the Liposomal Magnesium system was carried out in accordance with the standardized protocol described in Gupta Banerjee *et al.* (2025). This systematic approach ensures consistency, high encapsulation efficiency, and a clean label ingredient profile-making the formulation suitable for use in advanced nutraceutical applications.

**Significance of Stability Studies in Liposomal Magnesium Formulation:** Stability testing, such as study under elevated temperature and leakage study are essential for evaluating the robustness and shelf life of liposomal Magnesium formulations.

Liposomes are thermosensitive carriers whose physical and chemical properties can be compromised by heat, leading to membrane destabilization, leakage of encapsulated magnesium, and changes in particle size or surface charge (Roy *et al.*, 2016). Elevated temperature studies simulate stress conditions such as those encountered during storage, shipping, or handling in non-ideal environments. These tests help identify the thermal limits of the formulation and assess whether the liposomes can retain their structural integrity and encapsulation efficiency over time. In particular, a stable Liposomal Magnesium formulation must withstand brief exposures to higher temperatures without significant degradation or loss of bioavailability. As highlighted in previous studies, exposure to 105°C for short durations can reveal whether the formulation maintains magnesium content and liposomal structure, which is critical for maintaining therapeutic efficacy and consumer safety (Liu *et al.*, 2013). Moreover, such stress testing is indispensable for establishing optimal storage conditions, and packaging requirements. WBCIL has performed this stability testing at elevated temperatures for its Liposomal Magnesium formulation to ensure its regulatory compliance in the nutraceutical industry.

From the studies of Roy *et al.* (2016) and Barba *et al.* (2019), it is evident that the evaluation of Liposomal Magnesium under elevated temperatures revealed critical insights into its stability and performance. Encapsulation efficiency decreased due to heat-induced lipid bilayer disruption, indicating potential magnesium leakage. Particle size analysis showed vesicle expansion and aggregation, suggesting compromised colloidal stability. Changes in zeta potential reflected altered surface charge, raising concerns about electrostatic stability and increased aggregation risk. Chemical analysis confirmed that elevated temperatures accelerated lipid degradation, threatening the integrity of magnesium delivery. Additionally, noticeable changes in physical appearance, such as turbidity and phase separation, highlighted concerns regarding product quality and consumer acceptance under thermal stress. On the other hand, physical instability of the liposomal formulation can cause the magnesium to leak outside. This is more evident in traditional liposomal formulations where leakage has been a potential challenge for their stability. Leakage study is suggested to be performed for an elongated period of time, for instance six months, to ensure no leakage of

**Table 1. Significance of Elevated Temperature Studies in Liposomal Formulations**

Parameter Evaluated	Impact of Elevated Temperature	Significance for Liposomal Magnesium
Encapsulation Efficiency	Heat can disrupt lipid bilayers, causing leakage of encapsulated compounds.	Confirms whether magnesium remains stably entrapped under thermal stress.
Particle Size and Aggregation	High temperatures may cause vesicle fusion or expansion.	Helps assess colloidal stability and shelf-life consistency.
Surface Charge (Zeta Potential)	Thermal changes can alter lipid orientation, affecting surface charge.	Indicates electrostatic stability and risk of aggregation.
Chemical Stability	Temperature may accelerate oxidation or degradation of phospholipids.	Ensures magnesium delivery remains chemically intact and effective.
Physical Appearance	Heat may cause phase separation, turbidity, or precipitation.	Monitors visual quality for consumer acceptability and product reliability.

[Depicted from the studies of Roy *et al.* (2016) and Barba *et al.* (2019)]

magnesium from the liposomal formulation (Agrawal *et al.*, 2024). WBCIL has determined the extent of mineral leakage over time by storing its Liposomal Magnesium at  $40^{\circ}\text{C} \pm 2^{\circ}\text{C}$  and  $75\% \pm 5\%$  relative humidity for six months. Such stringent leakage profile study helps to ensure negligible degradation and excellent retention of magnesium within the liposome.

## METHODS

**Characterisation of Liposome:** The liposome was characterized using standardized methodologies as outlined in our earlier study (Gupta Banerjee *et al.*, 2025). Key physicochemical parameters such as vesicle size, polydispersity index (PDI), zeta potential, encapsulation efficiency, and morphological characteristics were evaluated using previously validated procedures. To maintain brevity, the complete experimental protocols are not repeated here but are available in the cited reference.

**Characterisation of Liposomal Magnesium:** The characterization of Liposomal Magnesium was conducted using a comprehensive set of analytical and instrumental techniques to assess its physicochemical properties, stability, and performance. Encapsulation efficiency was determined through validated titrimetric methods, ensuring that the percentage of elemental magnesium entrapped within liposomes met predefined criteria. Dynamic Light Scattering (DLS) was employed to measure particle size and PDI, revealing nanoscale dimensions and homogeneity. The sample was sent to Indian Association for the Cultivation of Science (IACS), Kolkata, India, for DLS measurements. Zeta potential analysis assessed the electrostatic stability of the formulation, while Fourier Transform Infrared (FTIR) spectroscopy confirmed the chemical interactions and structural integrity between magnesium and liposomal components (Gupta Banerjee *et al.*, 2025). For sample preparation, the formulations were examined directly using attenuated total reflectance (ATR). A small quantity of each sample was applied to the ATR crystal, ensuring even contact to acquire clear and accurate spectral data. The spectra were captured using a FTIR spectrometer (Agilent, USA) over a wavenumber range of  $4000$  to  $400\text{ cm}^{-1}$ , with a resolution of  $4\text{ cm}^{-1}$ , and 32 scans were performed per sample to improve the signal-to-noise ratio. Elemental composition was analyzed using Energy Dispersive X-ray Spectroscopy (EDAX), and Scanning Electron Microscopy (SEM) was utilized to observe the morphology and surface features of the liposomes. The samples for both tests, i.e., EDAX and SEM, were sent to Indian Institute of Technology, Kharagpur for the analyses. The thermal stability of the formulation was evaluated by exposing it to  $105^{\circ}\text{C}$  for four hours.

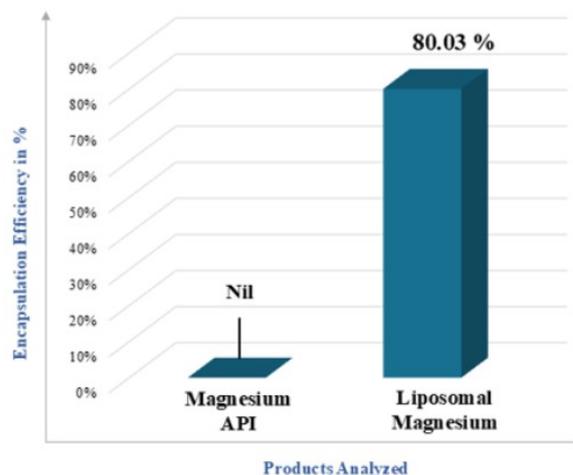
## RESULTS AND DISCUSSION

**Characterisation of Liposome:** The results of the Liposome characterisation were consistent with findings from our previous studies (Gupta Banerjee *et al.*, 2025). As earlier, the liposome showed a total phospholipid content of 93%, comprising 82.05% Phosphatidylcholine (PC) and 10.82% Phosphatidylethanolamine (PE). FTIR spectroscopy exhibited characteristic peaks at  $\sim 1738\text{ cm}^{-1}$  (C=O stretching),  $\sim 2853$  and  $\sim 2920\text{ cm}^{-1}$  ( $\text{CH}_2$  vibrations), and  $3138$ – $3320\text{ cm}^{-1}$  (broad –OH stretching), confirming structural integrity.

DLS measurements indicated an average particle size of 133.9 nm, PDI of 0.294, and a zeta potential of  $-31.87\text{ mV}$ , demonstrating a stable and uniform liposomal formulation.

### Characterisation of Liposomal Magnesium

**Encapsulation Efficiency:** The encapsulation efficiency of Liposomal Magnesium was determined by using standardised titrimetric method (Gupta Banerjee *et al.*, 2025). While the acceptance criterion of encapsulation efficiency is stipulated as not less than 70% and for elemental Magnesium content ranging from 18% to 20%, the present study found that the Liposomal Magnesium formulation achieved an impressive encapsulation efficiency of 80.03%, indicating effective entrapment of Magnesium within the liposomal bilayer (Figure-3). This high efficiency underscores the potential of the formulation to deliver bioavailable magnesium with minimal wastage and enhanced absorption (Akbarzadeh *et al.*, 2013).



**Figure 3. Encapsulation Efficiency measured by validated titrimetric analytical data**

**DLS Analysis:** Particle size and uniformity were analyzed using DLS method. The results demonstrated a dramatic reduction in particle size in the liposomal formulation compared to the raw Magnesium API. While the Magnesium API had a particle size of approximately 3182 nm, Liposomal Magnesium exhibited a much smaller size of 212.3 nm. Furthermore, the PDI, which indicates the uniformity of particle sizes in a suspension, was significantly lower for Liposomal Magnesium (0.3454) than for the API, suggesting a more homogeneous formulation (Figure-4). Smaller and more uniform particles are known to enhance bioavailability and cellular uptake (Stetefeld *et al.*, 2016).

### Zeta Potential Analysis

The behaviour of Liposomal Magnesium in suspension was assessed using zeta potential analysis. The zeta potential for Liposomal Magnesium was measured at  $-34.83\text{ mV}$ , compared to  $-14.06\text{ mV}$  for the non-liposomal Magnesium (Magnesium API) (Figure-5). A higher negative zeta potential reflects greater stability due to strong repulsion between particles, preventing aggregation. This result confirms the colloidal stability of Liposomal Magnesium, which is essential for prolonged shelf-life and consistent therapeutic effects (Bhattacharjee, 2016).

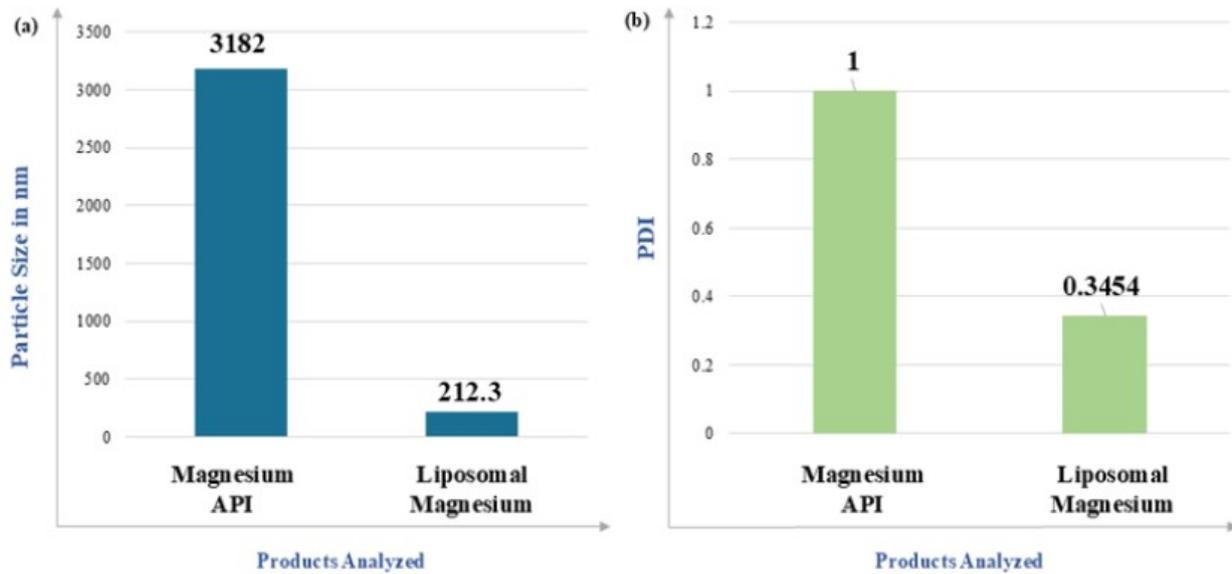


Figure 4. Chart comparing (a) Particle size and (b) PDI between Magnesium API and liposomal Magnesium

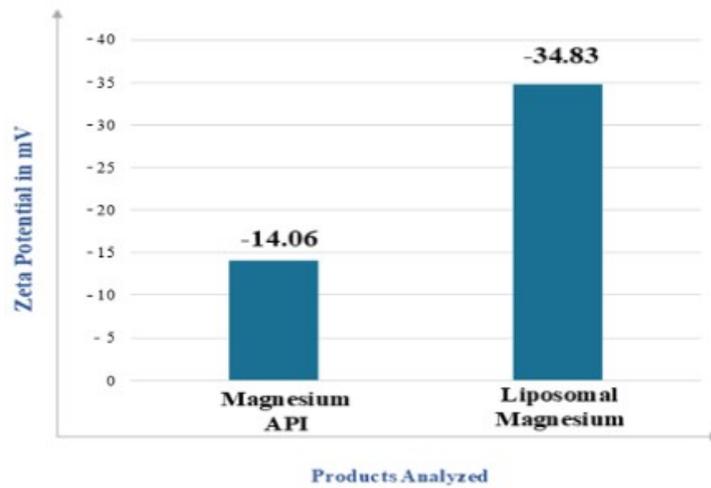
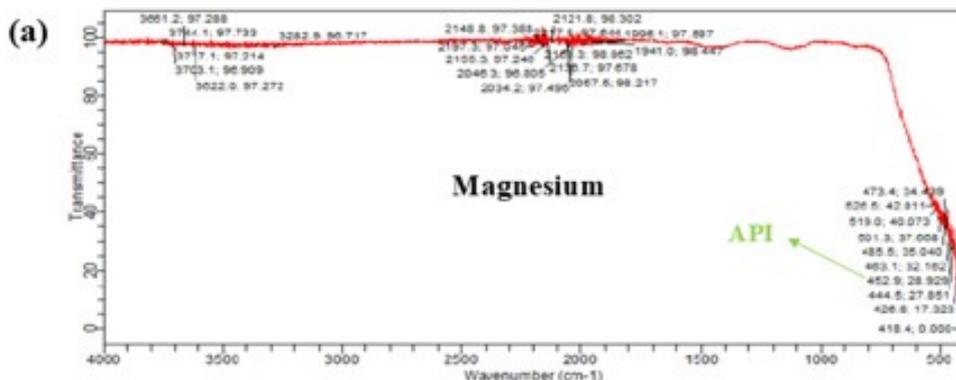


Figure 5. Chart comparing Zeta Potential between Magnesium API and liposomal Magnesium

FTIR Spectroscopy Analysis

Table FTIR Analysis Summary of Liposomal Magnesium

Wavenumber (cm <sup>-1</sup> )	Type of Vibration	Assignment / Functional Group	Interpretation
3410	O-H Stretching	Hydrophilic group (water, hydroxyl groups)	Indicates hydrophilic interactions within the liposomal structure.
2918	C-H Stretching	Lipid alkyl chains (hydrophobic tails)	Confirms presence of lipid bilayer and encapsulation stability.
2845	C-H Stretching	Lipid alkyl chains (hydrophobic tails)	Supports stable lipid tail organization.
1450	C-H Bending	Aliphatic chains	Associated with hydrophobic tail interactions in phospholipids.



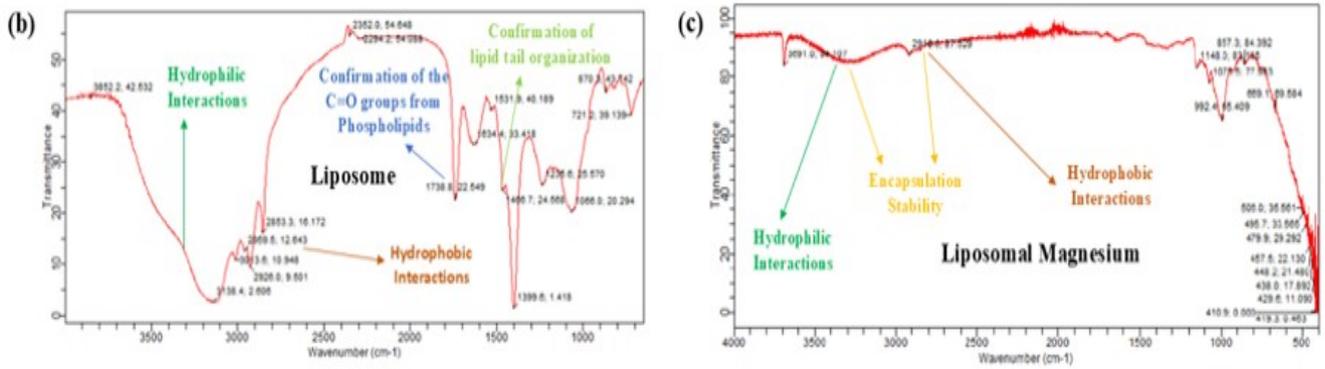


Figure 6. FTIR Transmission spectrum showing bands at different wavelengths of (a) Magnesium API, (b) Liposome and (c) Liposomal Magnesium

Elemental Composition

Table. Elemental Composition of Liposomal Magnesium

Element	Percentage (%)	Role / Interpretation
Carbon (C)	56.58%	Major component of phospholipids; forms the hydrophobic tails.
Oxygen (O)	25.85%	Involved in polar head groups and oxygen-containing functional groups.
Phosphorus (P)	0.24%	Indicates presence of phospholipids (e.g., phosphatidylcholine).
Nitrogen (N)	17.33%	Designates phosphatidylethanolamine or other nitrogen-containing head groups.

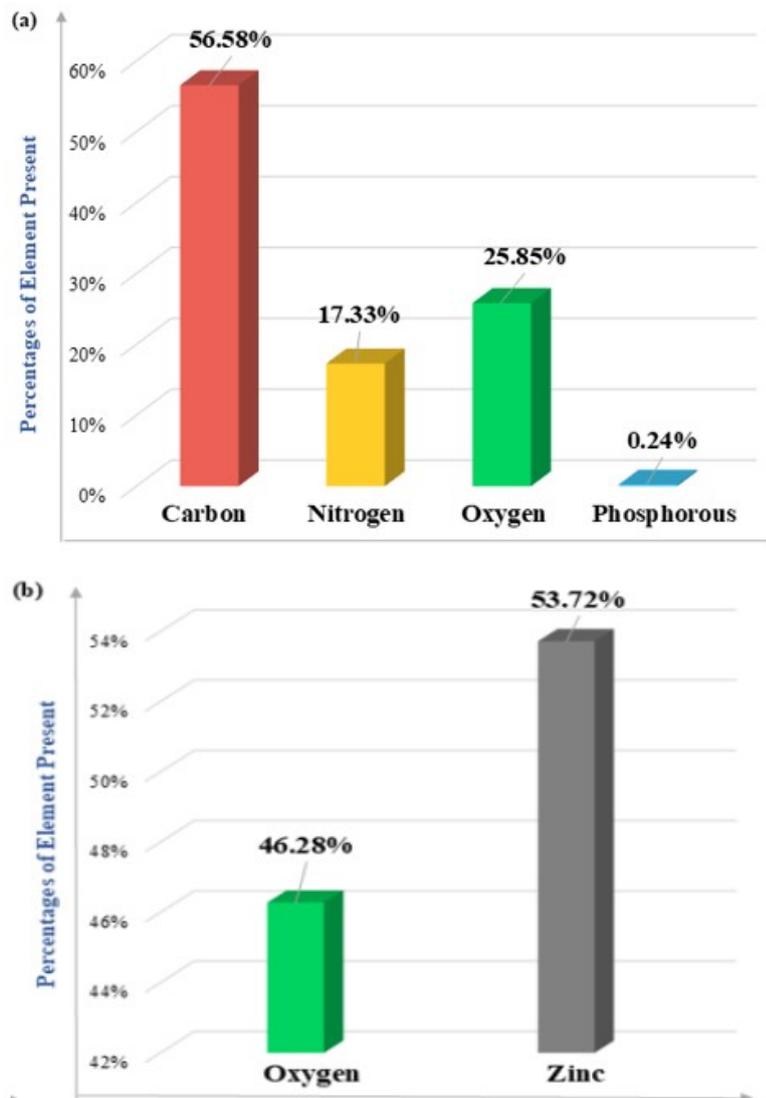


Figure 7. A graphical representation of the percentages of elements composing (a) Liposomal Magnesium and (b) Magnesium API

Elemental analysis via EDAX revealed the presence of oxygen (25.85%), carbon (56.58%), nitrogen (17.33%), and phosphorus (0.24%) in the Liposomal Magnesium formulation (Figure- 7). The high carbon is expected to be present as the phospholipids are primarily carbon-based compounds, oxygen is attributed to the hydroxyl and phosphate groups in the bilayer, nitrogen is primarily attributed to the phospholipid components of phosphatidylethanolamine or other nitrogen-containing head groups in the liposome structure, and phosphorus is correspondent to phosphate moieties of the phospholipids. The elements, such as carbon, oxygen, nitrogen and phosphorus are consistent with the expected composition of liposomal structures, particularly the phospholipid bilayer (Goldstein *et al.*, 2017). Contrarily, the non-liposomal Magnesium (Magnesium API) is characterized by a simple binary composition of magnesium and oxygen (magnesium oxide), confirming its mineral oxide nature. While Liposomal Magnesium shows a complex elemental profile with high carbon, moderate nitrogen and oxygen, and trace phosphorus, indicative of phospholipid-based encapsulation, the absence of a magnesium signal in the EDAX analysis of Liposomal Magnesium supports the fact that magnesium is fully encapsulated within the liposomal vesicles and not exposed on the surface, thus confirming the structural integrity and successful formulation of the liposomal delivery system.

**3-Month Real-Time Stability Study:** After three months, the elemental stability of Liposomal Magnesium was re-evaluated using EDAX following exposure to accelerated stability conditions ( $40\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$  and  $75\% \pm 5\%$  relative humidity). The analysis revealed that the elemental composition remained unchanged, with carbon at 49.32%, oxygen at 33.30%, nitrogen at 16.92%, and phosphorus at 0.46%. Importantly, no detectable surface Magnesium signal was observed, confirming that the mineral remained fully encapsulated within the liposomal matrix (Figure 8). These findings demonstrate that the formulation preserves its structural and compositional stability over time, ensuring continued protection and bioavailability of Magnesium.

**Morphology via SEM Imaging:** SEM imaging provided detailed insights into the physical structure of the Liposomal Magnesium. The images revealed well-defined, spherical liposomes uniformly distributed within the field of observation. The round, well-defined structure of liposomes is a hallmark of successful self-assembly of phospholipids into bilayer vesicles—a process driven by the amphiphilic nature of the lipid molecules. The spherical shape provides an ideal surface area-to-volume ratio, which enhances encapsulation efficiency and allows for more effective drug loading (Figure-8).

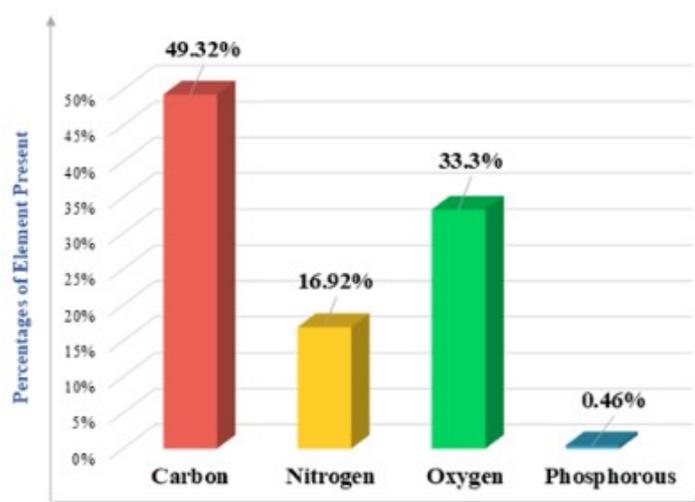


Figure 8. A graphical representation of the percentages of elements composing Liposomal Magnesium after 3 months

## Leakage Studies

### Table Leakage Study of Liposomal Magnesium over time

Duration (Months)	Magnesium Assay (%)	Encapsulation Efficiency (%)	Interpretation
0 (Initial)	20.00	80.03	Fresh sample; baseline magnesium content and high encapsulation.
1	20.50	80.00	Slight increase in assay; encapsulation efficiency remains stable.
2	20.00	80.50	Minor variation; negligible leakage observed.
3	19.50	81.00	Very slight decline, still within acceptable retention limits.
6	19.40	81.00	Minimal leakage after 6 months; confirms good long-term stability.

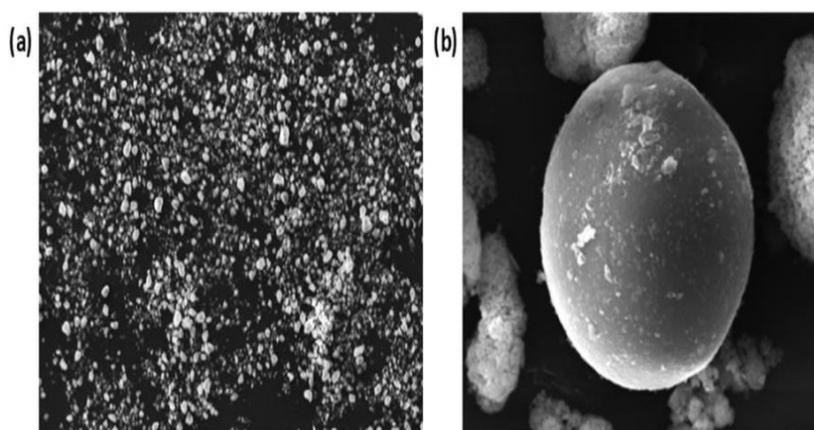
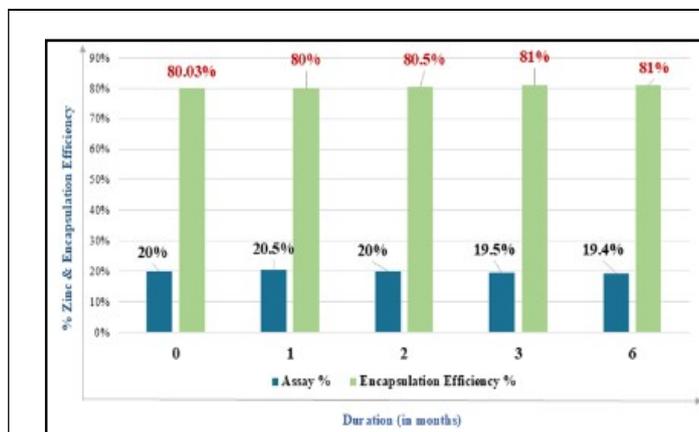


Figure 9: Morphology via SEM Imaging

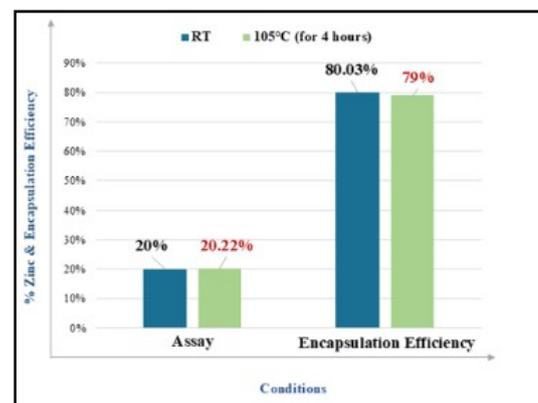
## Thermal Stability at Elevated Temperatures

**Table. Thermal Stability of Liposomal Magnesium**

Condition	Magnesium Assay (%)	Encapsulation Efficiency (%)	Interpretation
Room Temperature (RT)	20.00	80.03	Baseline control; stable at normal conditions.
105°C for 4 Hours	20.20	79.00	Slight reduction in encapsulation efficiency; magnesium content remains stable.



**Figure 10. Chart comparing the stability of Liposomal Magnesium stored over a period of 6 months at 40°C ± 2°C and a relative humidity of 75% ± 5%**



**Figure 11. Chart comparing the stability of Liposomal Magnesium both at room temperature (RT) and at 105°C for 4 hours of exposure**

This geometry maximizes internal volume, making it particularly suitable for accommodating hydrophilic compounds like magnesium ions. The presence of encapsulated Magnesium within the lipid bilayers was clearly visible, further validating the successful integration of Magnesium into the liposomal carriers (Yamashita *et al.*, 2009). Moreover, a total absence of aggregation strongly indicates that the formulation maintained its stability under the experimental conditions. To determine the extent of mineral leakage over time, Liposomal Magnesium was stored at 40°C ± 2°C and 75% ± 5% relative humidity for six months. Over the six-month period, EE% values showed minimal variation from 80% to 81%, indicating excellent stability and very limited leakage. This high retention confirms that the liposomal structure effectively prevents the loss of Magnesium even under stress conditions (Torchilin, 2005).

Similarly, the assay percentage confirmed that the liposomal Magnesium remained unchanged from 20% to 19.40% suggesting no degradation and excellent retention (Figure- 10). This high retention underscores the robustness of the liposomal carrier. Moreover, the consistency in assay percentage, maintaining between 20% and 19.40%, confirms that the active Magnesium content remained chemically stable and did not undergo degradation or loss during the storage period. These results are significant for nutraceutical applications, as they confirm the potential of liposomal formulations to enhance the shelf life, stability, and bioavailability of magnesium supplements. This stability under harsh conditions also suggests that the product is likely to remain effective during distribution and storage, ensuring consistent nutraceutical efficacy for end-users. Liposomal Magnesium was subjected to thermal stress at 105°C for 4 hours. Although a slight decrease in encapsulation efficiency was observed (dropping from 80.03% to 79%), the formulation maintained structural integrity and Magnesium content, demonstrating resilience to high-temperature conditions. Such stability is crucial for withstanding processing and transport environments. Likewise, the Magnesium assay values also remained stable, with measurements of 20.00% at room temperature and 20.22% following thermal stress (Figure 11).

These results collectively confirm the strong structural integrity along with sustained encapsulation of the liposomes under stress conditions, indicating their potential for consistent long-term storage (Zhao *et al.*, 2015). This test confirms an extended thermal limit of the formulation and suggests that the liposomes are able to retain their structural integrity and encapsulation efficiency over time. In particular, the Liposomal Magnesium formulation is fully stable to withstand exposures to higher temperatures without any degradation.

## CONCLUSION AND FUTURE ASPECTS

The Liposomal Magnesium formulation developed by WBCIL demonstrates significant advancements in magnesium supplementation technology. With its high encapsulation efficiency, nanoscale particle size, stable zeta potential, and robust bilayer structure enriched with phospholipids, the formulation ensures superior bioavailability and gastrointestinal tolerance compared to conventional magnesium supplements. Comprehensive physicochemical and stability assessments, including FTIR, SEM, elemental analysis, leakage studies, and thermal stress testing, confirm structural integrity and long-term storage potential of the formulation. Collectively, these findings highlight the effectiveness of Liposomal Magnesium as a stable, efficient, and well-tolerated supplement suitable for improving magnesium delivery in human health applications. Stability testing, i.e., elevated temperature study and leakage study confirm that the liposomes are able to retain their structural integrity and encapsulation efficiency over time. In particular, the Liposomal Magnesium formulation is fully stable to withstand exposures to higher temperatures without any leakage and degradation. The promising results of Liposomal Magnesium formulated by WBCIL open several avenues for future research and development. Further clinical studies are warranted to evaluate its bioavailability and efficacy in diverse health conditions associated with magnesium deficiency. Additionally, exploring its application in functional foods as well as personalized medicine could enhance its commercial viability. The phospholipid-based delivery system of the

formulation also presents opportunities for co-encapsulation of other micronutrients or bioactive compounds, potentially broadening its scope in targeted nutrient delivery and integrative health solutions.

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