Journal Homepage: - www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI: 10.21474/IJAR01/21331
DOI URL: http://dx.doi.org/10.21474/IJAR01/21331

RESEARCH ARTICLE

CONTINUOUS IMPROVEMENTS IN THE PRODUCT PORTFOLIO OF LIPOSOMAL IRONBY WBCIL TO OFFER ENHANCED NUTRACEUTICAL EFFICACY AND PUBLIC HEALTH SAFETY

Poulami Gupta Banerjee, Atanuka Paul and Argha Chakraborty

Manuscript Info

Manuscript History
Received: 06 May 2025
Final Accepted: 09 June 2025
Published: July 2025

Abstract

Liposomal Iron is an advanced delivery system designed to address the limitations of traditional Iron supplements, including low bioavailabilit y, gastrointestinal side effects, and oxidative instability. In our previous study, we established the basic nutraceutical potential of Liposomal Iron manufactured by West Bengal Chemical Industries Ltd., Kolkata, India (WBCIL). This article builds on that work using advanced analyti cal techniques to further characterize its physicochemical properties, structural interactions, and encapsulation efficiency. Advanced analyses using Dynamic Light Scattering (DLS), Zeta Potential Analysis, Fourier Transform Infrared (FTIR) Spectroscopy, Scanning Electron Microscopy (SEM), Energy Dispersive X Ray Analysis (EDA X), Differential Scanning Calorimetry (DSC), Thermogravimetric Analysis (TGA), and mineral loading studies were employed to comprehensively characterize the Liposomal Iron formulation. The results demonstrated that Liposomal Iron exhibited a high encapsulatio n efficiency of 89%, nanoscale particle size of 153.8 nm, and a zeta potential of -61.2 mV, indicating excellent colloidal stability. FTIR confirmed strong interactions between Iron and phospholipid components, while SEM revealed well-defined, spherical Liposomes with no aggregation. EDAX analysis showed complete encapsulation of Iron with no surface exposure. Stability studies under thermal and accelerated conditions confirmed minimal leakage and consistent assay values over time. DSC and TGA analyses demonstrated enhanced thermal stability and delayed decomposition, while the mineral loading capacity was determined to be 0.71 mg Iron/Mg Liposomal material, indicating efficient Iron incorporation. These findings confirm that Liposomal Iron formulated by WBCIL is a stable, efficient, and advanced delivery system with strong potential for nutraceutical applications.

Introduction:-

Liposomal Iron represents an advanced delivery system to overcome the common limitations of conventional Iron supplements, such as poor bioavailability, gastrointestinal discomfort, and oxidative instability. Encapsulation of

Corresponding Author:- Poulami Gupta Banerjee

Iron within phospholipid-based Liposomes offers several advantages, including enhanced absorption, reduced side effects, and targeted delivery, making it a promising candidate for improving Iron supplementation strategies in both clinical and nutraceutical contexts.

We have previously published a study on Liposomal Iron, establishing a foundational understanding of its preparation and therapeutic potential (Gupta Banerjee et al., 2024). Building upon that work, the present article explores the formulation using more advanced analytical techniques to achieve deeper insights into its physicochemical attributes, structural interactions, and encapsulation behavior.

Continuous improvements in the product portfolio should be the fundamental strategy of the manufacturer to enhance product quality, efficiency, and consumer benefit. The philosophy of Kaizen, or continuous improvement, demonstrates that even small, incremental enhancements—when applied consistently—can yield significant gains in reliability and performance over time (Imai, 1986). In manufacturing and healthcare sectors, structured methodologies such as Lean, Six Sigma, Total Quality Management (TQM), Quality by Design (QbD) and digitalized process optimization have been shown to reduce operational waste, standardize production, and elevate customer satisfaction (George, 2002; Antony et al., 2012). Lean methodology focuses on eliminating waste (anything that does not add value) in the production processes. Lean techniques help reduce unnecessary steps in Liposomal Iron production, ensuring faster and more cost-effective manufacturing. Six Sigma is a data-driven quality improvement methodology that aims to reduce variability and defects in processes. Six Sigma could ensure that every batch of Liposomal Iron meets consistent quality and encapsulation efficiency targets. TQM is a holistic approach to long-term success through customer satisfaction and continuous improvement. QbD is a systematic, science-based approach that emphasizes designing quality into products and processes from the outset, ensuring consistent performance and regulatory compliance (Alshaer et al., 2023). According to Bhuiyan and Baghel (2005), integrating these practices enables an organization to respond more flexibly to market demands and maintain a competitive edge. Specifically, in consumer healthcare product manufacturing, companies that have embraced lean strategies report impressive outcomes—higher product reliability, faster delivery, lower waste, and markedly improved customer relations (Dale et al., 2001; Yusuf et al., 2007). Thus, continual improvement of Liposomal Iron portfolio by WBCIL not only enhances product robustness and nutraceutical effectiveness but also serves broader public health interests by delivering safer, more reliable nutritional interventions.

WBCIL has employed techniques such as Dynamic Light Scattering, Zeta Potential Analysis, Fourier Transform Infrared Spectroscopy, Scanning Electron Microscopy, and Differential Scanning Calorimetry to comprehensively characterize the formulation at a molecular level. In addition to analytical characterization, this study places particular emphasis on the stability of the Liposomal Iron formulation. Rigorous stability studies, including thermal stress tests, long-term storage under accelerated conditions, leakage analysis, and thermogravimetric evaluations, were conducted to assess structural integrity and sustained encapsulation performance of the compound over time. The findings confirm the ability of the formulation to retain Iron effectively, resist degradation under stress, and maintain high encapsulation efficiency, thereby validating its potential for long-term application in nutraceutical settings. Through this integrated approach, the article advances our understanding of Liposomal Iron and highlights its suitability as a robust and efficient mineral delivery system.

Overall, WBCIL is focusing more on portfolio enhancements of its product Liposomal Iron through ongoing scientific innovation. While the Liposomal Ferric Pyrophosphate formulation of WBCIL has higher bioavailability compared to ferrous sulfate and plain ferric pyrophosphate, along with reduced oxidative stress and excellent gastrointestinal tolerability, the encapsulation efficiency exceeding 70 % underpin physical stability and minimal leakage of the formulation. In addition to lab-scale elegance, WBCIL emphasizes real-world reliability. Our stability studies demonstrate that the Liposomal formulation maintains structural integrity and encapsulation during thermal stress and extended storage, supporting long-term nutraceutical viability.

Past, Present, and Future Improvement Trajectory of Liposomal Iron by WBCIL

WBCIL has demonstrated a consistent commitment to enhancing the quality and effectiveness of its Liposomal Iron formulation through a phased and scientifically rigorous development process. In the initial phase (2024), the company established the fundamental encapsulation potential and basic physicochemical profile of the product, with an encapsulation efficiency of 89% and a zeta potential of -39.4mV (Gupta Banerjee et al., 2024). Building on this foundation, the present study integrates advanced analytical techniques and manufacturing refinements, which have led to a significantly reduced particle size, enhanced zeta potential, extended thermal stability, and comprehensive

structural and elemental analyses including TGA, EDAX, SEM, and mineral loading capacity evaluations. These upgrades align with Lean and Six Sigma principles, ensuring improved product reliability, stability, and consumer safety. Looking ahead, WBCIL has initiated and rigorously working on in-vitro studies in controlled lab environment to evaluate the biocompatibility and absorption behavior of Liposomal Iron within the human body. This future step marks a critical transition from formulation optimization to biological validation, further reinforcing the company's vision of delivering a scientifically robust, clinically relevant, and globally competitive nutraceutical product.

Methodology:-

Advanced characterization techniques used for Liposome

The Liposomal formulation was characterized following standardized and validated methodologies as detailed in our earlier publication (Gupta Banerjee et al., 2025). Core physicochemical attributes - including vesicle size, polydispersity index (PDI), zeta potential, encapsulation efficiency, and morphological features - were assessed using established procedures. To avoid redundancy, the full experimental protocols are not reiterated here but can be found in the referenced article. Notably, we have previously published a study on Liposomal Iron, which served as the foundational phase of the current research.

Formulation Advancements through Innovation and Quality Enhancement

To enhance the efficacy, stability, and overall quality of the Liposomal Iron formulation, WBCIL has developed new batches under a state-of-the-art ISO-certified manufacturing environment. These new formulations represent a significant technological upgrade, integrating advanced techniques to improve structural integrity, encapsulation fidelity, and long-term performance of the Liposomal system. The manufacturing process now employs high-pressure homogenization to achieve a more consistent and refined vesicle size distribution, which contributes to improved bioavailability and reduced variability in product behaviour.

Additionally, WBCIL has adopted a more advanced encapsulation techniques, allowing for more efficient and targeted incorporation of Iron into the Liposomal core. This innovation ensures that a higher proportion of Iron remains securely entrapped within the Liposomes, thereby reducing the likelihood of premature leakage and enhancing nutraceutical reliability. Furthermore, the company has prioritized the use of high-purity, non-GMO phospholipids, which offer superior bilayer stability, improved resistance to oxidative stress, and enhanced compatibility with biological systems (Gupta Banerjee et al., 2025).

Collectively, these technological advancements enable the production of a Liposomal formulation with greater uniformity, improved stability under thermal and storage conditions, and better alignment with international nutraceutical quality standards. Through this strategic enhancement of formulation design and production methodology, WBCIL reinforces its commitment to delivering a safer, more efficient, and scientifically robust Iron supplement capable of meeting the evolving demands of healthcare and wellness markets.

Advanced characterization techniques used for Liposomal Iron

The characterization of Liposomal Iron was carried out using a suite of advanced analytical and instrumental techniques to evaluate its physicochemical properties, structural integrity, and functional performance. The encapsulation efficiency was quantified using validated titrimetric methods, ensuring that the entrapped elemental Iron met the desired specifications. Dynamic Light Scattering (DLS) was employed to determine the average particle size and PDI, confirming nanoscale dimensions and a uniform particle distribution. These measurements were conducted at the Indian Association for the Cultivation of Science (IACS), Kolkata, India. Zeta potential analysis was performed to assess the electrostatic stability of the Liposomal suspension (Gupta Banerjee et al., 2025).

Fourier Transform Infrared (FTIR) spectroscopy, using Attenuated Total Reflectance (ATR) mode, was utilized to verify the chemical interactions between Iron and the Liposomal matrix. A small, uniform layer of each sample was placed on the ATR crystal for optimal contact, and spectra were collected using an Agilent FTIR spectrometer (USA) across the 4000–400 cm^{-1} range, at a resolution of 4 cm^{-1} with 32 scans per sample to enhance signal quality (Gupta Banerjee et al., 2025).

Elemental composition and surface morphology were examined through Energy Dispersive X-ray Spectroscopy (EDAX) and Scanning Electron Microscopy (SEM), respectively. These tests were performed at the Indian Institute

of Technology, Kharagpur. Thermal stability was tested by subjecting the formulation to a temperature of 105°C for four hours(Gupta Banerjee et al., 2025). Leakage study was done by storing Liposomal Iron over a period of 6 months at 40°C ± 2°C and a relative humidity of 75% ± 5%. Assay% and encapsulation efficiency% were measured to ensure negligible leakage of Iron from the Liposomal carrier.

Additionally, long-term elemental stability was evaluated subjecting the Liposomal Iron formulation to accelerated stability conditions (40°C ± 2°C, 75% ± 5% RH) over three months. The endothermic behaviour and phase transition characteristics were studied using Differential Scanning Calorimetry (DSC). DSC was used to analyse thermal transitions of Liposomal Iron in comparison with pure ferric pyrophosphate (Iron Active Pharmaceutical Ingredient or Iron API) and empty Liposomes. The sample of Liposomal Iron was sent to Sapala Organics (Secunderabad, India) for DSC. Thermogravimetric Analysis (TGA) was conducted to evaluate thermal decomposition behaviour and moisture content of the formulation. TGA characterized the heat stability and decomposition pattern of Liposomal Iron compared to empty Liposomes. Both samples were subjected to heat up to 830°C. The mineral loading capacity of the Liposomes was also quantified to determine the efficiency of Iron incorporation within the lipid bilayer system.

Results and Discussion:-

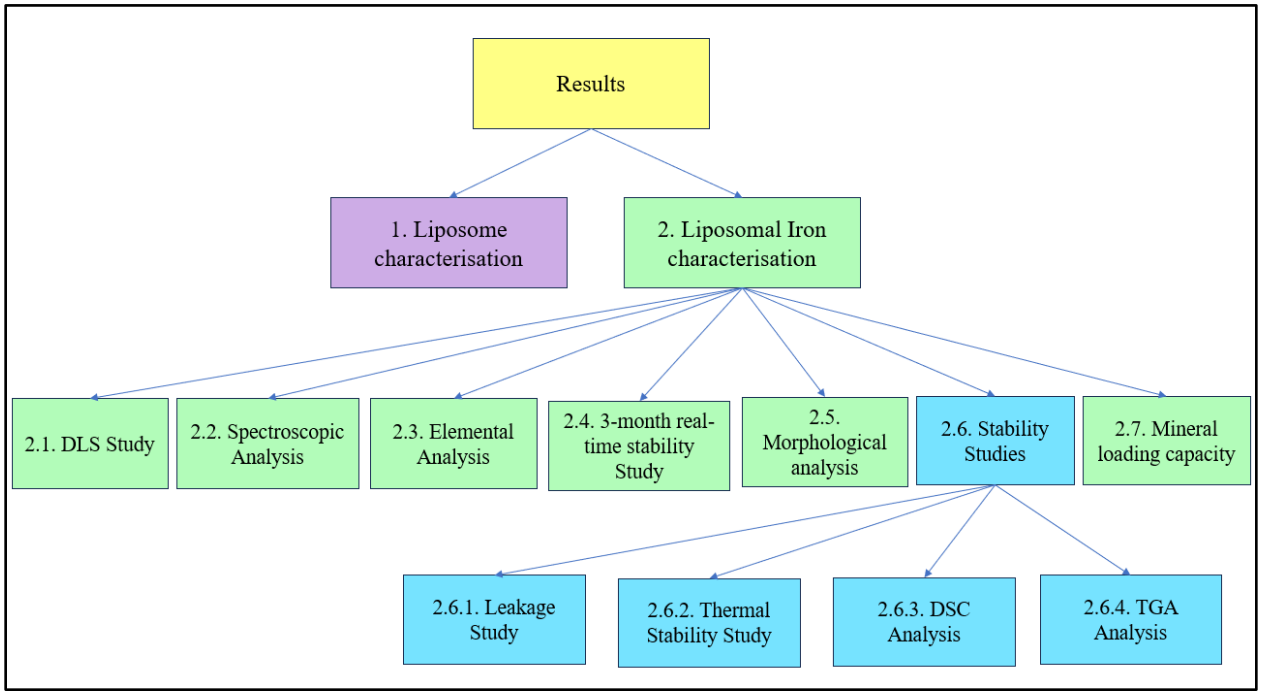

Figure 1:- Cladogram of Results.

Results of Liposome characterisation

Table 1:- Liposome Characterisation.

Parameter	Result	Interpretation
Total Phospholipid Content	93%	Indicates high lipid incorporation efficiency
Phosphatidylcholine (PC)	82.05%	Major lipid component
Phosphatidylethanolamine (PE)	10.82%	Secondary lipid component contributing to bilayer structure
FTIR Peaks	~1738 cm^{-1} (C=O stretching)	Confirms ester functional groups from lipids
	~2853 & ~2920 cm^{-1} (CH_2 vibrations)	Indicative of lipid hydrocarbon chain organization
	3138–3320 cm^{-1} (broad –OH stretching)	Reflects hydrogen bonding and water interactions
Average Particle Size (DLS)	133.9 nm	Confirms nanoscale size suitable for enhanced absorption
Polydispersity Index (PDI)	0.294	Suggests a moderately uniform size distribution
Zeta Potential	-31.87 mV	Indicates good colloidal stability and resistance

		to aggregation

The results obtained from the characterization of the Liposomal formulation aligned closely with those reported in our earlier research (Gupta Banerjee et al., 2025). Similar to previous findings, the Liposomes contained a total phospholipid concentration of 93%, with Phosphatidylcholine (PC) accounting for 82.05% and Phosphatidylethanolamine (PE) comprising 10.82%. FTIR analysis revealed distinctive spectral peaks around 1738 cm^{-1} (indicative of C=O stretching), 2853 and 2920 cm^{-1} (corresponding to CH_2 stretching vibrations), and a broad –OH stretching band between 3138 and 3320 cm^{-1}, all of which confirmed the preservation of structural integrity. DLS analysis showed an average particle size of 133.9 nm, a PDI of 0.294, and a zeta potential of -31.87 mV, indicating that the Liposomal formulation was both stable and well-dispersed (Gupta Banerjee et al., 2025).

Results of Liposomal Iron characterisation

Encapsulation Efficiency, DLS, and Zeta Potential Study

The encapsulation efficiency of Liposomal Iron was assessed using a standardized titrimetric method as previously described in our published article (Gupta Banerjee et al., 2025). According to established criteria, the minimum acceptable encapsulation efficiency is not less than 70%. In this study, the Liposomal Iron formulation demonstrated a notably high encapsulation efficiency of 89.01%, indicating effective incorporation of Iron within the Liposomal bilayer (Figure 2). This superior efficiency suggests the formulation's strong potential for delivering bioavailable Iron with minimal loss and improved absorption (Akbarzadeh et al., 2013).

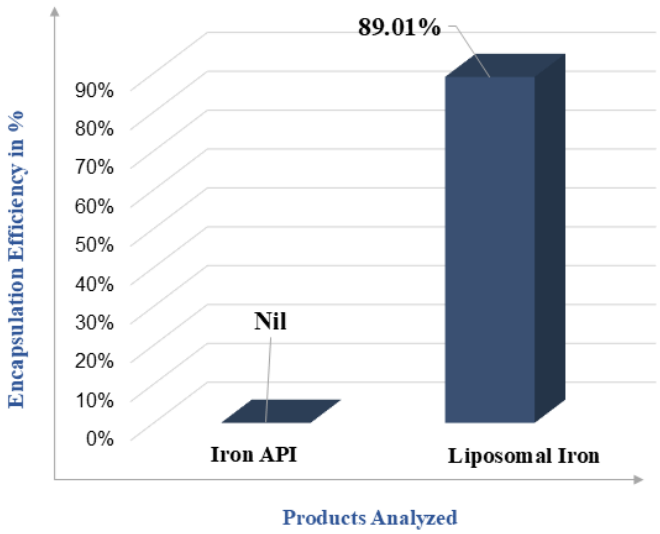

Figure 2:- Encapsulation Efficiency determined via validated titrimetric data.

DLS was employed to assess the particle size and distribution uniformity of various iron formulations. The analysis revealed a significant reduction in particle size upon encapsulation of Iron in Liposomes. As shown in Figure 3, the Iron API from our previously published journal reported an average particle size of 3696 nm, while the Iron API used in the current study exhibited a size of 1942.0 nm (Gupta Banerjee et al., 2025). In contrast, the previously formulated Liposomal Iron showed a reduced particle size of 1296.0 nm. Notably, the currently developed Liposomal Iron formulation by WBCIL demonstrated a drastic reduction to just 153.8 nm, highlighting a substantial improvement in nano-sizing and formulation efficiency. This reduction in particle size underscores WBCIL's commitment to improving the physicochemical properties of iron formulations, potentially enhancing absorption and therapeutic efficacy.

Furthermore, Figure 4 presents the Polydispersity Index (PDI), an indicator of size distribution uniformity. The Iron API from the published journal had a PDI of 0.3517, while the current Iron API measured 1.000, suggesting high heterogeneity. The previously formulated Liposomal Iron had a PDI of 0.4465, whereas the currently developed Liposomal Iron achieved a notably lower PDI of 0.2799, indicating a more homogeneous particle population. This

improved uniformity is crucial for enhancing bioavailability, cellular uptake, and formulation stability (Stetefeld et al., 2016).

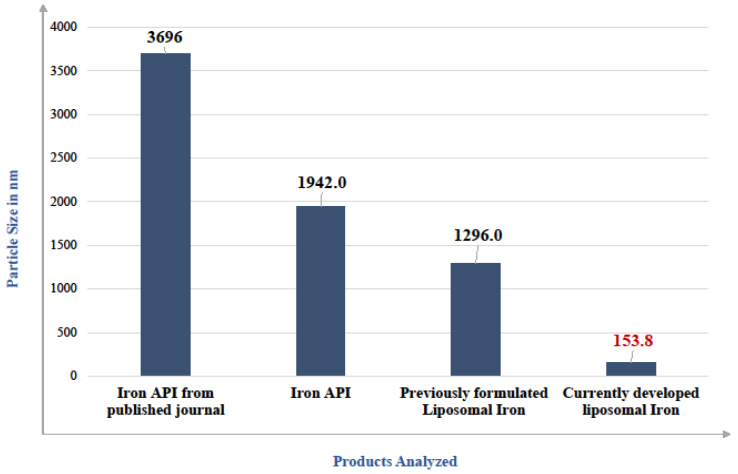

Figure 3:- Chart comparing Particle size between Iron API and Liposomal Iron for the previously published journal and the current work.

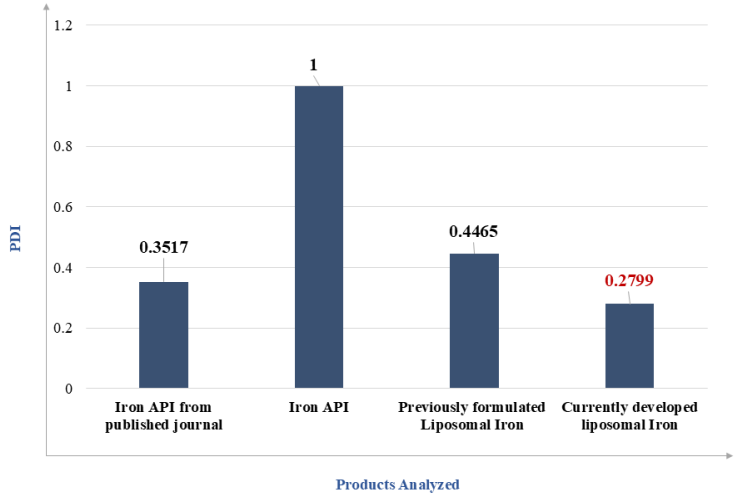

Figure 4:- Chart comparing PDI between Iron API and Liposomal Iron for the previously published journal and the current work.

The colloidal stability of the Iron formulations was assessed via zeta potential measurement. As presented in Figure 5, the currently developed Liposomal Iron by WBCIL demonstrated a highly negative zeta potential of -61.22 mV, indicating robust electrostatic repulsion among the particles. In contrast, the previously formulated Liposomal Iron exhibited a zeta potential of -39.47 mV (Gupta Banerjee et al., 2024). In case of Iron API, the zeta potential is -26.85 mV in the present study and it was -18.05 mV in our previously published article.

A more negative zeta potential is indicative of enhanced colloidal stability, as it prevents particle aggregation through stronger repulsive forces. The high zeta potential achieved in the current Liposomal Iron formulation confirms its superior stability in suspension, which is a critical parameter for ensuring prolonged shelf life, consistent dispersion, and improved therapeutic performance (Bhattacharjee, 2016). These findings further emphasize the advancements made by WBCIL in optimizing formulation parameters for better clinical outcomes.

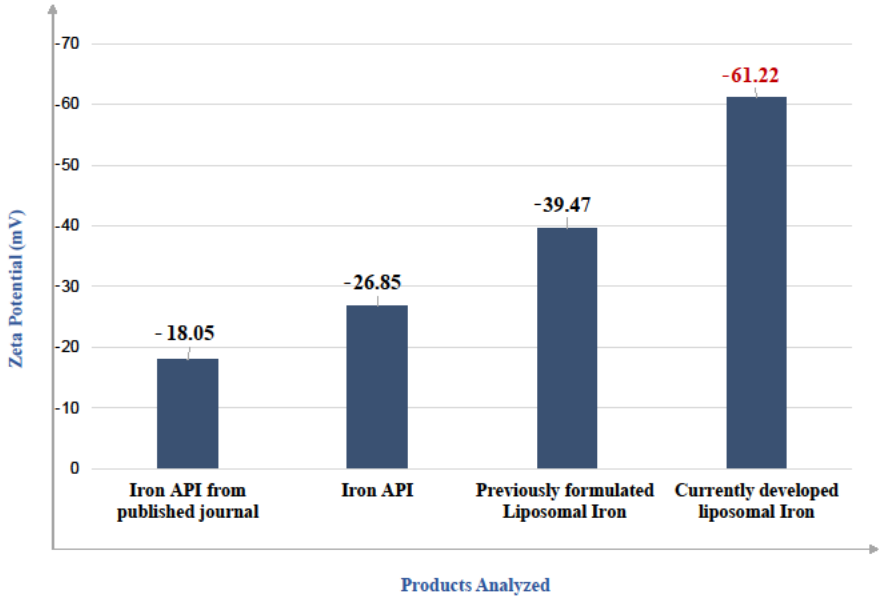

Figure 5:- Chart comparing Zeta Potential between Iron API and Liposomal Iron.

Spectroscopic Analysis

Table 2:- FTIR Spectroscopy Analysis.

Wavenumber (cm^{-1})	**Functional Group / Vibration**	**Component**	**Significance**
~1652.1	C=O stretching	Phospholipids / Ferric ion interaction	Indicates strong hydrogen bonding and encapsulation stability
~1024.1	PO_4^- stretching	Phospholipid headgroups	Confirms binding of ferric ions with phosphate groups
~3403.1–3418.0	Broad –OH stretching	Water / Hydrophilic domains	Suggests hydrophilic interactions and moisture presence in Liposomal structure
~2920 & ~2850	CH_2 asymmetric and symmetric stretching	Lipid tails	Confirms ordered packing and integrity of the lipid bilayer
~1738	C=O ester bond (carbonyl stretching)	Lipids (ester linkage)	Characteristic of phospholipid backbone
~410.0 & ~430.5	Fe–O vibrations	Ferric pyrophosphate	Verifies presence and encapsulation of ferric Iron within the Liposome

The FTIR analysis of Liposomal Iron offered valuable insights into the structural composition and molecular interactions within the formulation (Figure 6). A prominent absorption band around 1652.1 cm^{-1} corresponded to C=O stretching vibrations, while another significant peak near 1024.1 cm^{-1} was attributed to PO_4^- stretching. These findings indicate strong bonding interactions between ferric ions and the phospholipid matrix. Additionally, the presence of broad –OH stretching bands in the 3403.1–3418.0 cm^{-1} range pointed to hydrophilic interactions that enhance the formulation's stability in aqueous systems. Peaks at 2920 cm^{-1} and 2850 cm^{-1}, associated with CH_2 stretching, reflected the organized arrangement of lipid bilayers, supporting successful Liposome formation. Furthermore, distinct Fe–O vibrational bands detected at approximately 410.0 cm^{-1} and 430.5 cm^{-1} confirmed the incorporation of ferric pyrophosphate into the lipid core (Figure 6). Together, these spectral characteristics validate the chemical stability and effective encapsulation of Iron within the Liposomal Iron system, with both hydrophilic and hydrophobic interactions contributing to the robustness of the formulation.

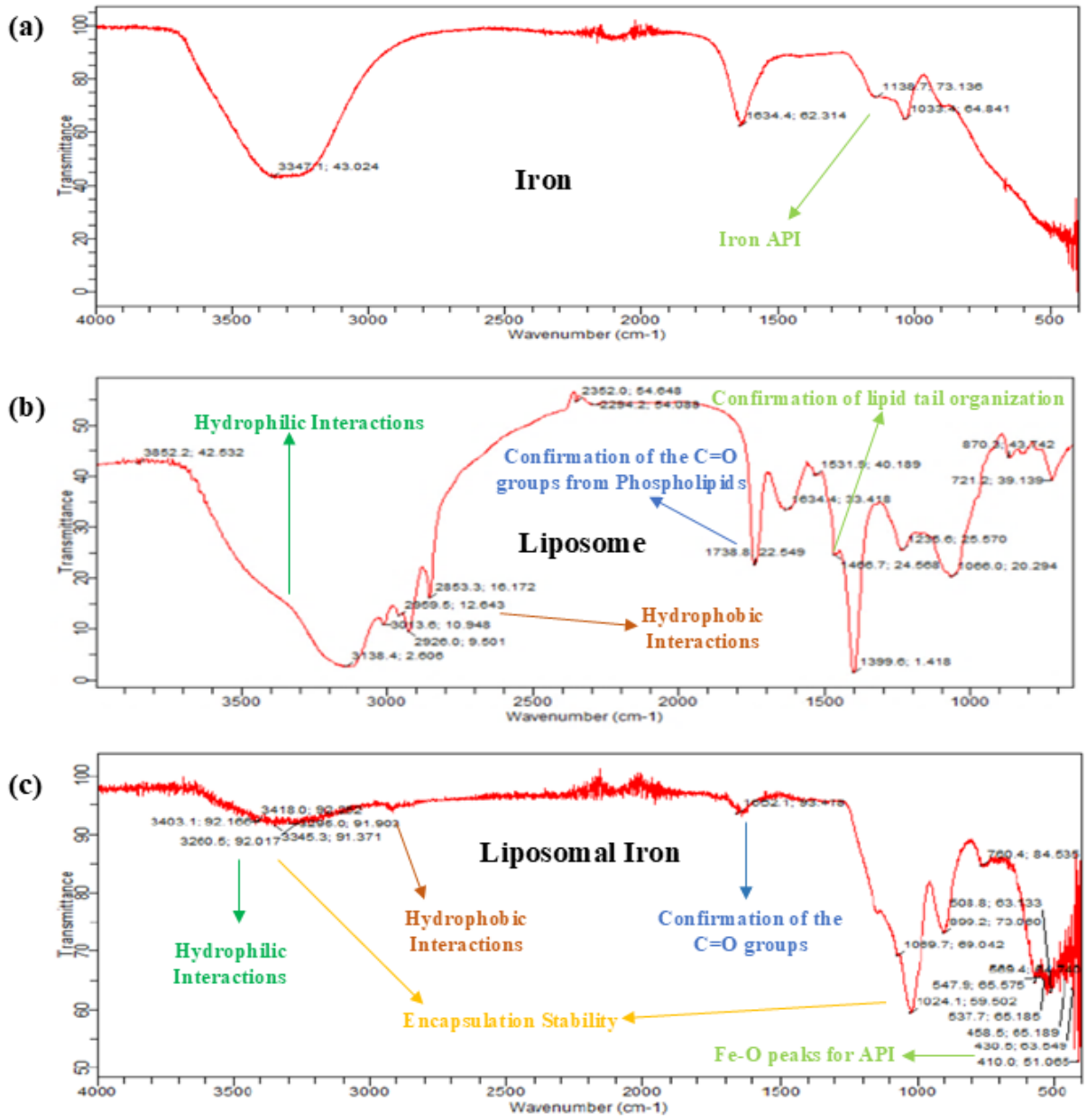

Figure 6:- FTIR Transmission spectrum showing bands atdifferent wavelengths of (a) IronAPI, (b) Liposome and (c) Liposomal Iron.

Elemental Analysis

Elemental analysis conducted using EDAX identified the presence of oxygen (37.80%), carbon (41.22%), nitrogen (19.78%), and phosphorus (1.20%) in the Liposomal Iron formulation (Figure 7). The elevated carbon content is attributed to the phospholipids, which are primarily composed of carbon-rich molecular structures. The detected oxygen corresponds to hydroxyl and phosphate groups present within the bilayer, while the phosphorus originates from the phosphate moieties of the phospholipid components. Nitrogen corresponds to the phospholipid components of phosphatidylethanolamine or other nitrogen-containing head groups in the Liposome structure. These elemental constituents—carbon, oxygen, nitrogen, and phosphorus—are consistent with the typical composition of Liposomal systems, especially the phospholipid bilayer (Goldstein et al., 2017). On the other hand, the elemental analysis of Iron API indicates that it constitutes oxygen (12.93%), carbon (16.76%), nitrogen (11.08%), phosphorus (26.76%), and Iron (32.48%) (Figure 7).

The elemental profile of Liposomal Iron, characterized by high carbon, moderate oxygen, and trace phosphorus, is indicative of successful phospholipid-based encapsulation. Notably, the absence of an Iron signal in the EDAX spectrum confirms that Iron is fully encapsulated within the Liposomal vesicles and is not exposed on the surface. In comparison to our previous study whereIron was present in measurable quantity on the surface though below threshold level (Gupta Banerjee et al., 2024), the present study confirms complete absence of Iron on the surface and thus full encapsulation of Iron within the Liposome. This finding validates the effective formulation and confirms the structural integrity of the Liposomal Iron delivery system.

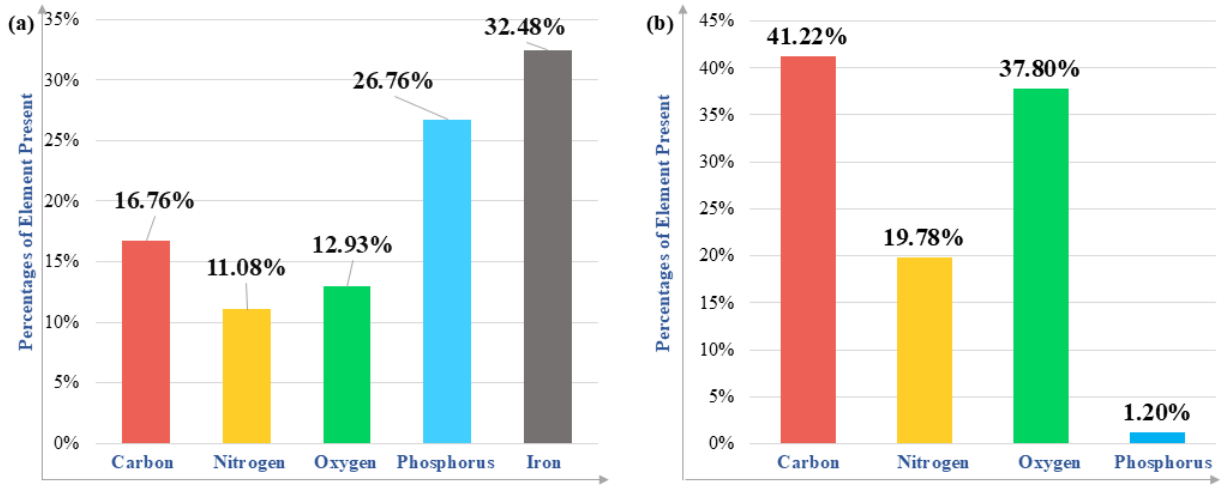

Figure 7:- A graphical representation of the percentages of elements composing (a) Iron API and (b) Liposomal Iron.

3-Month Real-Time Stability Study

The elemental stability of Liposomal Iron after three months was again assessed using EDAX after subjecting the samples to accelerated stability conditions (40°C ± 2°C and 75% ± 5% relative humidity). The results showed that the composition remained consistent, with carbon accounting for 34.47%, oxygen 38.33%, nitrogen 18.39%, and phosphorus 8.81%. Notably, no surface Iron signal was detected, confirming that the Iron remained fully encapsulated within the Liposomal structure (Figure 8). This indicates that the Liposomal formulation maintains its structural and compositional integrity over time, thereby ensuring sustained mineral protection and bioavailability.

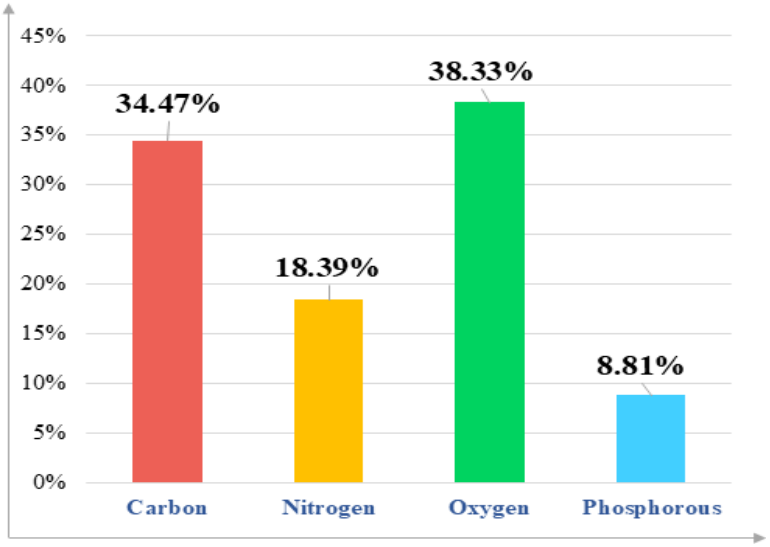

Figure 8:- A graphical representation of the percentages of elements composing Liposomal Iron after 3 months.

Morphological analysis

Table:- Morphological analysis via SEM Imaging.

Aspect	Observation	Implication
Morphology	Well-defined, spherical Liposomes uniformly distributed	Indicates successful self-assembly of phospholipids into bilayer vesicles
Shape	Spherical shape	Provides optimal surface area-to-volume ratio for encapsulation and drug loading
Internal Volume	Maximized internal volume	Suitable for accommodating Iron ions
Encapsulation	Encapsulated Iron clearly visible within lipid bilayers	Confirms successful integration of Iron into Liposomal carriers
Aggregation	No aggregation observed	Indicates good formulation stability under experimental conditions

The SEM imaging offered detailed visualization of the physical characteristics of the Liposomal Iron. The images showed uniformly distributed, well-defined spherical Liposomes throughout the observed area. This distinct round morphology is indicative of the successful self-assembly of phospholipids into bilayer vesicles, a process driven by the amphiphilic properties of the lipid molecules. The spherical shape optimizes the surface area-to-volume ratio, which improves encapsulation efficiency and facilitates more effective molecule loading (Figure 9). Such geometry also maximizes the internal volume, making it highly suitable for accommodating Iron ions. The encapsulated Iron within the lipid bilayers was clearly observed, further confirming the successful incorporation of Iron into the Liposomal carriers (Yamashita et al., 2009). Additionally, the complete absence of aggregation suggests that the formulation remained stable under the tested conditions.

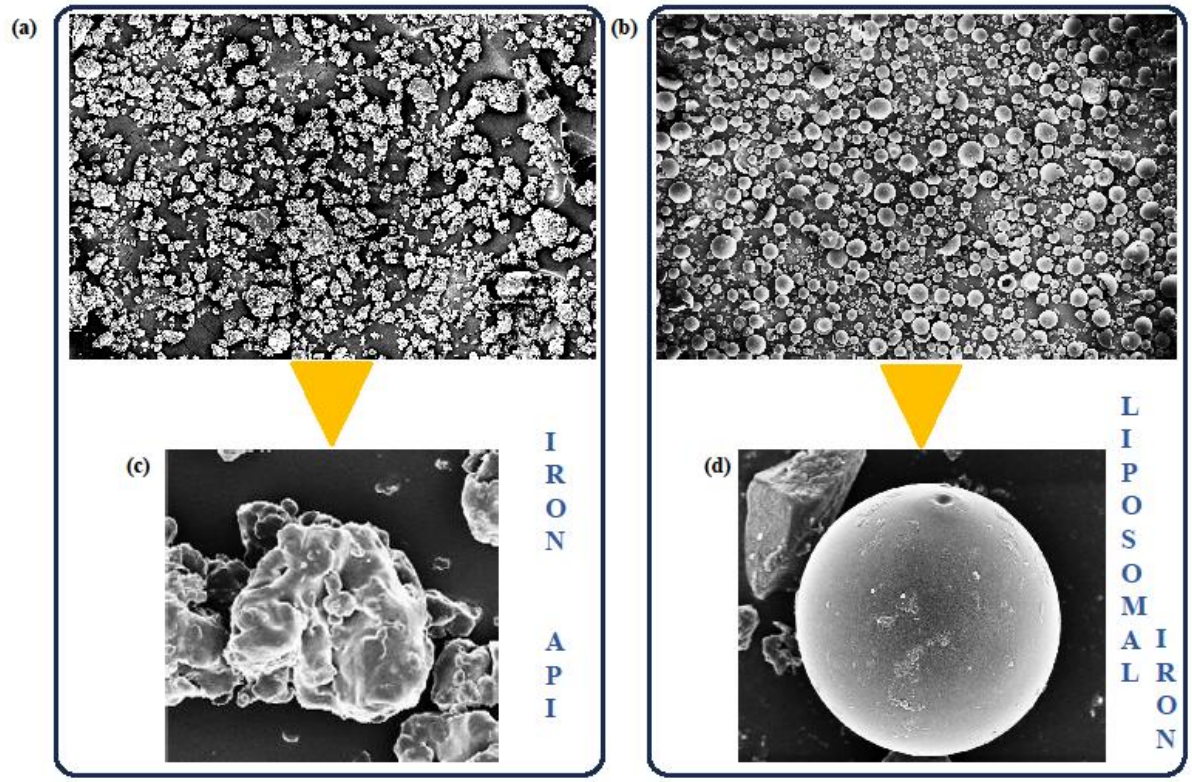

Figure 9:- SEM image of (a) non-encapsulated Iron API, (b) Encapsulated Liposomal Iron, (c) zoomed in image of Iron API and (d) zoomed in image of Liposomal Iron.

Stability Studies

Leakage Study

To assess the degree of mineral leakage over time, Liposomal Iron was stored at 40°C ± 2°C and 75% ± 5% relative humidity for a duration of six months. Throughout this period, the encapsulation efficiency percentage exhibited minimal change, ranging from 89.50% to 87%, demonstrating excellent stability and very limited leakage (Figure 10). This high level of retention confirms that the Liposomal structure effectively inhibits the loss of Iron even under stressful conditions (Torchilin, 2005). Likewise, the assay percentage showed that the Liposomal Iron remained nearly constant, shifting slightly from 8% to 7.91%, indicating no degradation and excellent retention.

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

Thermal Stability Study

In our previous study, we have measured the thermal stability of the product Liposomal Iron at 105°C for 10 min (Gupta Banerjee et al., 2024). In the present study, Liposomal Iron was exposed to thermal stress at 105°C for 4 hours. There was a slight increasein encapsulation efficiency, from 89.75%to91.27% which shows the capability of the formulation to preserve its structural integrity and Iron content (Zhao et al., 2015). It demonstrates robustness under high-temperature conditions. This stability is essential for enduring processing and transportation environment. Similarly, the Iron assay values remained consistent, measuring 7.87% at room temperature and 8.05% after thermal exposure (Figure 11). Together, these findings confirm the strong structural stability and sustained encapsulation of the Liposomes under stress, highlighting their potential for reliable long-term storage (Zhao et al., 2015).

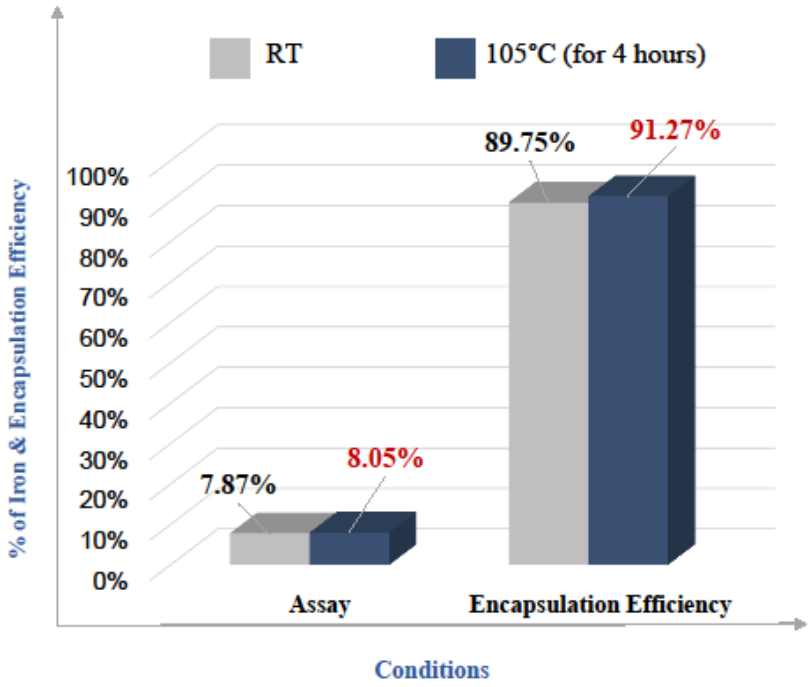

Figure 11:- Chart comparing the stability of Liposomal Iron both at room temperature (RT) and at 105°C for 4 hours of exposure.

DSC Analysis

DSC thermograms obtained for Iron API, Empty Liposome, and Liposomal Iron offer valuable insights into the thermal behaviour, phase transitions, and stability of each component and the final formulation (Figure 12).

The thermogram of Iron API exhibits two prominent endothermic peaks. The first transition begins at an onset temperature of approximately 203.97°C, reaching a peak at 221.71°C with an enthalpy change of 20.731 J/g, while the second peak occurs at 295.67°C with an enthalpy of 12.567 J/g. These sharp and well-defined peaks are characteristic of the crystalline nature of the raw Iron compound, likely corresponding to melting and subsequent structural decomposition. Such thermal behaviour indicates that the Iron salt exists in a stable, highly ordered state that requires significant energy input for disruption. However, these crystalline features may contribute to its limited solubility and poor bioavailability when used as a standalone supplement.

In contrast, the DSC curve for the Empty Liposome shows a broader and more complex profile, typical of lipid-based systems. The initial transition begins at 40.99°C and peaks at 98.25°C, with a significantly higher enthalpy of 384.57 J/g, reflecting the gel-to-liquid crystalline phase transition of the phospholipid bilayer. This is followed by a second broad endothermic transition peaking at 302.26°C, with an onset of 290.82°C and an enthalpy change of 182.00 J/g, likely associated with the thermal decomposition or rearrangement of lipid molecules at elevated temperatures. These thermal characteristics demonstrate that the phospholipids used in Liposome formation exhibit significant structural changes under heat, which is critical for assessing the thermal robustness and storage conditions of Liposomal products.

The DSC profile of Liposomal Iron displays a markedly different thermal pattern compared to its individual components, confirming the occurrence of strong physicochemical interactions between the encapsulated Iron and the lipid bilayer. The first transition, with an onset at 49.71°C and a peak at 93.65°C, has a normalized enthalpy of 118.44 J/g, indicating a modified lipid phase behaviour due to the presence of ferric pyrophosphate. This shift from the empty Liposome's original transition range implies integration of the Iron compound within the bilayer, affecting its thermal response. A second significant transition is observed at a higher temperature, with an onset at 350.67°C and a peak at 368.56°C, with an enthalpy of 47.308 J/g. This elevated and broadened peak is absent in both the Iron API and the empty Liposome profiles, suggesting that encapsulation confers enhanced thermal resistance and structural reinforcement to the formulation.

Collectively, the DSC thermograms reveal that Liposomal Iron has undergone successful encapsulation, leading to altered phase transitions and improved thermal properties. The disappearance of sharp crystalline peaks from the Iron API and the emergence of new thermal events at higher temperatures in the Liposomal form confirm chemical stabilization and physical entrapment of Iron within the lipid matrix. These thermal enhancements not only reflect improved product stability under heat stress but also support long-term shelf life and resistance during processing and storage, establishing Liposomal Iron as a reliable and thermally stable nutraceutical formulation (Wissing et al., 2004).

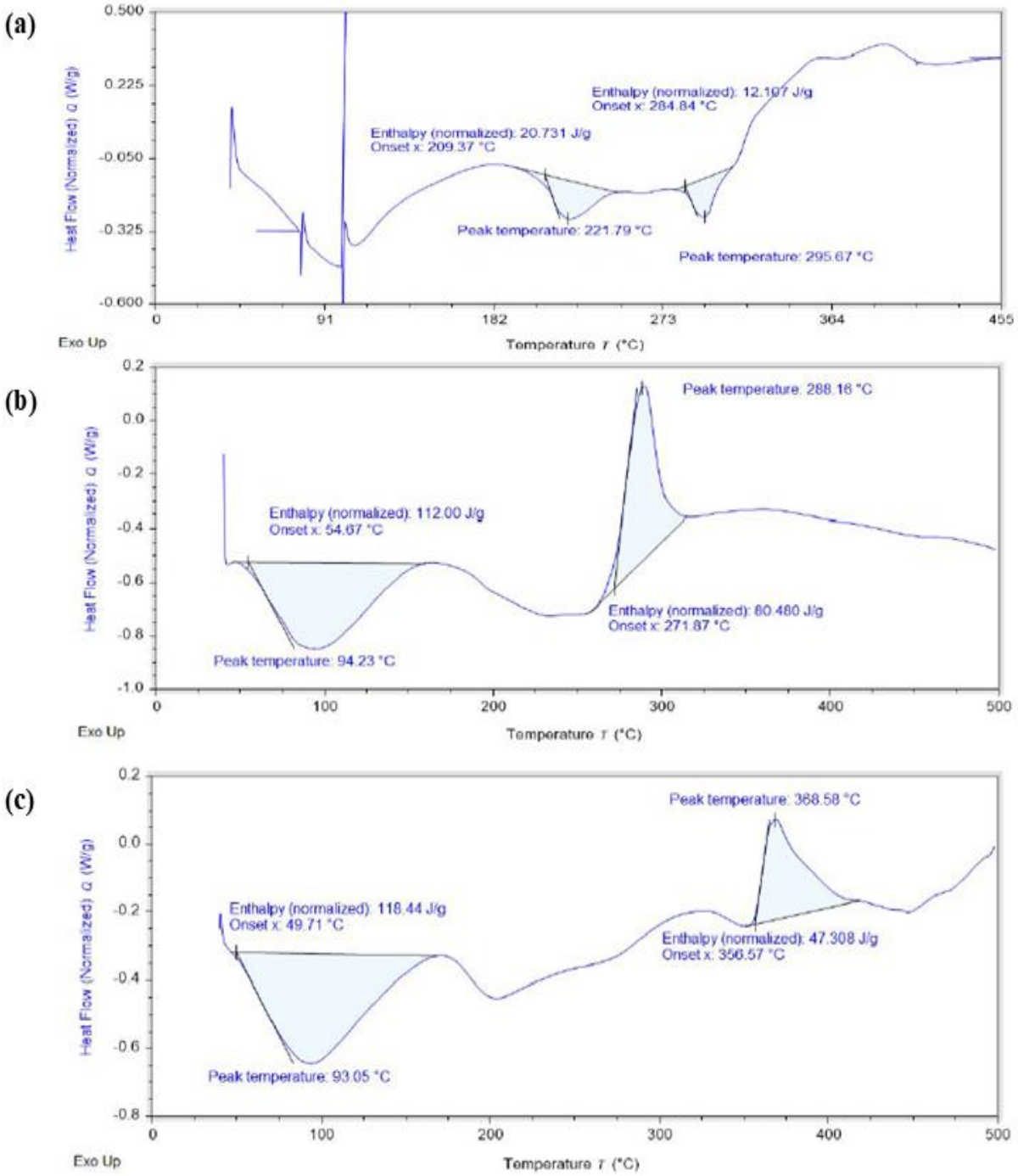

Figure 12:- DSC Thermogram of (a) Iron API, (b) empty Liposome and (c) Liposomal Iron.

TGA Analysis

TGA further characterized the heat stability and decomposition pattern of Liposomal Iron compared to empty Liposomes (Figure 13). Both samples were subjected to heat up to 830°C. The initial weight loss below 150°C in both cases was attributed to the evaporation of moisture or loosely bound water molecules. However, Liposomal Iron exhibited a delayed onset of thermal decomposition, beginning around 300–350°C, compared to earlier degradation observed in the unencapsulated form. DTA data showed an endothermic peak in Liposomal Iron at 130–140°C, associated with moisture loss, which was higher than that observed in the free API, indicating a protective effect conferred by the Liposomal encapsulation. These findings demonstrate that the Liposomal Iron is more thermally robust than conventional Iron compounds (D'Souza& DeLuca, 2006). The TGA data highlights several functional advantages of the Liposomal formulation. The enhanced thermal stability indicates better protection against degradation during storage and processing. Encapsulation effectively delays decomposition and reduces the exposure of Iron to environmental conditions, which in turn minimizes oxidation risks. This supports improved bioavailability and contributes to an extended-release profile. The multi-stage thermal events also suggest a more controlled degradation mechanism, which is beneficial for sustained Iron delivery in nutraceutical contexts (Sharma & Sharma, 1997). Furthermore, the encapsulated form reduces gastrointestinal irritation, enhancing overall patient compliance and safety.

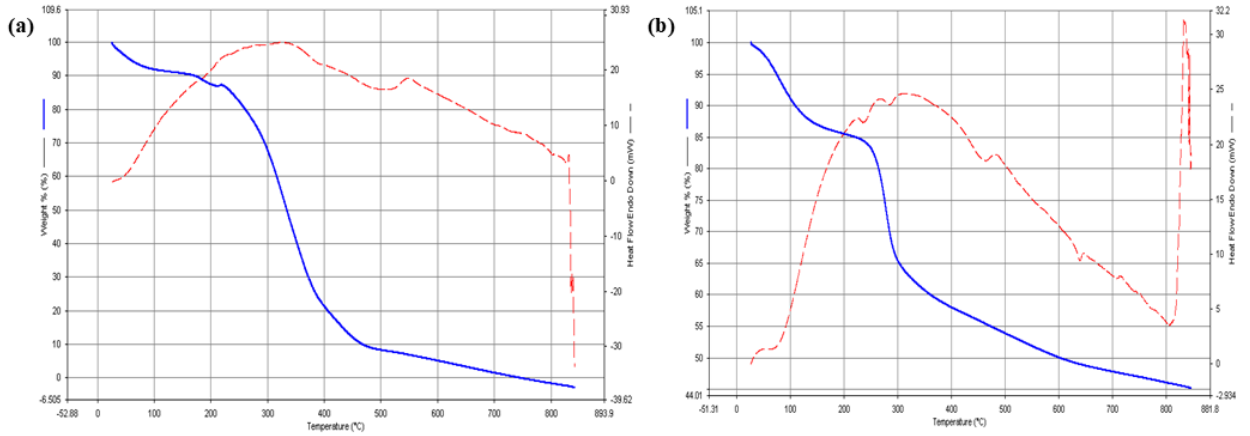

Figure 13:- A Thermogravimetric Analysis (TGA---) and Differential Thermogravimetric Analysis (DTA---) plots showing mass loss pattern upon application of heat up to 830ºC for (a) Empty Liposome and (b) Liposomal Iron.

A correlation analysis between DSC and TGA results provided deeper insights into the physical and thermal changes occurring in Liposomal Iron. Both techniques indicated moisture loss around 132–140°C, confirming a real material transition involving both thermal energy absorption (DSC) and mass loss (TGA). At 223°C, DSC indicated a phase transition, though no corresponding mass loss was observed in TGA, suggesting internal rearrangement rather than decomposition. A final correlated event was observed at ~288–300°C, where both DSC and TGA identified decomposition, confirming that structural degradation of the encapsulated compound was taking place[Tian & Chen, (2011); Lu& Park, (2013)]. These combined analyses validate the thermal resilience and structural fidelity of the Liposomal system.

Notably, the absence of abrupt or multiple degradation steps reflects the uniformity and purity of the Liposomal Iron. Furthermore, the synchronization of weight loss (TGA) with thermal events (DTA) provides a comprehensive understanding of thermal transitions of Liposomal Iron. Together, these findings confirm that the Liposomal Iron system is thermally stable, with well-defined degradation pathways and superior heat resistance compared to its unencapsulated counterparts. The data support the product's suitability for long-term storage and handling under variable temperature conditions, reinforcing its viability as a robust nutraceutical solution.

Mineral loading capacity

The mineral loading capacity of the Liposomal formulation was evaluated to determine the amount of Iron encapsulated relative to the total weight of the Liposomal product. This parameter is a crucial indicator of the formulation's efficiency, reflecting how effectively the active mineral is integrated within the lipid bilayer system. The analysis showed a loading capacity of 0.71 mg of Iron per mg of Liposomal material. A high loading capacity

ensures that a significant amount of the nutraceutical agent is delivered with minimal use of lipid excipients, enhancing the overall nutraceuticalpayload and reducing formulation bulk (Mozafari, 2005).

According to the U.S. FDA guidelines on Liposomal mineral products, mineral loading - defined as the ratio of encapsulated mineral to total lipid content - is considered as a critical quality attribute (CQA). It directly impacts the stability, bioavailability, and in vivo release kinetics of the molecule. The guidelines emphasize that variability in mineral loading can influence mineral leakage and affect the safety and efficacy of the final product. Hence, maintaining consistency in mineral loading across production batches is vital for ensuring nutraceutical reliability and regulatory compliance (USFDA, 2018).

Moreover, an optimal mineral-to-lipid ratio contributes to improved encapsulation efficiency, long-term storage stability, and reduced risk of aggregation or fusion of Liposomes. It also plays a role in the control of release profile—an essential factor in modulating absorption rates and minimizing gastrointestinal irritation associated with Iron supplementation. Therefore, the high mineral loading capacity observed in the Liposomal Iron formulation not only validates the robustness of the encapsulation technique but also aligns with global regulatory expectations for Liposome-based mineral products, ensuring a safe, effective, and scalable nutraceutical solution.

Comparative Analysis of Physicochemical and Stability Parameters of Liposomal Iron: Previous vs. Present Study

Table:- Betterment in Physicochemical and Stability Parameters of Liposomal Iron in thepresent Study in comparison to our previous study.

Parameter	Previous published results (2024)	Present Study (2025)
Zeta Potential (mV)	-39.47	-61.22
Particle Size (nm)	1296	153.8
Thermal Stability Test Conditions	105°C for 10 min	105°C for 4 hours
EDAX (Elemental Analysis)	Iron content measurable (surface-exposed)	No surface Iron detected, indicating complete encapsulation
TGA Decomposition	Not performed before	Performed in this study
Mineral Loading Capacity	Not performed before	Performed in this study

The comparative evaluation between the previous (2024) and present (2025) studies on Liposomal Iron reveals significant advancements in formulation stability and analytical thoroughness by WBCIL. The zeta potential improved markedly from -39.47 mV to -61.22 mV, indicating enhanced colloidal stability and reduced aggregation tendency in the current formulation. Likewise, the particle size dropped dramatically from 1296 nm to 153.8 nm, reflecting a more refined nanoscale formulation with better absorption potential. A major improvement is seen in the thermal stability assessment: previously limited to 10 minutes at 105°C, the current study extended this to 4 hours, demonstrating superior heat resistance. Crucially, EDAX analysis now confirms complete encapsulation of Iron with no surface exposure—an advancement over the earlier finding of detectable surface Iron. Moreover, the inclusion of TGA and mineral loading capacity analyses in the 2025 study represents a significant enhancement in quality control and characterization, highlighting WBCIL's proactive approach to optimizing the stability, safety, and efficacy of its Liposomal Iron formulation.

Application of Lean, QbD, and Six Sigma Principles in the Continuous Improvement of WBCIL's Liposomal Iron Formulation

The systematic improvements observed in WBCIL's 2025 Liposomal Iron study align closely with the principles of Six Sigma, QbD, and Lean Manufacturing—thewell-established continuous improvement methodologies widely adopted in pharmaceutical and nutraceutical industries. Six Sigma is a data-driven methodology that aims to reduce process variation and defects to achieve consistent, high-quality outcomes. The efforts of WBCIL to enhance encapsulation efficiency, ensure thermal and colloidal stability, and minimize surface Iron exposure and leakage demonstrate a clear focus on minimizing deviation from defined product specifications. By extending thermal stability tests and introducing new quality control checkpoints like TGA and mineral loading assays, WBCIL is essentially reducing the "defect rate" (i.e., poorly encapsulated or unstable batches), aligning with the DMAIC cycle (Define, Measure, Analyse, Improve, Control) of Six Sigma.

WBCIL has embraced QbD—a systematic, science- and risk-based approach to pharmaceutical development that builds quality into the product from the earliest stages. QbD principles have guided WBCIL in defining critical

quality attributes (CQAs) such as encapsulation efficiency, zeta potential, particle size distribution, and mineral loading capacity. By understanding and controlling the relationships between material attributes, processing parameters, and product performance, WBCIL ensures that the Liposomal Iron formulation meets predefined quality standards consistently across batches.

Lean focuses on eliminating waste and optimizing process flow. WBCIL's shift from a 1296 nm to a 153.8 nm particle size using high-pressure homogenization technique show a Lean-style focus on process refinement and resource efficiency. Additionally, their decision to use non-GMO, high-purity phospholipids improve product performance while reducing the risk of batch failure or recall—effectively cutting "waste" in the form of reprocessing or customer dissatisfaction. The longer thermal stability window (from 10 minutes to 4 hours) also suggests a more robust product that is less vulnerable to spoilage during transport or storage, another Lean value.

Conclusion and Future Aspects:-

The present study reinforces the potential of Liposomal Iron as a superior Iron delivery system, particularly in addressing the limitations of conventional Iron supplements. By employing a suite of advanced analytical techniques—ranging from DLS and Zeta Potential analysis to FTIR spectroscopy, SEM imaging, DSC, and TGA—the formulation was thoroughly characterized for its physicochemical attributes, molecular interactions, and encapsulation efficiency. The results demonstrated that the Liposomal system successfully encapsulated ferric pyrophosphate within stable bilayer vesicles, offering a nanoscale, uniform, and robust delivery mechanism.

Stability studies under thermal and accelerated conditions further validated the structural and functional resilience of the formulation. With minimal leakage over extended periods and consistent encapsulation efficiency and assay values, the Liposomal Iron formulation proved to be thermally stable and capable of withstanding stressful environmental conditions. These findings position Liposomal Iron as an effective and safe candidate for long-term nutraceutical applications, offering both enhanced bioavailability and improved human compliance.

Looking forward, WBCIL has initiated in-vitro studies to evaluate the clinical efficacy and bioavailability of the formulation in human subjects. Comparative studies with other delivery systems, such as polymeric nanoparticles, could also be valuable to further establish the superiority of Liposomal Iron. Moreover, expanding the application of this technology to encapsulate other essential micronutrients or synergistic compounds could lead to the development of multi-nutrient Liposomal platforms.

References:-

1. Akbarzadeh, A., Rezaei-Sadabady, R., Davaran, S., Joo, S. W., Zarghami, N., Hanifehpour, Y., ... & Nejati-Koshki, K. (2013). Liposome: classification, preparation, and applications. Nanoscale Research Letters, 8(1), 102. https://doi.org/10.1186/1556-276X-8-102
2. Alshaer, W., Nsairat, H., Lafi, Z., Hourani, O. M., Al-Kadash, A., Esawi, E., & Alkilany, A. M. (2023). Quality by design approach in liposomal formulations: Robust product development. Molecules, 28(1), 10. https://doi.org/10.3390/molecules28010010
3. Antony, J., Snee, R. D., & Hoerl, R. (2007). Lean Six Sigma: Yesterday, Today and Tomorrow. International Journal of Productivity and Performance Management, 56(7), 690–698.
4. Bhattacharjee, S. (2016). DLS and zeta potential–what they are and what they are not? Journal of Controlled Release, 235, 337–351. https://doi.org/10.1016/j.jconrel.2016.06.017
5. Bhuiyan, N., & Baghel, A. (2005). An overview of continuous improvement: from the past to the present. Management Decision, 43(5), 761–771.
6. D'Souza, S. S., & DeLuca, P. P. (2006). Methods to assess in vitro drug release from injectable polymeric particulate systems. Pharmaceutical Research, 23(3), 460–474.
7. Dale, B. G., Wu, P. Y., Zairi, M., Williams, A. R. T., & Van der Wiele, T. (2001). Total Quality Management and Theory: An Exploratory Study. International Journal of Production Research, 39(8), 1551–1570.
8. George, M. L. (2002). Lean Six Sigma: Combining Six Sigma Quality with Lean Production Speed. McGraw-Hill.
9. Goldstein, J., Newbury, D. E., Joy, D. C., Lyman, C. E., Echlin, P., Lifshin, E., ... & Michael, J. R. (2017). Scanning Electron Microscopy and X-ray Microanalysis (4th ed.). Springer. https://doi.org/10.1007/978-1-4939-6676-9

10. Gupta Banerjee, P., Paul, A., Mukhopadhyay, M., Lily, K., & Banerjee, U. (2024). Liposomal Iron: A novel approach for improved Iron supplementation, manufactured by West Bengal Chemical Industries Ltd.World Journal of Pharmaceutical Sciences. https://wjpsonline.com/
11. Gupta Banerjee, P., Paul, A., Chakraborty, A., & Kundu, S. (2025). Liposomal calcium: A novel nutraceutical delivery approach by West Bengal Chemical Industries Ltd., Kolkata, India. Acta Traditional Medicine, 4(1), Article 05. https://doi.org/10.51470/ATM.2025.4.1.05
12. Gupta Banerjee, P., Paul, A., Chakraborty, A., & Kundu, S. (2025). Enhancing the stability and bioavailability of alpha-lipoic acid: Development and evaluation of a Liposomal formulation by West Bengal Chemical Industries Ltd. International Journal of Pharmaceutical Science and Drug Analysis, 5(1), 39–48.https://www.wbcil.com/wp-content/uploads/2025/03/Enhancing-the-stability-and-bioavailability-of-alpha-lipoic-acid.pdf
13. Gupta Banerjee, P., Paul, A., Chakraborty, A., & Kundu, S. (2025). Liposomal glutathione: A breakthrough in cellular health by West Bengal Chemical Industries Ltd., Kolkata, India. The Pharma Innovation Journal, 14(2), 73–81.
14. Gupta Banerjee, P., Paul, A., Chakraborty, A., & Kundu, S. (2025). Advanced Liposomal CoQ10 formulation by WBCIL: A step forward in cardiovascular nutraceutical therapy. International Journal of Pharmaceutical Science Invention, 14(3), 140–155. https://doi.org/10.35629/6718-1403140155.
15. Gupta Banerjee, P., Paul, A., Chakraborty, A., & Kundu, S. (2025). Innovative Liposomal Vitamin C by West Bengal Chemical Industries Ltd., Kolkata, India: Enhancing nutraceutical effectiveness. Eastern Journal of Medical Sciences, 10(2).
16. Imai, M. (1986). Kaizen: The Key to Japan's Competitive Success. McGraw-Hill.
17. Lu, Y., & Park, K. (2013). Polymeric micelles and alternative nanonized delivery vehicles for poorly soluble drugs. International Journal of Pharmaceutics, 453(1), 198–214.
18. Mozafari, M. R. (2005). Liposomes: an overview of manufacturing techniques. Cellular & Molecular Biology Letters, 10(4), 711–719.
19. Sharma, A., & Sharma, U. S. (1997). Liposomes in drug delivery: progress and limitations. International Journal of Pharmaceutics, 154(2), 123–140.
20. Stetefeld, J., McKenna, S. A., & Patel, T. R. (2016). Dynamic light scattering: a practical guide and applications in biomedical sciences. Biophysical Reviews, 8(4), 409–427. https://doi.org/10.1007/s12551-016-0218-6
21. Tian, Y., & Chen, B. (2011). Thermal analysis and decomposition kinetics of lipid-based drug delivery systems. Journal of Thermal Analysis and Calorimetry, 106, 707–713.
22. Torchilin, V. P. (2005). Recent advances with Liposomes as pharmaceutical carriers. Nature Reviews Drug Discovery, 4(2), 145–160. https://doi.org/10.1038/nrd1632
23. U.S. Food and Drug Administration. (2018). Liposome drug products: Chemistry, manufacturing, and controls; Human pharmacokinetics and bioavailability; and labeling documentation: Guidance for industry. Center for Drug Evaluation and Research (CDER). https://www.fda.gov/media/70837/download
24. Wissing, S. A., Kayser, O., & Müller, R. H. (2004). Solid lipid nanoparticles for parenteral drug delivery. Advanced Drug Delivery Reviews, 56(9), 1257–1272.
25. Yamashita, S., Hashiguchi, T., Endo, H., Fujii, T., & Nagatomo, H. (2009). Observation of Liposome morphology by scanning electron microscopy. Journal of Electron Microscopy, 58(4), 205–209. https://doi.org/10.1093/jmicro/dfp030
26. Yusuf, Y. Y., Gunasekaran, A., & Dan, G. A. (2007). Agile Manufacturing: The Drivers, Concepts and Attributes. International Journal of Production Economics, 62(1), 33–43.
27. Zhao, Y., Wang, W., Zhang, W., & Zhang, Y. (2015). Thermal stability of Liposomes encapsulating curcumin. Food Chemistry, 175, 203–210. https://doi.org/10.1016/j.foodchem.2014.11.151.