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क्रम सं/SL No :033138888

पेटेंट कार्यालय,भारत सरकार | The Patent Office, Government Of India

पेटेंट प्रमाण पत्र | Patent Certificate

(पेटेंट नियमावली का नियम 74) | (Rule 74 of The Patents Rules)

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आवेदन सं. / Application No.	:	202531003321
फाइल करने की तारीख / Date of Filing	:	15/01/2025
पेटेंटी / Patentee	:	WEST BENGAL CHEMICAL INDUSTRIES LTD

प्रमाणित किया जाता है कि पेटेंटी को, उपरोक्त आवेदन में यथाप्रकटित *LIPOSOMAL IRON: A NOVEL APPROACH FOR IMPROVED IRON SUPPLEMENTATION WITH ENHANCED STABILITY AND MANUFACTURING THEREOF* नामक आविष्कार के लिए, पेटेंट अधिनियम, 1970 के उपबंधों के अनुसार आज तारीख जनवरी 2025 के पंद्रहवें दिन से बीस वर्ष की अवधि के लिए पेटेंट अनुदत्त किया गया है।

It is hereby certified that a patent has been granted to the patentee for an invention entitled *LIPOSOMAL IRON: A NOVEL APPROACH FOR IMPROVED IRON SUPPLEMENTATION WITH ENHANCED STABILITY AND MANUFACTURING THEREOF* as disclosed in the above mentioned application for the term of 20 years from the 15th day of January 2025 in accordance with the provisions of the Patents Act,1970.

पेटेंट कार्यालय,भारत सरकार
The Patent Office, Government Of India
सत्यमेव जयते

अनुदान की तारीख :
Date of Grant : 05/08/2025

पेटेंट नियंत्रक
Controller of Patents

टिप्पणी - इस पेटेंट के नवीकरण के लिए फीस, यदि इसे बनाए रखा जाना है, जनवरी 2027 के पंद्रहवें दिन को और उसके पश्चात प्रत्येक वर्ष मे उसी दिन देय होगी।

Note. - The fees for renewal of this patent, if it is to be maintained, will fall / has fallen due on 15th day of January 2027 and on the same day in every year thereafter.

569578

Indian Patent

Patent Number: 569578
Date of Patent: 05 August, 2025

Liposomal Iron: A Novel Approach for Improved Iron Supplementation with Enhanced Stability and Manufacturing Thereof

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Application: **202531003321**

Filed: **15 January, 2025**

ABSTRACT:

The present invention discloses a novel liposomal ferric pyrophosphate composition and its manufacturing process, addressing critical challenges in iron supplementation. This invention utilizes liposome-coated liposomes to enhance iron delivery, achieving high encapsulation efficiency (>89%). The manufacturing process involves optimized emulsification and spray drying techniques, ensuring consistent particle morphology and stability under varied storage conditions. The composition and manufacturing process is remarkable which in turn demonstrates better efficacy across populations, including pregnant women, children, and the elderly, with minimal side effects. Key innovations include compatibility with dietary nutrients, resistance to gastric degradation, and advanced reactor designs for scalability. The simplicity of the process without use of any organic solvent and complicated technology gives the height to the innovation. Applications extend to anemia management and nutritional supplementation. This robust and scalable formulation offers a superior alternative to conventional iron therapies, aligning with global health needs.

08 Claims, No Drawings

Claims

1. A liposomal ferric pyrophosphate composition with encapsulation efficiency exceeding 85%, comprising:
   a) ferric pyrophosphate;
   b) liposome;
   c) citric acid;
   d) soda ash; and
   e) maize starch or lactose;
   f) wherein the composition having particle size in the range of 500-2000 nm, PDI less than 1, and zeta potential exceeding ±30 mV range and the composition having sufficient surface charge for stability in suspension and colloid which maintain the composition quality during storage and improve effectiveness within body.
2. A process for preparing the liposomal ferric pyrophosphate claimed in claim 1 using a liposome coating, comprising:
   a) dissolving tetra sodium pyrophosphate in water;
   b) precipitating ferric pyrophosphate by adding ferric chloride;
   c) adjusting pH to achieve desired solubility of ferric pyrophosphate;
   d) adding starch or lactose to obtain desired mass of ferric pyrophosphate;

e) coating with liposome using emulsification at high speed RPM and stirring time of 0.5 hour using specific reactor design; and
f) spray drying to obtain the final product.

3. The process claimed in claim 2 wherein desired solubility of ferric pyrophosphate includes both soluble ferric pyrophosphate and insoluble ferric pyrophosphate.
4. The process claimed in claim 2 wherein adjusting pH refers to the soluble ferric pyrophosphate which includes adding citric acid and 5 soda ash to bring pH about 2 to 6.5.
5. The process claimed in claim 2 wherein step (d) of adding starch to bring the iron content of insoluble ferric pyrophosphate about 7 to 9 %.
6. The process claimed in claim 2 wherein step (d) of adding lactose to filtrate soluble ferric pyrophosphate.
7. The process claimed in claim 2 wherein emulsification includes heating liposome in separate reactor at high speed RPM for 0.5 hour and mixing with colloidal mass of soluble ferric pyrophosphate obtained from claim 7 or insoluble ferric pyrophosphate obtained from claim 6 and stirring at room temperature.
8. The process claimed in claim 2 wherein liposome heated to 60^{o}C under emulsification in separate reactor.

TITLE OF THE INVENTION

LIPOSOMAL IRON: A NOVEL APPROACH FOR IMPROVED IRON SUPPLEMENTATION WITH ENHANCED STABILITY AND MANUFACTURING THEREOF

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PREAMBLE TO THE DESCRIPTION

The following specification particularly describes the invention and the manner in which it is to be performed.

TITLE: LIPOSOMAL IRON: A NOVEL APPROACH FOR IMPROVED IRON SUPPLEMENTATION WITH ENHANCED STABILITY AND MANUFACTURING THEREOF

FIELD OF THE INVENTION

The present invention is related to the field of nutraceutical and pharmaceutical formulations. More specifically, it relates to a novel liposomal iron manufacturing process, designed to improve iron supplementation with enhanced stability.

BACKGROUND OF THE INVENTION

Iron deficiency is one of the most common nutritional deficiencies worldwide, contributing to anemia and various health complications. Conventional iron supplements, such as ferrous sulfate or ferric citrate, are widely used to address this deficiency. However, these supplements often result in poor bioavailability, gastrointestinal side effects, and oxidative stress, which can negatively impact patient compliance.

Iron supplements play a critical role in treating iron deficiency anemia (IDA), which affects millions globally. However, poor absorption, adverse effects, and interactions with dietary inhibitors limit the effectiveness of traditional iron therapies. To overcome these challenges, liposomal technology has emerged as a promising solution. Liposomes are lipid-based bilayer vesicles capable of encapsulating both hydrophilic and lipophilic molecules, thus enhancing drug delivery and absorption.

The prior art WO2013141139disclose the Iron pyrophosphate composition where iron (III) pyrophosphate is coated with polyoxyethylene sorbitan fatty acid ester or glycerin fatty acid ester and enzymatically decomposed lecithin. The obtained product from the prior is silent on poly dispersity index which mean that particle size distribution and their pattern is unknown whether distribution is homogenous or heterogeneous. The prior art also silent on the encapsulation efficiency.

The prior art IN2895/CHENP/2009 disclose the method for producing iron-and vitamin-enriched rice or barley where emulsifying agent is selected from the group consisting of a sucrose fatty acid ester, a glycerol fatty acid ester, a propylene glycol fatty acid ester, a sorbitan fatty acid ester and an enzymatically decomposed lecithin. The prior art does not disclose the stability of obtained product and also silent on the encapsulation efficiency and free assay iron.

The prior art JP1998225263 disclose the method for producing iron-enriched fermented milk by liposome coating process where enzymatically decomposed lecithin used with ferric pyrophosphate with sour milk through solid-liquid separation by centrifugation method. The prior art produced fermented milk enriched with Iron but the process involves complexity and any type of discrepancy in the process may hazard the final product of milk and may lost the original flavor of milk. The prior art silent on the stability parameter and encapsulation efficiency.

The existing prior art related to rice, barley, milk etc. which is just fortification of food and has limited role in the pharmaceutical and nutraceutical industry. The problem lies in the prior art that people may lactose intolerant, might have allergy or issue with consuming rice or barley. If liposomal fortified with food, then very difficult to bypass the gastrointestinal tract which show poor absorption of liposomal iron in the body.

The existing prior art in liposomal iron, there remains a need for an iron supplement with improved encapsulation efficiency, stability, and reduced side effects. The present invention aims to address these unmet needs by providing a novel liposomal ferric pyrophosphate composition and their process of preparation.

The present invention offers a novel liposomal preparation that provides a scalable manufacturing process for consistent product quality.

The present invention utilize advanced delivery technology, presents a promising solution to enhance iron uptake and mitigate adverse effects. The

liposomal protection allows the iron to overcome the free gastric environment, preventing early degradation of the substance and/or its inactivation and to be absorbed directly. Consequently, this method of iron supplementation is associated with high gastrointestinal absorption, high bioavailability, and a low incidence of side effects.

The present invention provides encapsulation procedure offers several advantages such as higher encapsulation efficiency; ability to load lipophilic and hydrophilic drugs together; biocompatibility; provision of receptor-mediated site-specific targeting and triggering of controlled release. Liposomes are typically suitable for such drug delivery since they delay the clearance duration and increase the intravascular circulation time of encapsulated drugs, thereby altering their bio-distribution Liposomal iron represents a promising advancement in iron supplementation, offering improved efficacy, tolerability, and suitability across diverse populations.

Some parts of the present invention published in World Journal of Pharmaceutical Sciences on 26/07/2024 with title of "LIPOSOMAL IRON: A NOVEL APPROACH FOR IMPROVED IRON SUPPLEMENTATION, MANUFACTURED BY WEST BENGAL CHEMICAL INDUSTRIES LTD".
Available at:
https://wjpsonline.com/index.php/wjps/article/view/1602.

OBJECTIVE OF THE INVENTION

The objective of the present invention is to develop a novel liposomal ferric pyrophosphate composition and a robust scalable manufacturing process which have a diverse stability of iron supplementation. The invention aims to overcome the limitations associated with conventional iron supplements, such as poor absorption, gastrointestinal side effects, and oxidative stress.

The present invention focuses on providing a highly stable and efficient liposomal iron formulation, leveraging advanced encapsulation techniques using liposome- coated liposomes. The objective is to ensure improved iron delivery, compatibility with other nutrients, and resistance to dietary inhibitors. Additionally, the invention addresses critical challenges in iron supplementation by minimizing iron ion leakage, reducing oxidative stress, and enhancing patient compliance across diverse populations, including pregnant women, children, and the elderly.

Key goals include:

- Achieving high encapsulation efficiency exceeding 85%.
- Enhancing storage stability and reducing iron ion degradation.
- Mitigating adverse gastrointestinal effects through advanced liposomal technology.
- Providing a scalable and robust manufacturing process that ensures consistent quality and effectiveness.

SILENT FEATURE OF THE INVENTION

Liposomal is a drug delivery system. Microencapsulated iron pyrophosphate in liposomal form is a novel advancement in management of iron deficiency anemia. Liposome technology is a special kind of microencapsulation technique, which has been extensively investigated and developed in the biomedical field as a drug delivery system. An important aspect of this application is the protection afforded by encapsulation, against potentially damaging conditions in the extracapsular environment.

Preparation of soluble or insoluble Liposomal ferric pyrophosphate is tricky and faces challenges in scale up. It requires fridge drying or repeated cooling at very low temperature or centrifugation with high RPM or Thin-film hydration (TF) or Thin-film and sonication (TFS) or Freeze-thawing (FT) or Reverse-phase evaporation (REV).

- The process of present invention does not involve any such complicated technique. Emulsification is a critical step which can be achieved by simple reactor design.
- Refrigerated Centrifuge with High RPM or ultrahigh centrifuge or use of rotavapor or microwave radiation does not involve in the present process.
- In present invention process, it does not involve the use of any kind of organic solvent like alcohol or ether or chloroform.
- In the present invention process, the final product can be isolated by spray drying.

SUMMARY OF THE INVENTION

The present invention provides a liposomal ferric pyrophosphate composition with high encapsulation efficiency. The composition employs advanced emulsification techniques and liposome-based coatings to achieve superior particle morphology and stability. The invention also provides a robust manufacturing process optimized for large-scale production. Key phyco-chemical features include:

1. A novel encapsulation method using liposome as a coating material.
2. Optimized particle size and morphology to ensure efficient iron delivery.
3. Stability across varying storage conditions and reduced iron ion leakage.
4. Enhanced compatibility with other nutrients and reduced interaction with dietary inhibitors.
5. Applicability across diverse populations, including pregnant women, children, and the elderly.

Figures and Tables

- **Figure 1:** SEM images showing particle morphology.
- **Figure 2:** Stability analysis under accelerated and real-time conditions.
- **Figure 3:** DSC diagram of Ferric pyrophosphate, liposome and liposomal ferric pyrophosphate.
- **Table 1:** Elemental composition under varying conditions.
- **Table 2:** Particle size and zeta potential data with PDI.
- **Table 3:** Colloidal stability with zeta potential
- **Table 4:** Particle size study where Assay element iron, assay of free iron and Encapsulation efficiency in respect of mesh size.
- **Table 5:** Leakage rate of liposomal ferric pyrophosphate.
- **Table 6:** Phase transition temperatures via DSC.

DETAILED DESCRIPTION OF THE INVENTION

The present invention addresses several limitations associated with traditional iron supplements, including:

- Poor absorption of iron salts.
- Gastrointestinal discomfort and side effects.
- Oxidative stress caused by free iron ions.
- Interaction with dietary inhibitors like phytic acid.

By encapsulating ferric pyrophosphate in liposomes, the present invention enhances iron uptake, minimizes side effects, and improves patient compliance. The use of liposome-coated liposomes provides additional benefits, such as increased stability and resistance to degradation in the gastrointestinal tract.

COMPOSITION

The liposomal ferric pyrophosphate composition comprises the following components:

- **Ferric Pyrophosphate:** The primary iron source, encapsulated within liposomes.
- **Liposome:** A phospholipid employed as a coating material to enhance stability and bioavailability.
- **Additional Ingredients:** Optional excipients such as citric acid, soda ash, maize starch and lactose for emulsifier and pH adjustment.

The composition is optimized to achieve a particle size range of 1200-1600 nm, ensuring efficient iron delivery and stability in suspension.

CHARACTERIZATION

1. <u>Particle Size and Morphology</u>

Cell membranes are built from fatty molecules called phospholipids, one type being liposome. Liquid state scanning electron microscopy (SEM) analysis at 7500x magnification (Fig-1) revealed significant differences between uncoated and liposome-coated ferric pyrophosphate particles. Uncoated particles appeared with non-uniform crystals (4-7 microns). As the liposome coating increased (2-10%), particles became more spherical and uniform, growing in size. The particle size increased from 12-13 micron (2% Soy Liposome coated) to 15-16 micron (10% Soy Liposome Coating). This suggests successful encapsulation of the cargo within the liposome coated liposomes, potentially influencing stability as well. The Fig 1 clearly represents the transition to a regular spherical and uniform structure by means of liposome coating.

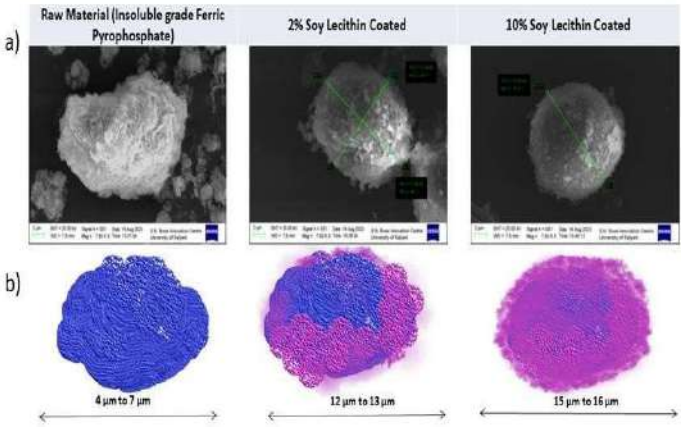

Figure1: SEM Magnification View (7500X) (a) Single Particle view (b) 2-D representation

2. Morphology and Surface Characterization

The shape and surface features of iron-containing liposomes (insoluble grade) were examined to see how they affect stability. The use of energy-dispersive X- ray spectroscopy (EDX) to analyze the elements in two samples: one stored under accelerated stability conditions for 3 months and another under real-time conditions for 3 months. EDX found carbon, nitrogen, and oxygen as the main elements in both samples, likely due to liposome, a key component of the liposome membrane. Iron was also present, but at much lower levels (Table1). Liposomes are comprised mainly of phospholipids, rich in these detected elements. Secondly, the iron pay load is encapsulated within the liposome structure, resulting in a lower overall abundance compared to the bulk membrane components. Which ultimately assures the higher EE. This suggests successful iron encapsulation within the liposomes. Interestingly, no major differences were found between the two storage conditions. This finding suggests that the storage conditions do not markedly influence the stability of the liposomes, potentially indicating their robustness across varying storage scenarios. The successful encapsulation of iron within the liposomes, coupled with their uniform spherical morphology and minimal impact from storage conditions (Fig-2), suggests a well- designed and stable drug delivery system with unique and well established robust scalable process.

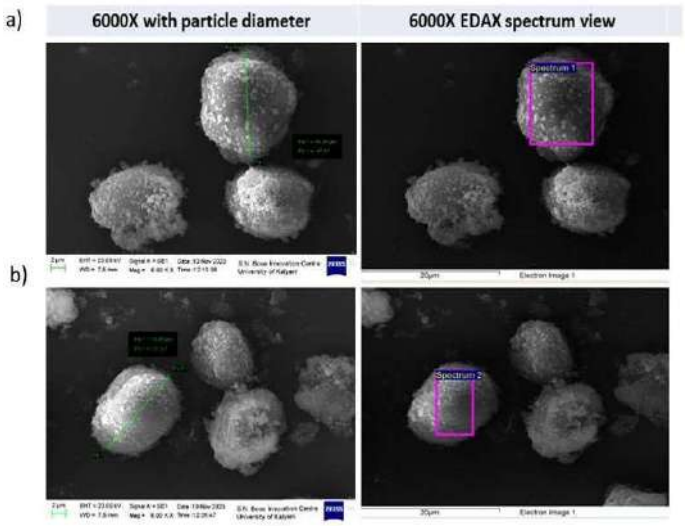

Figure 2: Ferric Pyrophosphate in (a) 3 months accelerated stability (b)Real time stability

Table 1: Elemental composition 3 months accelerated stability, Real time stability

	ACCELERATED STABILITY		REALTIME STABILITY	
Element	**Weight %**	**Atomic %**	**Weight %**	**Atomic %**
C K	31.28	36.83	32.51	38.49
N K	21.28	22.09	15.75	15.99
O K	46.28	40.91	50.78	45.13
P K	0.10	0.04	0.69	0.32
FeK	0.47	0.12	0.27	0.07
Total	**100.00**		**100.00**	

3. Encapsulation Efficiency

The results indicate successful production of Liposomal Ferric Pyrophosphate that meets quality standards. Encapsulation efficiency reached 89.01%, exceeding the minimum acceptable level of 85%. An elemental iron assay confirmed 8.12% iron in the final product, which falls within the acceptable range of 7.8% to 9.0% (Ref: USP Food Chemical Codex). The high encapsulation efficiency suggests most iron particles are trapped within the liposomes, potentially leading to better absorption and fewer digestive issues compared to traditional iron supplements (Table 5).

4. Zeta potential and Colloidal stability

The electrical charge (zeta potential) on the surface of liposomal iron particles were measured to assess their stability in suspension. Established guidelines suggest higher absolute zeta potential (positive or negative) indicates greater stability, with values exceeding +/- 30 mV considered ideal. The liposomal formulation was dispersed in a neutral (pH7.0) solution. The change in surfacechargefrom-18.05mV to a more stable-39.47mV (Table2), exceeding there commended threshold. This successful formulation with a zeta potential of -39.47 mV indicates sufficient surface charge for stability in suspension. This improved stability is essential for maintaining product quality during storage and potentially improving its effectiveness within the body.

Table2: Characteristic features of the coated and non-coated materials.

	pH 7.0 (Zeta Potential in mV)	Particle size in Liquid particulate (nm)	Poly dispersity index in liquid state
Ferric Pyrophosphate API	-18.05	3896	0.3517
10% coating	-39.47	1296	0.4465
Limit	±30	500-2000	Less than 1

Zeta Potential Values and Stability

Zeta Potential (mV)	Colloidal Stability
> +30 or < -30	Highly stable (good repulsion).
Between +10 and -10	Unstable (prone to aggregation).
Between +10 and +30 / -10 and -30	Moderately stable.

Table 3: Colloidal stability with zeta potential

Why Zeta Potential > +30 mV or < -30 mV Indicates Stability

1. **Electrostatic Repulsion:**
   a) Particles in a colloidal suspension typically acquire an electric charge due to ionization, adsorption of ions, or surface reactions.
   b) The Zeta potential reflects the magnitude of this charge and the resulting electrostatic forces between particles.
   c) A high positive (> +30 mV) or high negative (< -30 mV) Zeta potential means that particles strongly repel each other due to like charges, preventing them from coming close enough to aggregate.

2. **Threshold for Stability:**
   a) When the Zeta potential is within the range of -30 mV to +30 mV, the repulsive forces are weaker, making the particles more susceptible to van der Waals attractions, leading to aggregation or flocculation.
   b) Beyond ±30 mV, the repulsive electrostatic forces dominate, maintaining a stable dispersion.

3. **Energy Barrier:**
   a) The repulsive forces create an energy barrier that prevents particles from approaching each other. This barrier is strong when the Zeta potential exceeds ±30 mV, minimizing collisions that could lead to aggregation.

5. Particle size distribution and Poly dispersity index (PDI)

Liposomal iron formulation size and distribution were characterized using Dynamic Light Scattering (DLS). DLS measures scattered light fluctuations to determine particle size. The analysis revealed a mean hydrodynamic diameter of 1296 nm, consistent with the predefined acceptable range for this application (Table 2). Polydispersity Index (PDI), a measure of size distribution, was also assessed. Ideally, PDI values closer to 1 indicate a more uniform population. The formulation exhibited a PDI of 0.4465 (Table 2), suggesting moderate poly dispersity.

6. Particle size and Encapsulation efficiency (EE)

This study investigated the relationship between particle size and encapsulation efficiency (EE) of iron microspheres. EE, expressed as a percentage, reflects the amount of iron successfully encapsulated. Particle size was categorized by mesh size, with lower numbers indicating larger particles. A decreasing trend in EE was observed with decreasing particle size within the #18 to #200 mesh range (approximately 1000 µm to 75 µm sieve size). EE dropped from 93.91% (mesh#18) to 86.27% (mesh#200), but remained above 85% across this range.

This suggests efficient encapsulation for these sizes. However, a significant decrease to 74.35% EE was observed for the smallest particles (#325 mesh) (Table 4). Two factors might explain this trend. Firstly, smaller particles offer limited internal volume to house iron molecules, potentially restricting encapsulation capacity. Secondly, their increased surface area to volume ratio exposes encapsulated iron to the external environment, possibly leading to leakage. These findings suggest a trade-off between particle size and EE for iron microspheres. In this invention, a particle size between 18 and 200 mesh seems optimal for high Encapsulation Efficiency (EE).

Table 4: Particle Size study

Sl. No	Mesh Size	Assay Elemental Iron	Assay of free Iron	Encapsulation efficiency
1	Mesh#18	9.42	0.57	93.91
2	Mesh#35	9.52	0.64	93.30
3	Mesh#40	8.93	0.89	90.00
4	Mesh#60	9.63	1.15	88.08
5	Mesh#80	9.19	1.34	85.41
6	Mesh#140	9.75	1.53	85.01
7	Mesh#200	9.30	1.28	86.27

7. Leakage rate and shelf-life stability

A stability study assessed iron leakage from liposomes over a simulated 6-month shelf life under accelerated conditions (Table-5). Two key measures were assessed: encapsulation efficiency and elemental iron content. Encapsulation efficiency refers to the percentage of medication protected within the spheres. The acceptable level was at least 85%, and throughout the study, the medication remained above 87%.

Elemental iron content refers to the amount of iron within the medication. The acceptable range was 7.8% to 9.0%, and the medication stayed within this range throughout the entire study. In conclusion, based on the findings from this investigation, liposomal ferric pyrophosphate appears to be stable even when stored at 40°C for 6 months. This suggests it's likely to remain stable at room temperature for an extended period of time.

Table 5: Leakage rate of drug from the liposomes (shelf-life period)

Period completed in month	Encapsulation Efficiency	Elemental Iron Assay
Initial (0 month)	89.5%	8.12%
1 month	88.0%	8.02%
2 months	87.5%	7.95%
3 months	87.5%	7.90%
6 months	87.0%	7.91%

8. Phase Transition Behavior via Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry (DSC) was used to investigate the phase transition behavior of liposomal iron. In liposomes, this refers to the temperature-dependent transformation from a gel like to a more fluid state, impacting stability and drug delivery. The liposome thermogram displayed multiple endothermic peaks, consistent with its known phase transitions. The "Liposomal Ferric Pyrophosphate" curve, reflecting the entire formulation, exhibited a more complex profile with several peaks at distinct temperatures (Fig-3). This suggests potential multi-step phase transitions within the liposomal iron. API is breaking at 2 different segments in 2 different temperature 112 and 177, but this pattern is missing in 10% formulation. 1st stage breakage at 112 degrees and 2nd stage breakage at 177 degrees. These two fragments breakage is absent in 10% coating, which is indicative of Coating (Table 6).

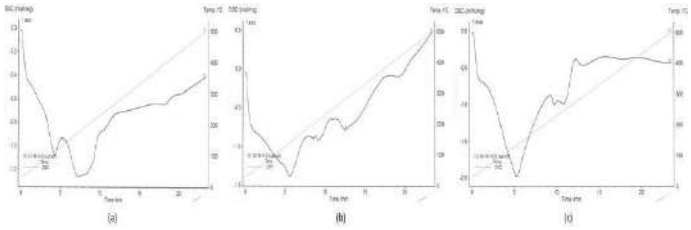

Figure 3: DSC diagram of (a) Ferric Pyrophosphate API, (b) Liposome, (c) Liposomal Ferric Pyrophosphate.

Table 6: Phase Transition Temperature

	Temp (°C)	Time (min)	DSC (mW/mg)
Ferric Pyrophosphate API	112.23	4.25	-1.07886
	177.67	7.5	-1.26281
Liposome	136.85	5.5	-1.37756
	212.78	9.25	-0.92002
	278.42	12.5	-0.78655
Liposomal Ferric Pyrophosphate	132.30	5.25	-1.97411
	223.04	9.75	-1.00296
	288.63	13	-0.46391

9. Temperature Exposure and Stability of Liposomal Iron (Insoluble Grade):

An accelerated stability test exposed the liposomal iron to 105°C for 10 minutes (commercial batch FPPLI092306A), simulating harsher storage conditions to predict long-term stability. Iron content (assay % w/w) remained stable, with values of 7.87% at room temperature and 8.05% after heat exposure. This slight increase falls within expected variability, suggesting minimal impact. Encapsulation efficiency (%) even showed a potential benefit. The initial value of 89.75% increased to 91.27% after treatment; the observed changes in iron content and encapsulation efficiency

were minimal. This suggests potential resistance to thermal degradation within the tested parameters.

This coating significantly improved particle shape, yielding uniform spheres compared to uncoated particles. Importantly, the coating-maintained stability across various storage conditions. The formulation achieved a high encapsulation efficiency (89.01%), exceeding requirements and indicating successful iron entrapment. A trade-off between particle size and encapsulation efficiency was observed. Encouragingly, the formulation exhibited minimal leakage throughout a simulated shelf-life study. While the complex phase transition profile warrants minimal impact from high-temperature exposure suggests promising stability under broader conditions. Overall, these findings demonstrate the large-scale production, potential of developed liposome-coated liposomal iron and characterization thereof.

Manufacturing Process

Preparation of Insoluble Liposomal Ferric Pyrophosphate:

1. Process water in a clean reactor with suitable stirring arrangement.
2. Add tetra sodium pyrophosphate into the above reactor under stirring.
3. Precipitate insoluble ferric pyrophosphate by addition of clear solution of ferric chloride
4. Wash sodium chloride with process water to bring the chloride content below 3%.
5. Add Maize starch to bring the iron content about 7 to 9%. At the end, a colloidal mass was obtained.
6. Add liposome in separate reactor with process water and heated to 60 deg C under emulsification.
7. The mixture obtained from step 6 was added to the colloidal solution of step 5.
8. The resulting mixture was stirred 3 to 6 hours at room temperature followed by isolation by spray drying to get desired product.

Preparation of Soluble Liposomal Ferric Pyrophosphate:

1. Follow steps 1-4 from the insoluble preparation process.
2. Adjust the pH to 2 to 6.5 more specifically 3 to 6 and precisely 4 to 5 using citric acid and soda ash.
3. Add lactose to the solution to filtrate soluble ferric pyrophosphate.
4. Add liposome in separate reactor with process water and heated to 60 deg C under emulsification.
5. Combine the liposome emulsion with the solution of ferric pyrophosphate and stir for 3 to 6 hours at room temperature.
6. Isolate the final product by spray drying.

Critical process parameters include:

- Emulsification speed: high speed RPM.
- Stirring time: about 0.5 hours during emulsion formation.
- Reactor impeller design: Preferably Pitch Blade Turbine Impeller (PBTI) for large-scale production.

The present invention is novel process to synthesis the liposomal ferric pyrophosphate and the step of emulsification is the critical process parameter. The RPM has to be high speed for the period of about 0.5h.

A limited colloidal stability is a potential problem of all emulsions. A prerequisite of good emulsion stability is that small droplets and a narrow droplet size distribution are obtained in the emulsification process. If these requirements are attained, the stability will depend on the ability of the emulsifier to prevent coalescence and flocculation of the droplets. The higher the stirring speed and the longer the stirring time, the density and viscosity values will decrease. The stability of the emulsion is increasing, where the higher the emulsifier concentration of liposome, the greater the stability obtained. Similarly, density and viscosity increase as solid concentrations increase. The optimum results obtained in this study for the homogenization process of emulsions are at high speed RPM and stirring time of 0.5 h.

The impeller types including the flat-blade turbine, especially good for emulsification, the pitched-blade turbine, well-suited for solids suspension, and the anchor-style, which is used to improve heat transfer for viscous liquids. These needs to be taken into consideration for proper emulsification for scale up and scale down. The design of reactor blade is also a critical attribute of this process.

The Pitch Blade Turbine Impeller (PBTI) is one of the most widely used impellers and one of the oldest designs of the mixing technology. The design of the PBT impeller provides a combination of both radial and axial flow, generates higher shear levels for reactions, and provides excellent mixing ability while providing

easy cleanup. Because of the simple design, it is also very cost effective in large applications and higher viscosity applications. While useful in most applications, this design excels in aggressive mixing requiring high power input per volume as well as flow, having geometry with a blade angle of 45 to 32 degrees.

For the preparation of Liposomal Ferric Pyrophosphate, the above specific design needs to be followed i.e., Pitch Blade Turbine Impeller (PBTI) or Flat Blade Impeller (FBI) or Vertical Impeller (VI) or Spiral Impeller (SI) for up to 3000 kg, preferably Pitch Blade Turbine Impeller (PBTI) and Impeller for up to 50 kg of production is must.

For the preparation of soluble Liposomal Ferric Pyrophosphate, the pH adjustment is also the critical process parameter. It has to be mild acidic. The pH to be adjusted to 2 to 6.5 more specifically 3 to 6 and precisely 4 to 5. Without proper adjustment the complexation of citric acid with ferric pyrophosphate will be incomplete. This will end up to mechanical mixture of soluble ferric pyrophosphate and citric acid.

Example 1: Preparation of Insoluble Liposomal Ferric Pyrophosphate:

a) Charged 3000 Lit of process water in a clean reactor with suitable stirring arrangement.
b) Charged 1000 kg of tetra sodium pyrophosphate into the above reactor under stirring.
c) The insoluble ferric pyrophosphate was precipitated out with addition of clear solution of ferric chloride (525 kg in 800 lit process water).
d) The sodium chloride was repeatedly washed with process water to bring the chloride content below 3%.
e) Maize starch (800 kg) was added lot wise to bring the iron content about 7 to 9%. At the end, a colloidal mass was obtained.
f) In a separate reactor 60 kg of liposome was added in 200 lit of process water and heated to 60 deg C under emulsification.
g) The mixture obtained from step f was added to the colloidal solution of step e.
h) The resulting mixture was stirred 6 hours at room temperature for followed by isolation by spray drying to afford about 1675 kg of desired product.

Example 2: Preparation of Soluble Liposomal Ferric Pyrophosphate:

a) Charged 3000 Lit of process water in a clean reactor with suitable stirring arrangement.
b) Charged 1000 kg of tetra sodium pyrophosphate into the above reactor under stirring.
c) The insoluble ferric pyrophosphate was precipitated out with addition of clear solution of ferric chloride (525 kg in 800 lit process water).
d) The sodium chloride was repeatedly washed with process water to bring the chloride content below 3%.
e) Citric acid (775 kg) and soda ash (about 400 kg) was added lot wise to bring the pH about 4 to 5. At the end,it was filtered to get a clear solution.
f) 20 kg of lactose was added to the above filtrate.
g) In a separate reactor 215 kg of liposome was added in 650 lit of process water and heated to 60 deg C under emulsification.
h) The mixture obtained from step g was added to the solution of step f.
i) The resulting mixture was stirred at room temperature for 6 hours followed by isolation by spray drying to afford about 1850 kg of desired product.

Enhanced Features of Liposomal ferric pyrophosphate

Better Efficacy:
Liposomal ferric pyrophosphate reaches peak plasma lev els faster than conventional iron salts. In controlled studies, it reaches the maximum level after 2 hours of supplement consumption which guarantees greater efficacy of the element for all metabolic processes in comparison to traditional iron. Liposomal iron can be given in low quantity as it is 2.7 and 3.5 times more bio-available than ferrous sulphate and plain ferric pyrophosphate, respectively.

Better tolerability:
Since it is Liposomal Iron, it can be given with other nutrients; hence, no interaction is present compared to conventional iron. Liposomal iron is almost devoid of all the common side effects associated with conventional iron, such as gastric irritation, nausea, constipation, etc. While iron supplements potentially interfere with the up- take of other nutrients such as calcium, zinc, and magnesium, liposomal iron has little impact on the efficacy of these nutrients, making it a more versatile option.

No Oxidative Stress:
Iron can induce oxidative stress in the body that can be harmful. Liposomal delivery help to mitigate the risk by minimizing the release of iron ions contributory to oxidative damage. The conventional iron is known to

increase the oxidative damage by altering the levels of malondialdehyde (MDA) as well as super-oxide dismutase (SOD). The present liposomal iron composition is related to decreased levels of MDA and increased levels of SOD. This can help in decreasing the oxidative damage otherwise found with conventional iron supplements. The liposomal formulation minimizes the release of free iron ions, reducing oxidative damage.

No Interaction with Dietary Inhibitors:
The uptake of traditional non-haem iron might be prevented by several factors, such as dietary inhibitors. Phytic acid is one of such inhibitors. Phytic acid present in cereals and legumes-based diet has been shown to inhibit iron uptake in-vivo as well as in cell culture models. However, in case of liposomal iron delivery it provides a better delivery system for iron in the sense that iron uptake occurs without getting affected by dietary inhibitors.

Target Special Populations

- Pregnancy and Lactation: The present invention demonstrated higher hemoglobin improvement and reduced anxiety in women supplemented with liposomal iron compared to ferrous sulfate.
- Children: Enhanced tolerability and long-term improvements in hemoglobin levels.
- Elderly: Safe and effective treatment of secondary anemia with no significant gastrointestinal side effects.

Significance of present invention

The present invention of coating covering substances and nutrients with a membrane formed by polymers of various nature, natural or synthetic. Polymers consist of proteins, polysaccharides, polyesters, phospholipids, etc. Microencapsulation (understood as the preparation of both microcapsules and microspheres) can allow the change of color, shape, volume, solubility, reactivity, resistance, and stability of the trapped substance. The main applications of micro encapsulation allow:

- The increase in the stability of a substance;
- Overcoming the incompatibilities;
- Masking of unpleasant smells and tastes;

The present invention used for iron up-take is significantly different from normal intestinal up-take of iron. Liposomal iron absorption involves a sophisticated technology that uses liposome as a carrier, where iron without coming in contact to gastro intestinal mucosa gets directly absorbed in the intestine. In case of normal intestinal iron up-take, gastric acid is required to convert dietary iron into absorbable form, while the iron up-take is largely facilitated by a protein located on the surface of enterocytes, i.e., cell lining of the small intestine; iron is then released into the bloodstream and binds to the transport protein transferrin, which delivers it to various organs. Contrary to this, liposomal iron supplements that are consumed orally are tissues resistant to the acidic environment of the stomach, shielding the encapsulated iron from degradation and oxidation. Liposome encapsulated iron reach the small intestine, precisely the duodenum. In the duodenum, liposome releases iron into the intestinal lumen.

The specialty of having liposomal iron supplements includes that liposomal iron is absorbed more efficiently in comparison to traditional iron supplements as it strongly bypasses gastric digestion. The absorbed liposomal iron is then released to the bloodstream and utilized by the body, alike to iron obtained from a normal diet. Although, there are reports existing on the beneficial aspects of liposomal delivery mechanism as well as suitability of ferric pyrophosphate as iron source separately; still, our current product that delivers ferric pyrophosphate iron source through liposomal coating technology provides a novel insight in considering this supplement in minimizing the release of iron ions that contribute to significant side effects. Microencapsulated iron pyrophosphate in liposomal form is a novel advancement in management of iron deficiency anemia. This salt is “generally recognized as safe (GRAS)” by United States Food and Drugs Administration (USFDA) Code of Federal Regulation. Furthermore, European Food Safety Authority (EFSA) has also declared iron pyrophosphate to be a safe food additive. Comparatively to 5 conventional oral iron salts, micro encapsulated liposomal iron has the highest bioavailability. It leads to quicker increase in serum hemoglobin levels, its taste has better palatability, and it doesn’t have unwanted effects such as heart burn, Gastrointestinal upset, and constipation.

We Claims,

1. A liposomal ferric pyrophosphate composition with encapsulation efficiency exceeding 85%, comprising:
    a) ferric pyrophosphate;
    b) liposome;
    c) citric acid;
    d) soda ash; and
    e) maize starch or lactose;
    f) wherein the composition having particle size in the range of 500-2000 nm, PDI less than 1, and zeta potential exceeding ±30 mV range and the composition having sufficient surface charge for stability in suspension and colloid which maintain the composition quality during storage and improve effectiveness within body.

2. A process for preparing the liposomal ferric pyrophosphate claimed in claim 1 using a liposome coating, comprising:
    a) dissolving tetra sodium pyrophosphate in water;
    b) precipitating ferric pyrophosphate by adding ferric chloride;
    c) adjusting pH to achieve desired solubility of ferric pyrophosphate;
    d) adding starch or lactose to obtain desired mass of ferric pyrophosphate;
    e) coating with liposome using emulsification at high speed RPM and stirring time of 0.5 hour using specific reactor design; and
    f) spray drying to obtain the final product.

3. The process claimed in claim 2 wherein desired solubility of ferric pyrophosphate includes both soluble ferric pyrophosphate and insoluble ferric pyrophosphate.
4. The process claimed in claim 2 wherein adjusting pH refers to the soluble ferric pyrophosphate which includes adding citric acid and 5 soda ash to bring pH about 2 to 6.5.
5. The process claimed in claim 2 wherein step (d) of adding starch to bring the iron content of insoluble ferric pyrophosphate about 7 to 9 %.
6. The process claimed in claim 2 wherein step (d) of adding lactose to filtrate soluble ferric pyrophosphate.
7. The process claimed in claim 2 wherein emulsification includes heating liposome in separate reactor at high speed RPM for 0.5 hour and mixing with colloidal mass of soluble ferric pyrophosphate obtained from claim 7 or insoluble ferric pyrophosphate obtained from claim 6 and stirring at room temperature.
8. The process claimed in claim 2 wherein liposome heated to 60^{o}C under emulsification in separate reactor.

LIPOSOMAL IRON: A NOVEL APPROACH FOR IMPROVED IRON SUPPLEMENTATION WITH ENHANCED STABILITY AND MANUFACTURING THEREOF

ABSTRACT

The present invention discloses a novel liposomal ferric pyrophosphate composition and its manufacturing process, addressing critical challenges in iron supplementation. This invention utilizes liposome-coated liposomes to enhance iron delivery, achieving high encapsulation efficiency (>89%). The manufacturing process involves optimized emulsification and spray drying techniques, ensuring consistent particle morphology and stability under varied storage conditions. The composition and manufacturing process is remarkable which in turn demonstrates better efficacy across populations, including pregnant women, children, and the elderly, with minimal side effects. Key innovations include compatibility with dietary nutrients, resistance to gastric degradation, and advanced reactor designs for scalability. The simplicity of the process without use of any organic solvent and complicated technology gives the height to the innovation. Applications extend to anemia management and nutritional supplementation. This robust and scalable formulation offers a superior alternative to conventional iron therapies, aligning with global health needs.