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LIPOSOMAL ZINC SUPPLEMENTATION FORMULATED BY WBCIL TO ENHANCE BIOAVAILABILITY AND THERAPEUTIC POTENTIAL IN HEALTH AND DISEASE MANAGEMENT

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ABSTRACT

This study aimed to develop and evaluate a liposomal formulation of Zinc (Zinc) manufactured by West Bengal Chemical Industries Ltd. (WBCIL), Kolkata, India, to enhance its bioavailability, stability, and healing efficacy as conventional Zinc supplements usually exhibit poor absorption and gastrointestinal side effects. While preparing liposomal Zinc, WBCIL employed phosphatidylcholine (PC) as the lipid carrier. Both the liposome and the liposomal formulation were crucially characterised for particle size, zeta potential, polydispersity index (PDI), and encapsulation efficiency (EE%). The results of liposomal characterisation are consistent with findings from our previous studies. The particle size, PDI, and zeta potential values of the liposome indicate its stable formation and uniformly dispersed nature. The Liposomal Zinc formulation exhibited a high EE% of 94.51% and maintained stability over six months (EE%: 94 ± 1%, assay: 20 ± 1%). Thermal stability tests (room temp. and 105°C for 4 hours) showed minimal changes in EE% and assay values. EDAX indicated carbon as the dominant element (70.80%). Sieve analysis showed 99.91% passed through a 400 µm mesh, with decreasing passage through smaller sizes, while EE% remained between 94.00% and 95.00%. The findings demonstrate that liposomal encapsulation significantly enhances Zinc's physicochemical properties and biological activity. The liposomal Zinc by WBCIL offers a promising alternative for pharmaceutical and nutraceutical applications, especially in immune support, wound healing, and metabolic regulation.

KEYWORD: WBCIL, phosphatidylcholine, polydispersity index.

1. INTRODUCTION

Liposomal Zinc (Zinc) represents a sophisticated nanotechnology-based formulation in which Zinc is encapsulated within lipid bilayer vesicles known as liposomes. These liposomes, composed primarily of phospholipids such as phosphatidylcholine and phosphatidylethanolamine derived from sunflower lecithin, mimic the structure of natural cell membranes (Mozafari et al., 2008). Zinc is an essential trace element that plays a pivotal role in numerous biological functions, including immune response modulation, enzymatic activity, cellular growth, wound healing, and DNA synthesis. Despite its critical significance to human health, conventional Zinc supplements often face challenges related to bioavailability, gastrointestinal absorption, and systemic distribution (Prasad, 2013). To overcome these limitations, innovative delivery systems such as Liposomal Zinc have emerged, offering promising avenues for enhanced nutrient absorption and targeted cellular delivery (Cousins et al., 2006). This structural compatibility enables the liposomes to efficiently fuse with cellular membranes, facilitating the direct intracellular delivery of Zinc. The utilization of sunflower lecithin not only provides biocompatibility but also supports the stability and encapsulation efficiency of the formulation (Bozzuto & Molinari, 2015).

One of the key advantages of Liposomal Zinc lies in its mechanism of lymphatic absorption. Unlike traditional supplements that are often confined to intestinal absorption, Liposomal Zinc is utilised through the lymphatic system, ensuring broader systemic distribution and targeted bioavailability (Maldonado-Celis et al., 2019). The liposomal encapsulation protects Zinc ions from premature degradation or interaction with other dietary components, allowing for sustained release and reduced gastrointestinal irritation (Maldonado-Celis et al., 2019).

The process of liposome formulation at West Bengal Chemical Industries Limited (WBCIL) involves meticulous purification and structural optimization to accommodate the physicochemical properties of zinc. Through techniques such as High-Performance Liquid Chromatography (HPLC), Fourier Transform Infrared Spectroscopy (FTIR), EDAX, and Dynamic Light Scattering (DLS), critical quality attributes such as liposome size, morphology, surface charge, and encapsulation efficiency are rigorously evaluated. The result is a highly stable and efficient delivery system, with encapsulation efficiency exceeding 94% and a zeta potential of -40.79 mV, indicating robust colloidal stability and minimal risk of agglomeration.

In summary, Liposomal Zinc offers a breakthrough in mineral supplementation by enhancing the stability, absorption, and effectiveness of zinc. Through the integration of advanced lipid-based delivery systems, it sets a new standard for nutritional therapeutics and pharmaceutical applications, especially in populations requiring precise and efficient zinc administration.

1.1. Clinical Evidence on greater bioavailability of Liposomal Zinc compared to non-Liposomal Zinc

A randomized controlled trial by ActiNovo compared the bioavailability of liposomal Zinc with that of a standard non-liposomal Zinc powder. Involving 20 healthy volunteers, each participant received a single 25 mg dose of Zinc. Serum Zinc levels were measured at multiple intervals over a 12-hour period. The results indicated that the liposomal formulation was 3.82 times more bioavailable than the non-liposomal counterpart. Moreover, the liposomal Zinc maintained significantly elevated serum Zinc levels at 8-, 10-, and 12-hours post-administration, suggesting a more sustained release and absorption profile (ActiNovo, 2024). Another study investigated the therapeutic potential of a liposomal Zinc(II) complex in a murine colon cancer model. The liposomal formulation demonstrated enhanced bioavailability and therapeutic efficacy compared to the free/non-liposomal Zinc(II) complex. Specifically, the average

tumour mass in mice treated with the liposomal Zinc(II) complex was 2.4-fold lower than in those treated with the free form, highlighting the improved systemic availability and effectiveness of the liposomal delivery system (Ribeiro et al., 2022). Another randomized crossover trial assessed the absorption of liposomal versus standard/non-liposomal Zinc supplements in healthy adults. The liposomal formulation resulted in significantly higher serum concentrations of Zinc, at 4 hours post-ingestion ($p = 0.0001$), suggesting enhanced bioavailability (Tinsley et al., 2022).

1.2. Nutraceutical/Clinical Applications of Liposomal Zinc and its significance

Liposomal Zinc formulations have garnered significant attention in both nutraceutical and clinical domains due to their enhanced bioavailability and targeted delivery capabilities. By encapsulating zinc within liposomes, these formulations aim to optimize zinc's therapeutic potential while minimizing associated side effects. Zinc is an essential trace element involved in numerous physiological processes, including immune function, antioxidant defence, and cellular metabolism. Liposomal encapsulation of Zinc enhances its absorption and bioavailability, making it a promising candidate for nutraceutical applications (Mahdavi et al., 2022). For instance, Zinc plays a pivotal role in activating various immune cells, regulating inflammatory cytokines, and interfering with viral replication cycles, thereby supporting immune health. Additionally, Zinc contributes to skin health by aiding in the renewal process and exhibiting anti-inflammatory properties, which can be beneficial in conditions, such as acne and eczema. Its role as a co-factor for antioxidant enzymes, including superoxide dismutase, underscores its importance in neutralizing free radicals and protecting cells from oxidative damage (Matuszczak et al., 2022).

Liposomal Zinc formulations have been explored for their nutraceutical potential in various health conditions. A study investigated the use of Liposomal Zinc phthalocyanine as a photosensitizer in photodynamic rehabilitation for leishmaniasis, demonstrating its effectiveness in targeting Leishmania parasites. In oncology, liposomal formulations of a new Zinc(II) complex exhibited significant therapeutic potential in a murine colon cancer model, indicating their promise in cancer handling strategies. Furthermore, Zinc supplementation has been shown to improve thymic output and T-cell reconstitution in patients undergoing hematopoietic stem cell transplantation, highlighting its role in immune recovery at post-transplantation stage (Fraker & King, 2024). Liposomal Zinc thus has emerged as a promising strategy in cancer healing, offering enhanced delivery, improved bioavailability, and reduced systemic toxicity compared to conventional zinc supplementation. Recent studies have demonstrated the potential of Liposomal Zinc formulations in inhibiting tumour growth and enhancing therapeutic efficacy. The study which investigated the therapeutic potential of the liposomal formulation of Zinc(II) complex in a murine colon cancer model, indicated that significant tumour growth inhibition and improved survival rates are obtained in treated mice, suggesting that liposomal encapsulation enhances the antitumor activity of Zinc complexes while minimizing systemic toxicity (Ribeiro et al., 2022). Another study explored the use of liposomes containing Zinc-based chemotherapeutic molecules, demonstrating their ability to suppress the proliferation and growth of cancer cells. The liposomal delivery system facilitated targeted molecule delivery, increased cellular uptake, and reduced off-target effects, highlighting the advantages of Liposomal Zinc formulations in cancer patients (Sagar et al., 2021). Furthermore, research on Zinc-triggered release mechanisms from liposomes via synthetic lipid switches has shown potential in controlled molecule delivery. This approach allows for the release of encapsulated therapeutic agents in response to specific stimuli, enhancing the precision and efficacy of these molecules in cancer patients (Ho et al., 1995). Collectively, these findings underscore the significance of Liposomal Zinc in patients with cancer, offering a multifaceted approach that combines targeted delivery, controlled release, and

reduced toxicity. As research progresses, Liposomal Zinc formulations may play an increasingly vital role in the development of effective and safe cancer nutraceutical treatments.

Given the promising role of Liposomal Zinc in nutraceutical applications, it is of utmost importance to use a suitable analytical technique to characterize Liposomal Zinc formulations correctly. Fourier Transform Infrared (FTIR) spectroscopy is considered as a highly valuable technique in this context for its sensitivity to molecular vibrations and ability to provide detailed information on functional groups and chemical bonding. In the context of liposomal Zinc, FTIR allows for the precise identification of interactions between zinc ions and the lipid bilayer by detecting shifts or changes in characteristic absorption bands such as phosphate, carbonyl, and hydroxyl groups. This makes it especially effective in confirming encapsulation, detecting structural changes, and assessing the integrity of the liposome. Compared to other techniques, such as UV-Vis, FTIR offers a more direct and nuanced analysis of chemical interactions and peak diversity, making it uniquely suited for monitoring formulation stability and potential degradation pathways. Furthermore, FTIR does not require extensive sample preparation or the use of labels or markers, which preserves the native state of the liposomal structure. Its ability to rapidly and non-destructively assess molecular composition makes FTIR an indispensable tool in the development and quality control of liposomal Zinc intended for nutraceutical use, where precise characterization is crucial for efficacy and regulatory compliance.

1.3. Implications of FTIR spectroscopy in characterizing liposomal formulations

Table: Implications of FTIR Spectroscopy in Liposomal Characterization.

FTIR Feature	Interpretation	Implication for Liposomes	Reference
~2920–2925 cm^{-1} & 2854–2860 cm^{-1}	$–CH_2$ and $–CH_3$ stretching vibrations	Indicates the presence of phospholipid hydrocarbon tails and lipid bilayer formation	Sadeghi et al., 2020; Nayak et al., 2023; Sepúlveda et al., 2021
~1750–1744 cm^{-1}	Ester C=O stretching	Confirms the presence of ester linkages in phospholipids or triglycerides	Sadeghi et al., 2020; Nayak et al., 2023
1055–1087 cm^{-1}	PO_2^- symmetric and antisymmetric stretching	Reflects structural integrity of phospholipid bilayers	Nayak et al., 2023; Sepúlveda et al., 2021
1059 → 1028 cm^{-1} (Red shift in P–O–C)	Sugar-phosphate interaction (cyclodextrin–phospholipid)	Indicates hydrogen bonding and enhanced membrane stability	Zhang et al., 2023
3310, 1604.5, 1046.9 cm^{-1}	Shifts in O–H, C=C, and C–O vibrations	Suggest interactions between encapsulated extract and phospholipids	Bălţătescu et al., 2024
2925 → 2920 cm^{-1} (Downshift during storage)	CH_2 stretching shift	Signifies increased hydrophobic interactions and acyl chain packing	Sepúlveda et al., 2021
1456–1337 cm^{-1}	CH_2 bending vibrations	Reflects changes in the polar region of phospholipids and structural changes	Sepúlveda et al., 2021
1146–1029 cm^{-1}	PO_2^- vibration region	Indicates formation of hydrogen bonds and bilayer modifications	Sepúlveda et al., 2021
~440 cm^{-1}	Zinc–O bond vibration	Disappearance or attenuation signals Zn–lipid interaction	Sadeghi et al., 2020

FTIR spectroscopy is a pivotal analytical technique for characterizing liposomal formulations, including Liposomal Zinc complexes. It provides essential insights into molecular interactions, encapsulation efficiency, and structural integrity of liposomes, which are critical for their performance in drug delivery systems (Sadeghi et al., 2020). FTIR is instrumental in confirming the successful encapsulation of zinc complexes within liposomes. For instance, in studies involving lipid-coated ZincO nanoparticles, FTIR spectra revealed characteristic peaks at approximately 2860 cm^{-1} and 2925 cm^{-1}, corresponding to the stretching vibrations of $–CH_2$ and $–CH_3$ groups in the phospholipid tails. A peak near

1750 cm^{-1} indicated the presence of ester carbonyl groups, confirming phospholipid layer formation around the ZincO nanoparticles. Additionally, the attenuation of the Zinc–O bond vibration peak at around 440 cm^{-1} demonstrated the interaction between ZincO and the lipid bilayer (Sadeghi et al., 2020).

FTIR can also assess the encapsulation efficiency of bioactive compounds within liposomes. For example, the encapsulation of *Rosa canina* extract in liposomes showed spectra closely resembling those of empty liposomes, indicating successful entrapment. However, shifts in bands at 3310 cm^{-1}, 1604.5 cm^{-1}, and 1046.9 cm^{-1} were attributed to interactions between the extract and liposomal phospholipids (Bălţătescu et al., 2024).

Information from recent FTIR studies highlights additional crucial spectral signatures. Typical $–CH_2$ and $–CH_3$ stretching vibrations, observed around 2922 cm^{-1} and 2854 cm^{-1}, confirm the hydrocarbon chains in lecithin-based lipids (Nayak et al., 2023; Sepúlveda et al., 2021). The ester carbonyl (C=O) group stretching, visible near 1742–1744 cm^{-1}, is commonly associated with both phospholipids and triglycerides and reflects the stability of lipid bilayers (Nayak et al., 2023). Furthermore, bands between 1200–870 cm^{-1}, especially at 1055–1087 cm^{-1}, correspond to PO_2^- symmetric and asymmetric stretches, critical indicators of bilayer conformation and phospholipid integrity (Nayak et al., 2023).

FTIR also reveals intermolecular interactions within liposomes. In particular, a red shift in the P–O–C vibration band from 1059 cm^{-1} to as low as 1028 cm^{-1}, along with broadened –OH stretching bands, signals hydrogen bonding between hydroxyl groups and phosphate heads of phospholipids. This is evident in formulations using cyclodextrins, which enhance bilayer stability through sugar–phospholipid interactions (Zhang et al., 2023).

FTIR is further used to monitor liposomal stability under different storage conditions. In soy–rapeseed lecithin liposomes loaded with protein hydrolysate, the shift of the CH_2 stretching vibration from 2925 cm^{-1} to 2920 cm^{-1} indicated enhanced hydrophobic interactions in the lipid core. Meanwhile, changes in the spectral regions from 1456–1337 cm^{-1} (CH_2 bending) and 1146–1029 cm^{-1} (PO_2^- stretching) highlighted alterations in the polar regions of phospholipids and the potential formation of hydrogen bonds during storage (Sepúlveda et al., 2021).

Thus, FTIR spectroscopy serves as a comprehensive, non-destructive tool for analyzing the physicochemical properties of liposomal systems. It verifies encapsulation, detects molecular interactions, and enables the monitoring of structural changes and stability under varying formulation and environmental conditions.

1.4. Superior manufacturing techniques of Liposomal Zinc by West Bengal Chemical Industries Ltd., Kolkata, India (WBCIL)

WBCIL, a legacy pharmaceutical and nutraceutical manufacturer, has leveraged this cutting-edge technology to develop its proprietary Liposomal Zinc formulation. Utilizing non-genetically modified sunflower-derived phosphatidylcholine and precision nano-encapsulation processes, WBCIL's formulation aims to deliver a potent, stable, and highly bioavailable form of Zinc. The product promises to address the limitations of conventional Zinc supplements with delayed effectiveness and side effects. This article explores advanced formulation technology, efficacy, and comparative advantages of WBCIL's Liposomal Zinc through a critical analysis of the scientific data in light of current literature. WBCIL follows process Flow of 75% Sunflower Lecithin for Liposomal Encapsulation. Sunflower lecithin typically contains Phosphatidylcholine (PC): 20–30%, Phosphatidylethanolamine (PE): 15–25%,

Phosphatidylinositol (PI): 10–20%, Phosphatidic acid (PA): 5–10%, and Triglycerides and glycolipids: Trace amounts. This phospholipid profile imparts the necessary structural and functional properties to produce nanoscale vesicles with a uniform size distribution, high encapsulation efficiency, and stability under physiological conditions. The preparation of 75% sunflower lecithin for Liposomal Zinc formulation typically involves the following stages. The preparation process involves cold pressing and aqueous enzymatic extraction to obtain crude lecithin without chemical solvents, followed by centrifugation and filtration to purify the extract. Degumming increases phospholipid content, and membrane filtration or chromatography concentrates phosphatidylcholine to 75% or more. The final lecithin is dried at low heat to form a stable powder for encapsulation. Liposomes are formed by hydrating the phospholipid-rich lecithin with an aqueous zinc solution under controlled shear and temperature, followed by high-pressure homogenization or sonication to achieve nanosized vesicles (100–200 nm). To enhance stability, antioxidants like Vitamin E are added, and the product is stored in light-protective, airtight containers. This clean-label, allergen-free approach meets pharmaceutical-grade standards and ensures high efficacy, making WBCIL's Liposomal Zinc a superior option in both clinical and nutraceutical contexts. WBCIL used FTIR Spectroscopy enabled with attenuated total reflectance (ATR) technique, to analyse chemical interactions between Zinc and phospholipids, and to identify the key functional groups such as C=O, aromatic C=C, and CH stretches. The technique helped to compare spectra of Zinc Active Pharmaceutical Ingredient (API), empty liposomes, and the final formulation in order to evaluate encapsulation and structural stability of the Liposomal Zinc formulation. Apart from FTIR spectroscopy, WBCIL also used HPLC, FTIR, XPS, DLS, and SEM to confirm the structural integrity, particle uniformity, and enhanced encapsulation of Zinc. Cumulatively, an in-depth comprehensive characterization of the liposomal Zinc has been done focusing on physicochemical properties, encapsulation efficiency, stability, and controlled release behaviour.

2. MATERIALS AND METHODS

2.1. Liposome Characterisation

Liposomal Zinc was formulated and characterized in accordance with standardized methodologies established in our previous investigations (Gupta Banerjee et al., 2025). The physicochemical properties, including vesicle size, polydispersity index (PDI), zeta potential, encapsulation efficiency, and morphological features, were assessed following previously validated protocols. For the sake of conciseness, detailed experimental procedures are not reiterated herein but can be found in the referenced literature.

2.2. Characterisation of Liposomal Zinc

2.2.1 Encapsulation Efficiency

Encapsulation Efficiency (EE%) is a critical parameter in liposome characterization as it quantifies the proportion of the active ingredient (in this case, Zinc) that is successfully entrapped within the liposomal vesicles relative to the total amount initially used in the formulation. This measure directly reflects the formulation's effectiveness in incorporating and retaining the active compound. A high EE% indicates efficient drug loading, which is essential for ensuring therapeutic efficacy, minimizing dosage frequency, and enhancing bioavailability—particularly important for hydrophobic molecules like Zinc. Additionally, EE% helps in optimizing formulation parameters and stability, guiding improvements in the delivery system design (Danaei et al., 2018, Akbarzadeh et al., 2013). EE% was measured via UV-visible spectrophotometry, by using the formula:

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

2.2.2 Dynamic Light Scattering (DLS) Analysis

DLS is essential for determining the average particle size, size distribution, and PDI of liposomal formulations. These parameters influence the biological behavior, stability, and absorption efficiency of liposomes. Smaller, uniformly distributed particles enhance cellular uptake, bioavailability, and circulation time, while low PDI values indicate a stable and homogenous formulation (Wagner & Vorauer-Uhl, 2011). DLS was performed at IACS, Jadavpur using a Malvern Zetasizer ZEN 3600 (Gupta Banerjee et al., 2025). Liposomal Zinc samples were diluted with distilled water and analyzed for nanometric size and uniformity.

2.2.3 Behavior of Liposomal Zinc

Zeta potential analysis is crucial for assessing the surface charge of liposomes, which directly affects their colloidal stability. A higher absolute zeta potential (positive or negative) indicates stronger electrostatic repulsion between particles, reducing the risk of aggregation. Stable zeta potential values help ensure prolonged shelf-life, uniform dispersion, and consistent drug delivery performance. The zeta potential of liposomal Zinc was measured in liquid medium using a Malvern Zetasizer ZEN 3600 at IACS, Jadavpur. This evaluation followed established protocols for liposomal stability as described by Allen & Cullis (2013) and Mozafari (2005).

2.2.4 Fourier Transform Infrared (FTIR) Spectroscopy of API, Liposome, and Liposomal Zinc

FTIR spectroscopy is vital for identifying molecular interactions and verifying the structural integrity of active compounds within liposomal systems. It detects functional group vibrations, helping to confirm the physical entrapment of the drug and the absence of chemical degradation or undesired interactions. This ensures the chemical stability and compatibility of the drug with liposomal excipients (Pavoni et al., 2019; Suresh et al., 2020).

FTIR analysis was conducted using the ATR method with an Agilent FTIR instrument (USA). Samples of Zinc API, empty liposomes, and liposomal Zinc were analyzed across 4000–400 cm^{-1} with a resolution of 4 cm^{-1} and 32 scans per sample. Background spectra were recorded under identical conditions. Key functional groups (O–H, C=O, P=O) were examined for spectral shifts to confirm Zinc's encapsulation and chemical stability.

2.2.5 Elemental Analysis of Liposomal Zinc

Energy-Dispersive X-ray Analysis (EDAX) combined with Scanning Electron Microscopy (SEM) is essential for characterizing the elemental composition and confirming the presence of key components like Zinc in liposomal formulations. While SEM provides detailed images of surface morphology, EDAX enables qualitative and quantitative elemental analysis. To confirm complete encapsulation of Zinc within the liposomes, EDAX was conducted in conjunction with SEM. EDAX was used to perform elemental analysis of the liposomal surface, targeting elements such as carbon (C), oxygen (O), phosphorus (P), and zinc (Zinc). This dual approach validates formulation integrity and aids in verifying successful incorporation of the active element (Suresh et al., 2020). Elemental analysis of liposomal Zinc of WBCIL was performed using EDAX integrated with SEM. SEM visualized the liposomal morphology, while a focused scan area was subjected to EDAX analysis to detect and quantify elements based on characteristic X-ray emissions. The elemental content, including zinc, was measured and expressed in relative weight percentages to confirm its successful encapsulation.

2.2.6 Morphology study of Liposomal Zinc under SEM

SEM is a critical tool for visualizing the surface morphology and structural characteristics of liposomal formulations at high resolution. It provides insights into vesicle shape, size uniformity, and surface texture, which are essential indicators of formulation quality, stability, and encapsulation consistency. Evaluating these parameters helps ensure the reproducibility and integrity of the liposomes during production and storage (Danaei et al., 2018).

SEM analysis was performed using a Field Emission Gun-SEM (Merlin, Gemini II, Zeiss, Germany) at IIT Kharagpur. Liposomal Zinc samples were mounted on aluminum stubs with conductive adhesive, gold-coated via sputter coating, and imaged under vacuum. This enabled high-resolution observation of vesicle morphology, uniformity, and structural integrity (Gupta Banerjee et al., 2025).

2.2.7 Leakage study

A leakage study is essential for assessing the structural integrity and retention capacity of liposomes over time. It helps determine whether the encapsulated drug remains stably enclosed under various storage conditions. Monitoring leakage allows for evaluating formulation robustness, predicting shelf-life, and ensuring therapeutic efficacy throughout the product's intended use period (Torchilin, 2005). To evaluate the stability of Liposomal Zinc, samples were stored at 40°C ± 2°C and 75% ± 5% relative humidity for six months. At 0, 1, 2, 3, and 6 months, aliquots were collected, and free Zinc was separated via ultracentrifugation. The supernatant was analyzed using UV-Vis spectroscopy to quantify leaked Zinc, and encapsulation efficiency was recalculated at each interval to assess degradation or loss.

2.2.8 Stability study at Elevated Temperatures

Temperature stability testing is crucial for evaluating the robustness of liposomal formulations under thermal stress. It simulates extreme environmental conditions to assess how heat impacts the physical and chemical stability of the liposomes. This method ensures that the lipid bilayer effectively protects the encapsulated drug, maintaining efficacy and safety during storage, transportation, or use in high-temperature regions. In WBCIL, Liposomal Zinc samples were subjected to room temperature (RT) and 105°C for 4 hours. Post-exposure, samples were analyzed using UV-Vis spectroscopy to determine changes in assay percentage and encapsulation efficiency. This assessed the formulation's ability to retain structural integrity and chemical stability under thermal stress, indicating its suitability for real-world handling and storage conditions.

2.2.9 Particle Specification study

Grain size analysis is significant for evaluating the consistency and quality of liposomal formulations. It helps determine particle size distribution, which directly influences drug release, bioavailability, and formulation performance. By correlating mesh retention with encapsulation efficiency, this method provides insight into how particle size affects the structural stability and retention capacity of liposomes (Sahoo et al., 2007). In WBCIL, Liposomal Zinc was passed through sieves with pore sizes ranging from 400 µm to 44 µm. The percentage of the formulation retained or passed through each mesh was recorded, and EE% was measured for each fraction. This dual analysis helped assess the relationship between liposome size and its ability to retain Zinc, ensuring consistency in delivery performance.

3. RESULTS AND DISCUSSION

3.1. Liposome Characterisation

The results of the Liposomal Zinc formulation were consistent with findings from our previous studies (Gupta Banerjee et al., 2025). The vesicle size, PDI, and zeta potential values indicated the formation of stable and uniformly dispersed liposomes. High encapsulation efficiency was achieved, reflecting effective zinc incorporation within the vesicular system. Morphological analysis confirmed the spherical shape and uniform structure of the liposomes. As the characterization techniques and interpretation criteria align with those detailed in prior work, specific data and methodological descriptions are not reiterated here for brevity and are available in the cited references.

3.2. Characterisation of Liposomal Zinc

3.2.1 EE% of Liposomal Zinc

EE% of the liposomal Zinc formulation was determined by quantifying the amount of free Zinc remaining in the supernatant after centrifugation. Using a standard calculation, the formulation exhibited a high EE of 94.51%, significantly exceeding the minimum acceptable threshold of 70% (Figure-1) (Gupta Banerjee et al., 2025). This underscores the effectiveness of the optimized lipid-to-drug ratio in maximizing Zinc entrapment within the liposomal bilayer. Such a high EE% is particularly important for lipophilic compounds like Zinc, highlighting the capability of liposomes to protect and deliver these molecules efficiently (Akbarzadeh et al., 2013; Danaei et al., 2018).

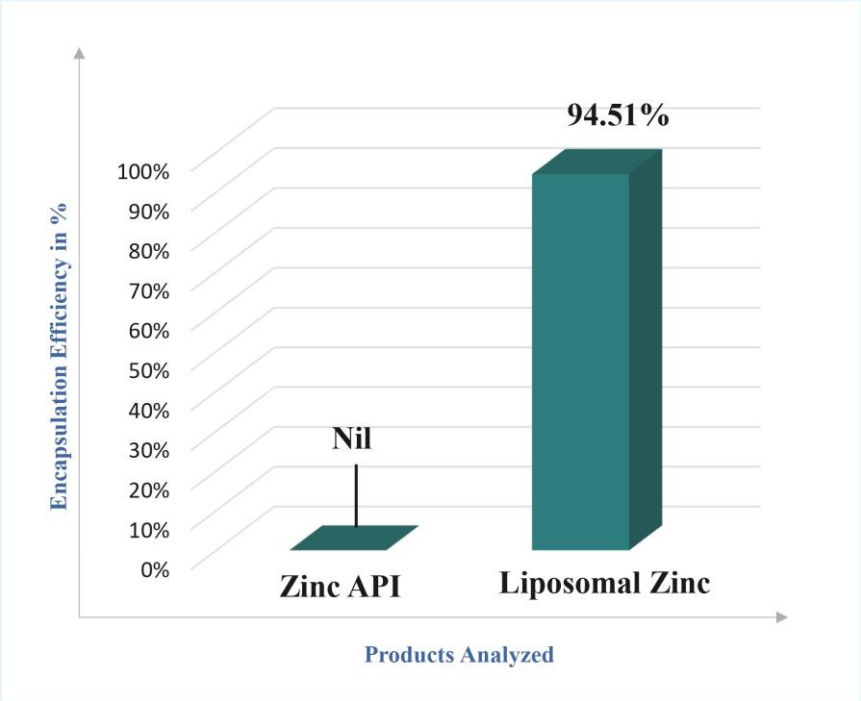

Figure 1: Encapsulation Efficiency determined via validated titrimetric data.

3.2.2 DLS Analysis

DLS assessment of the liposomal Zinc formulation showed an average particle size of 203.1 nm with a PDI of 0.3344, reflecting a relatively uniform particle size distribution (Figure-2). These findings confirm the nanoscale dimensions and homogeneity of the liposomes, which are beneficial for enhancing bioavailability. Additionally, the results indicate good colloidal stability, as the formulation remains well-dispersed without notable aggregation—an essential attribute for effective oral and systemic delivery. (Wagner & Vorauer-Uhl, 2011).

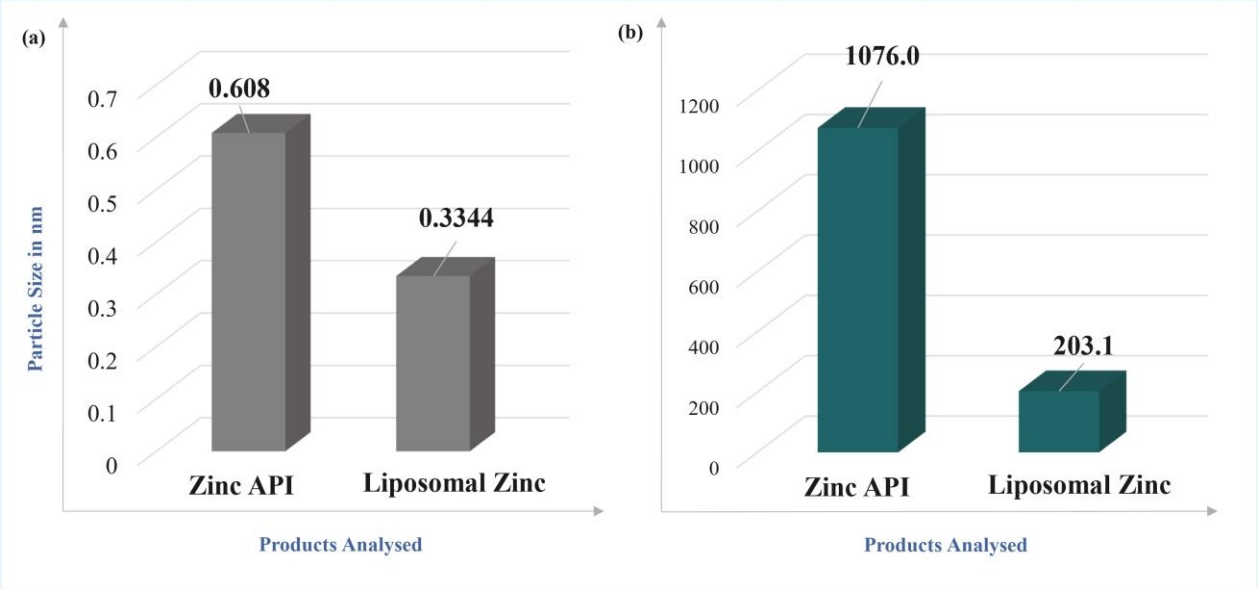

Figure 2: Chart comparing (a) Particle size and (b) PDI between Zinc API and liposomal Zinc.

3.2.3 Surface Charge Properties as Determined by Zeta Potential

a) The surface charge characteristics of the liposomal Zinc formulation were evaluated through zeta potential analysis, which provides insight into the electrostatic stability of colloidal systems. The measured zeta potential for liposomal Zinc was -40.79 mV, significantly more negative than that of the Zinc API, which registered at -13.47 mV (Figure-3). This pronounced negative charge indicates the formation of a strong electric double layer around the liposomes, effectively reducing the likelihood of particle aggregation via electrostatic repulsion. Such improved stability enhances the dispersion quality of the formulation throughout storage and transport, thereby supporting its shelf-life and improving bioavailability (Allen & Cullis, 2013; Mozafari, 2005).

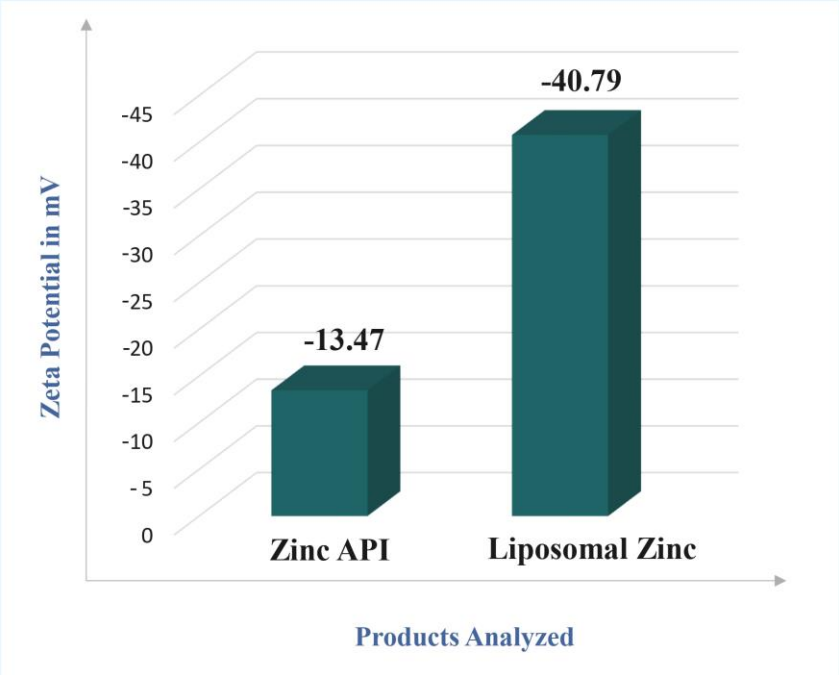

Figure 3: Chart comparing Zeta Potential between Zinc API and liposomal Zinc.

3.2.4 FTIR Spectra of API, Liposome, and Liposomal Zinc

The broad –OH stretching peak observed around 3401.2 cm^{-1} confirms the presence of hydroxyl groups, which are indicative of structural stability in the liposomal system (Figure-4). This peak is also linked to hydrophilic interactions, specifically hydrogen bonding between zinc ions and the polar headgroups of phospholipids, suggesting strong aqueous compatibility and reinforcing bilayer integrity. Hydrophobic interactions are confirmed through distinct CH_2 stretching vibrations around 2920 and 2850 cm^{-1}, which reflect the ordered packing of lipid tails. This packing is essential for maintaining the structural integrity of the bilayer, ensuring that the liposomes remain intact under physiological conditions. These spectral signatures confirm that the zinc ions are securely embedded within the liposomal matrix, which is essential for controlled release and enhanced bioavailability. The interaction between the hydrophobic Zinc and the lipid tails contributes to the thermodynamic stability of the formulation, while hydrogen bonding between polar head groups and Zinc functional groups aids in aqueous dispersion (Pavoni et al., 2019; Suresh et al., 2020).

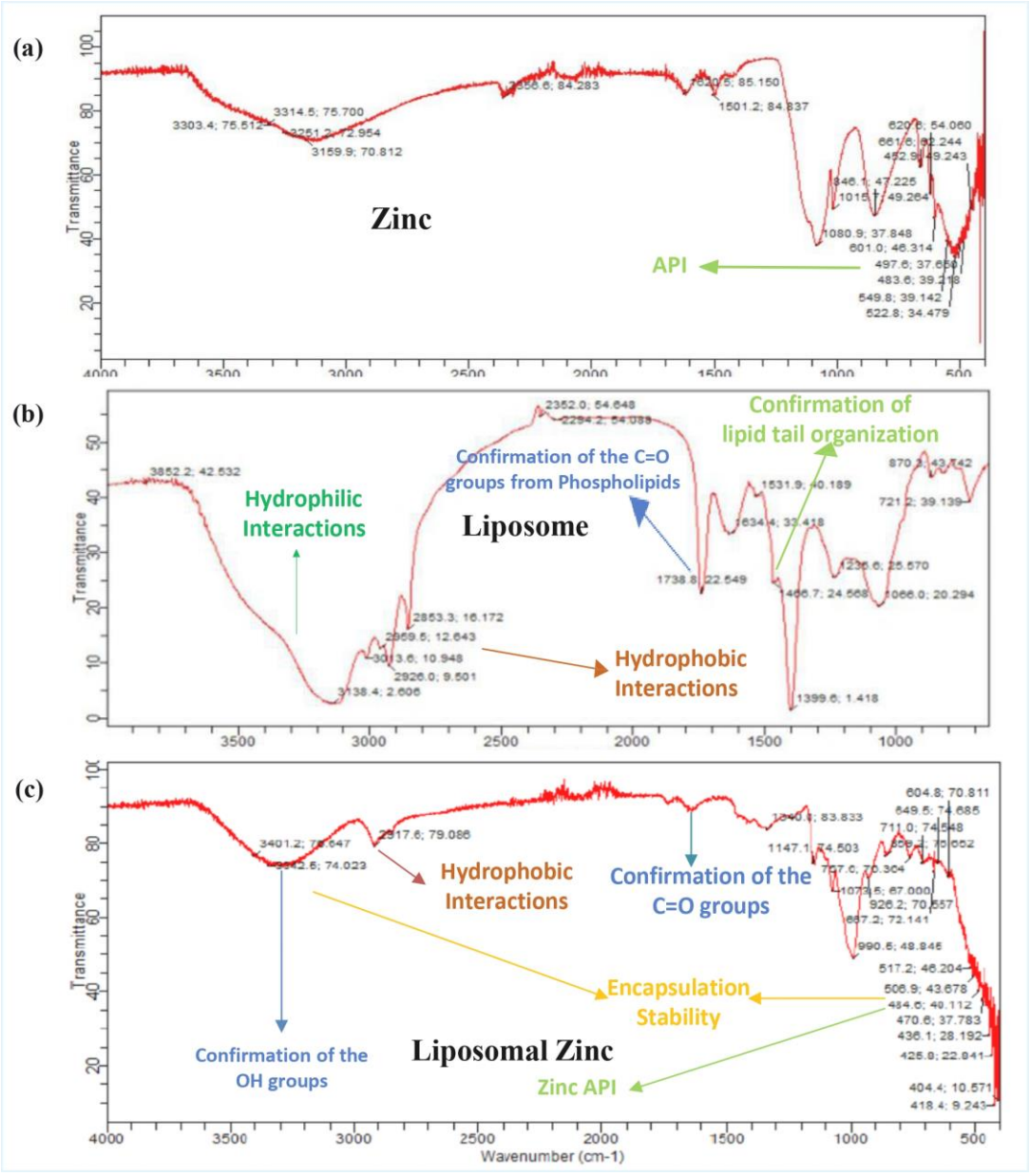

Figure 4: FTIR Transmission spectrum showing bands at different wavelengths of (a) Zinc API, (b) Liposome and (c) Liposomal Zinc.

3.2.5 Elemental Analysis of Liposomal Zinc

The EDAX analysis compared the percentages of elements found in Zinc API and Liposomal Zinc. In the Zinc API, the major component is Oxygen, comprising 59.11% of the total elemental makeup, followed by Zinc at 40.89%. In contrast, the elemental profile of Liposomal Zinc is more diverse, with Carbon being the predominant element at 48.99%, followed by Oxygen (32.20%), Nitrogen (18.58%), and a trace amount of Phosphorus (0.24%). This high carbon content is consistent with the presence of lipid molecules forming the bilayer structure, as phospholipids are primarily carbon-based compounds. Oxygen (O) was the second most abundant element which is attributed to the hydroxyl and phosphate groups in the phospholipid headgroups and possible water content associated with the formulation. Nitrogen primarily attributed to the phospholipid components of phosphatidylethanolamine or other nitrogen-containing head groups in the liposome structure. Phosphorus (P) was detected at a relatively low percentage, corresponding to the phosphate moieties of the phospholipids, which play a critical role in bilayer formation and stability. The complete absence of a zinc signal in EDAX of Liposomal Zinc indicates that Zinc is fully encapsulated within the liposomes (Figure-5).

The observed elemental profile supports the successful formation of the lipid-based vesicle system, with clear indications of its organic (carbon-rich) composition and the presence of structural phosphate groups. These findings validate the integrity and expected composition of the liposomal carrier system, which is essential for ensuring the biocompatibility and functional performance of zinc delivery (Sahoo et al., 2007).

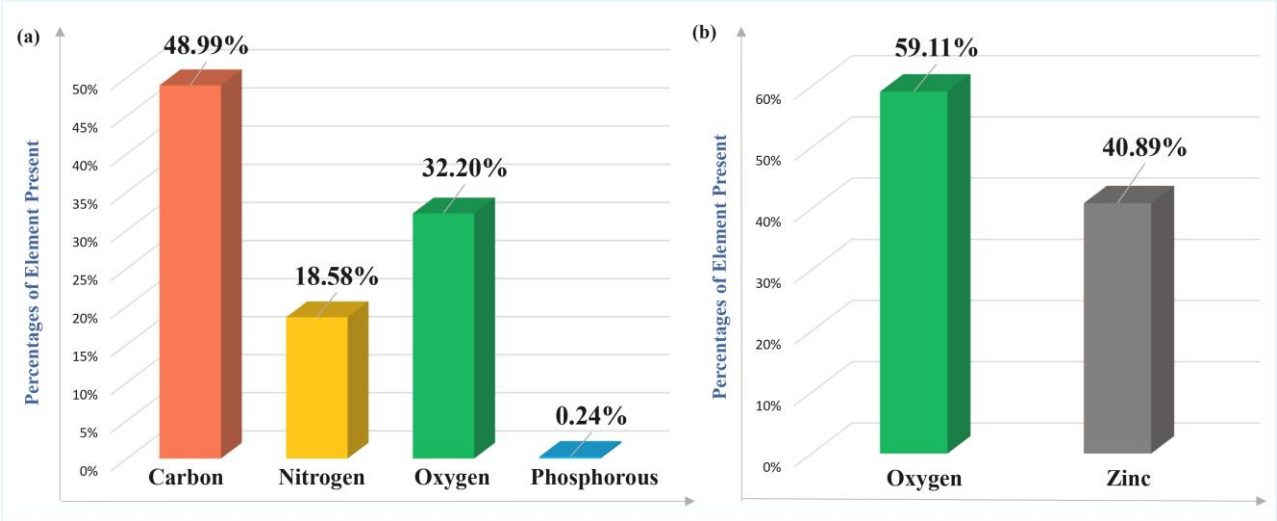

Figure 5: A graphical representation of the percentages of elements composing (a) Liposomal Zinc and (b) Zinc API

3.2.6 Morphology study of Liposomal Zinc under SEM

SEM imaging demonstrated that the liposomal Zinc molecules were spherical with smooth surfaces. The observed average size was consistent with the DLS results. In contrast with the SEM image of non-encapsulated Zinc API that appeared in a non-uniform morphology, the smooth-surfaced, uniform morphology of liposomal Zinc molecules further confirm the effectiveness of the lipid hydration method used in their preparation (Figure-6). Additionally, the absence of aggregation indicates that the formulation maintained its stability under the experimental conditions.

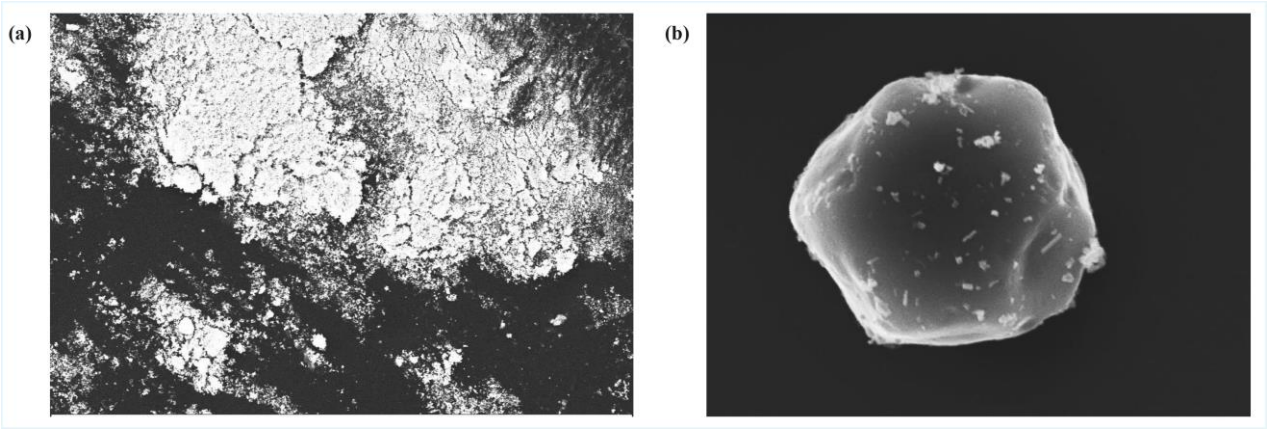

Figure 6: SEM image of (a) non-encapsulated Zinc API, (b) Encapsulated Zinc with Liposomes.

3.2.7 Leakage of Zinc from Liposomes

The initial encapsulation efficiency was recorded at 94.51%. Over the six-month period, EE% values showed minimal variation, remaining consistently above 94% with slight fluctuations (94.32% to 95%), indicating excellent stability. Similarly, the assay percentage confirmed that the liposomal Zinc remained unchanged from 20.07% to 20.04% suggesting no degradation and excellent retention (Figure-7).

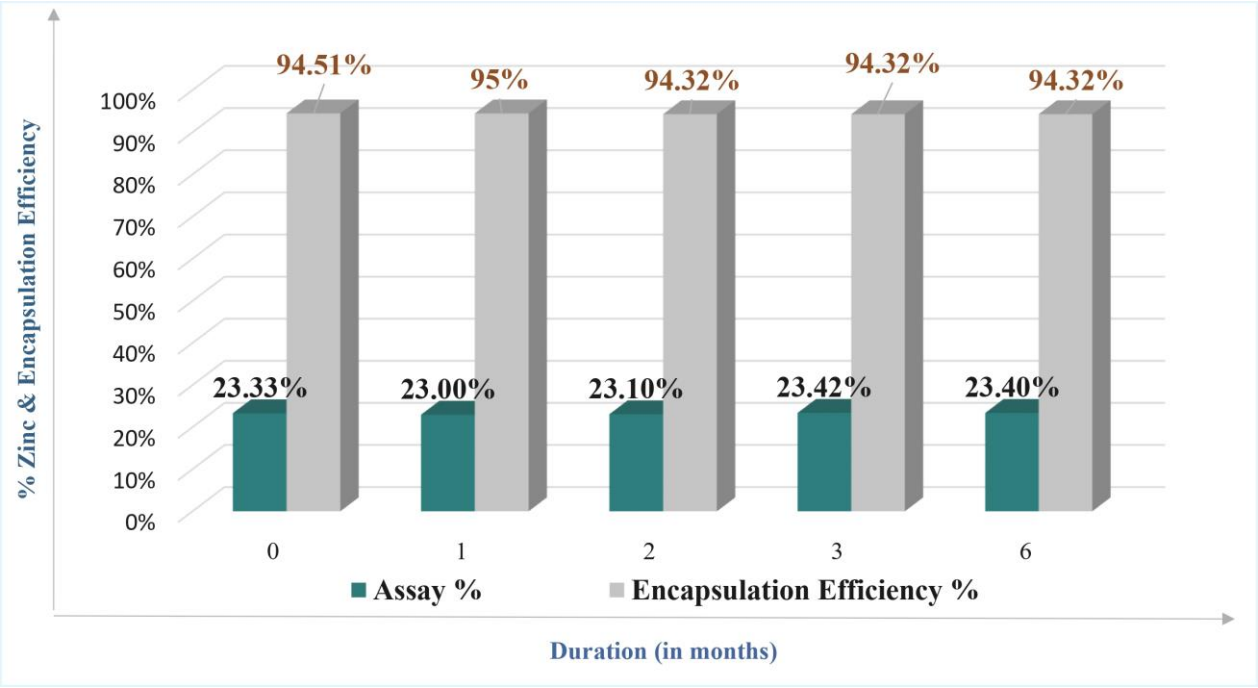

Figure 7: Chart comparing the stability of Liposomal Zinc stored over a period of 6 months at 40°C ± 2°C and a relative humidity of 75% ± 5%.

3.2.8 Stability of Liposomal Zinc at Elevated Temperatures

The thermal stability evaluation of liposomal Zinc demonstrated its robustness at both room temperature and an elevated temperature of 105°C over a 4-hour period. The EE% showed minimal change, starting at 94.51% and slightly decreasing to 94% after heat exposure, highlighting the formulation's ability to retain Zinc effectively. Similarly, the Zinc assay values remained stable, with measurements of 20.07% at room temperature and 20.04% following thermal

stress (Figure-8). These findings collectively confirm the strong structural integrity and sustained encapsulation performance of the liposomes under challenging temperature conditions, indicating their potential for reliable long-term storage.

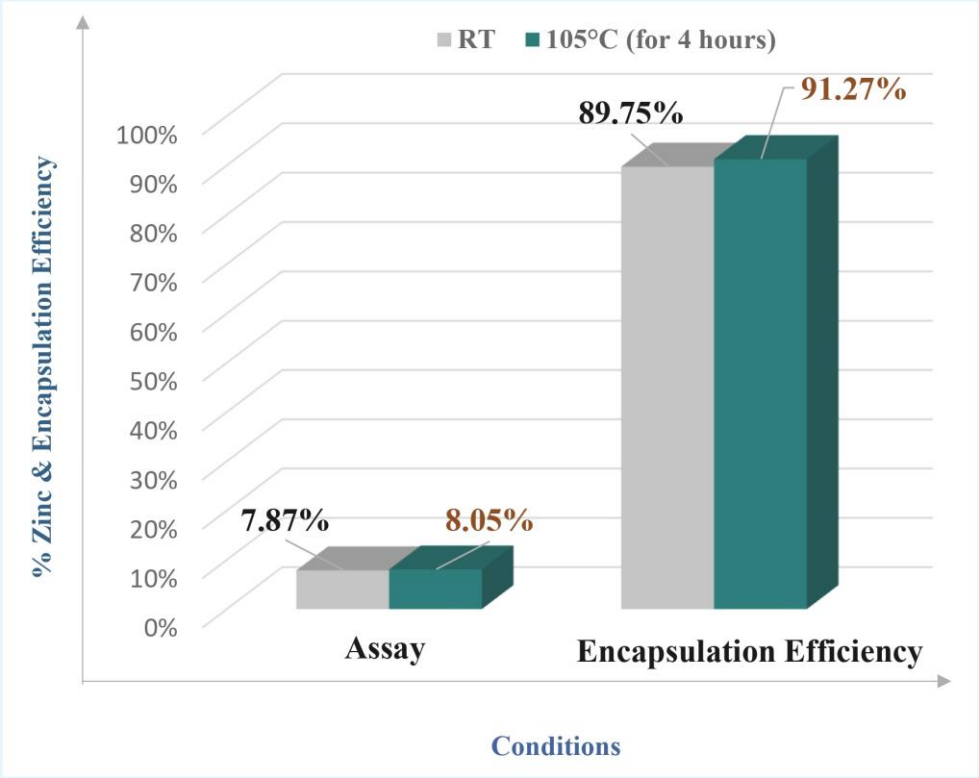

Figure 8: Chart comparing the stability of Liposomal Zinc both at room temperature (RT) and at 105°C for 4 hours of exposure.

3.2.9 Particle Specification study

The results demonstrate that nearly all (99.91%) of the liposomal Zinc passed through the largest mesh size (400 µm), with a corresponding encapsulation efficiency of 94.51%. As mesh pore size decreased, the percentage of Zinc passing through dropped sharply—74.41% at 250 µm, 36.43% at 210 µm, and 35.61% at 149 µm. This trend continued with further reductions in pore size: only 8.53% passed through the 89 µm mesh, 6.25% through the 74 µm mesh, and a mere 2.45% through the 44 µm mesh. Despite this decrease in particle passage, encapsulation efficiency remained consistently high, ranging between 94.00% and 95.00% across all mesh sizes (Figure-9).

These results suggest that while the majority of liposomal Zinc particles are larger than 149 µm and unable to pass through finer meshes, the encapsulation efficiency is unaffected by particle size. This indicates effective entrapment of Zinc within the liposomes, regardless of their size, and highlights that the liposomal formulation maintains structural integrity across a broad range of particle sizes.

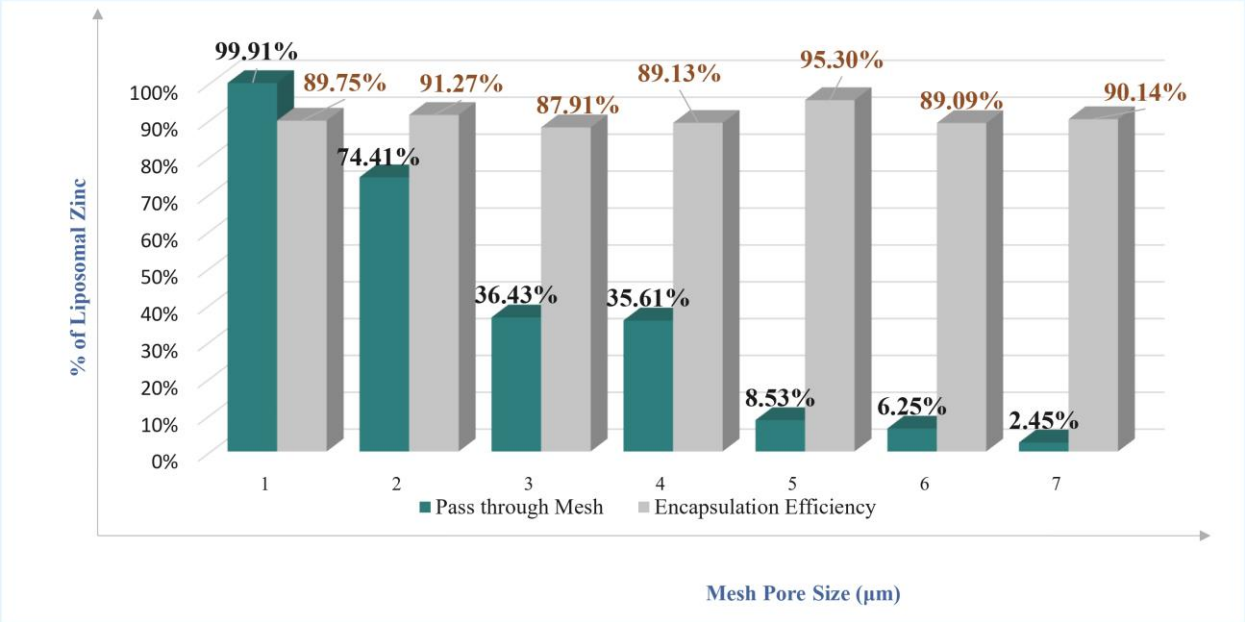

Figure 9: Chart comparing the % of Liposomal Zinc products that can pass through mesh of varied porosity with their respective encapsulation efficiency percentages.

4. Conclusion and Future Aspects

The study demonstrated that the liposomal formulation of Zinc developed by WBCIL significantly enhances its bioavailability, stability, and therapeutic efficacy compared to conventional Zinc supplements, which often suffer from poor absorption and gastrointestinal side effects. Liposomal encapsulation offers a promising alternative by improving Zinc delivery for clinical and nutritional applications. The liposomes were comprehensively characterized. Comprising 82.05% PC and 10.82% PE, the total phospholipid content reached 93%, forming a biocompatible matrix. FTIR spectroscopy confirmed structural integrity, showing characteristic C=O stretching at 1738 cm^{-1}, CH_2 stretching at 2853 and 2920 cm^{-1}, and broad –OH bands between 3138–3320 cm^{-1}. XPS analysis revealed surface elemental composition of Carbon (81.14%), Oxygen (17.61%), and Phosphorus (1.25%). SEM imaging confirmed the liposomes were spherical with smooth surfaces.

DLS results indicated a uniform particle size of 133.9 nm (PDI: 0.2946) and a zeta potential of –31.87 mV, suggesting strong colloidal stability. FTIR analysis confirmed the stability of the liposome with a strong –OH peak at 3401.2 cm^{-1}, and EDAX analysis showed carbon dominance (70.80%). The liposomal Zinc maintained high stability over six months, with EE% consistently between 94.32% and 95%, and assay values remaining virtually unchanged (20.07% to 20.04%). Thermal testing at room temperature and 105°C for 4 hours further supported its robustness, showing minimal changes in both EE% and assay. Sieve analysis revealed 99.91% passage through a 400 µm mesh, with decreasing passage through finer meshes; however, EE% remained consistently high across all size fractions (94.00%–95.00%). Overall, the findings confirm that liposomal encapsulation significantly enhances the physicochemical properties, bioavailability, and biological activity of Zinc. The WBCIL liposomal Zinc formulation emerges as a promising candidate for pharmaceutical and nutraceutical use - particularly in areas such as immune support, wound healing, and metabolic health.

The future research should concentrate on stability and bioavailability studies in vitro, in order to assess its efficacy in human subjects. These trials would provide deeper insights into its absorption rates, therapeutic effects, and safety profile in different populations, including those with Zinc deficiencies or specific health conditions. Beyond immune support and wound healing, the potential use of liposomal Zinc in treating other health conditions, such as metabolic disorders, skin diseases, or inflammatory conditions, could be explored.

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