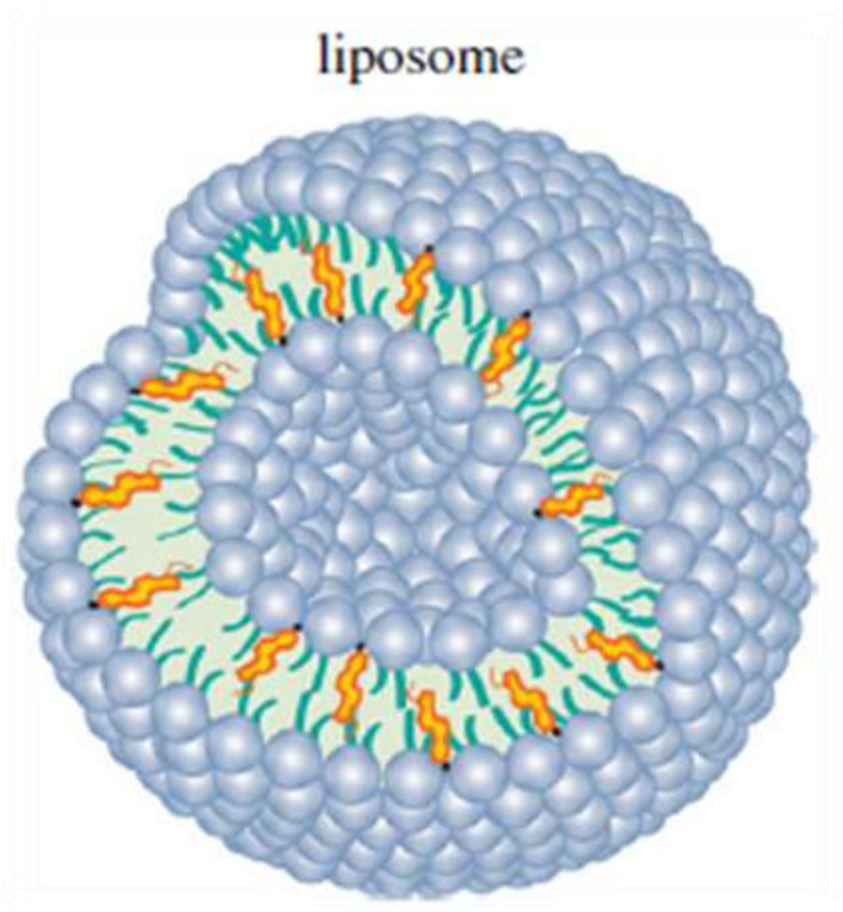
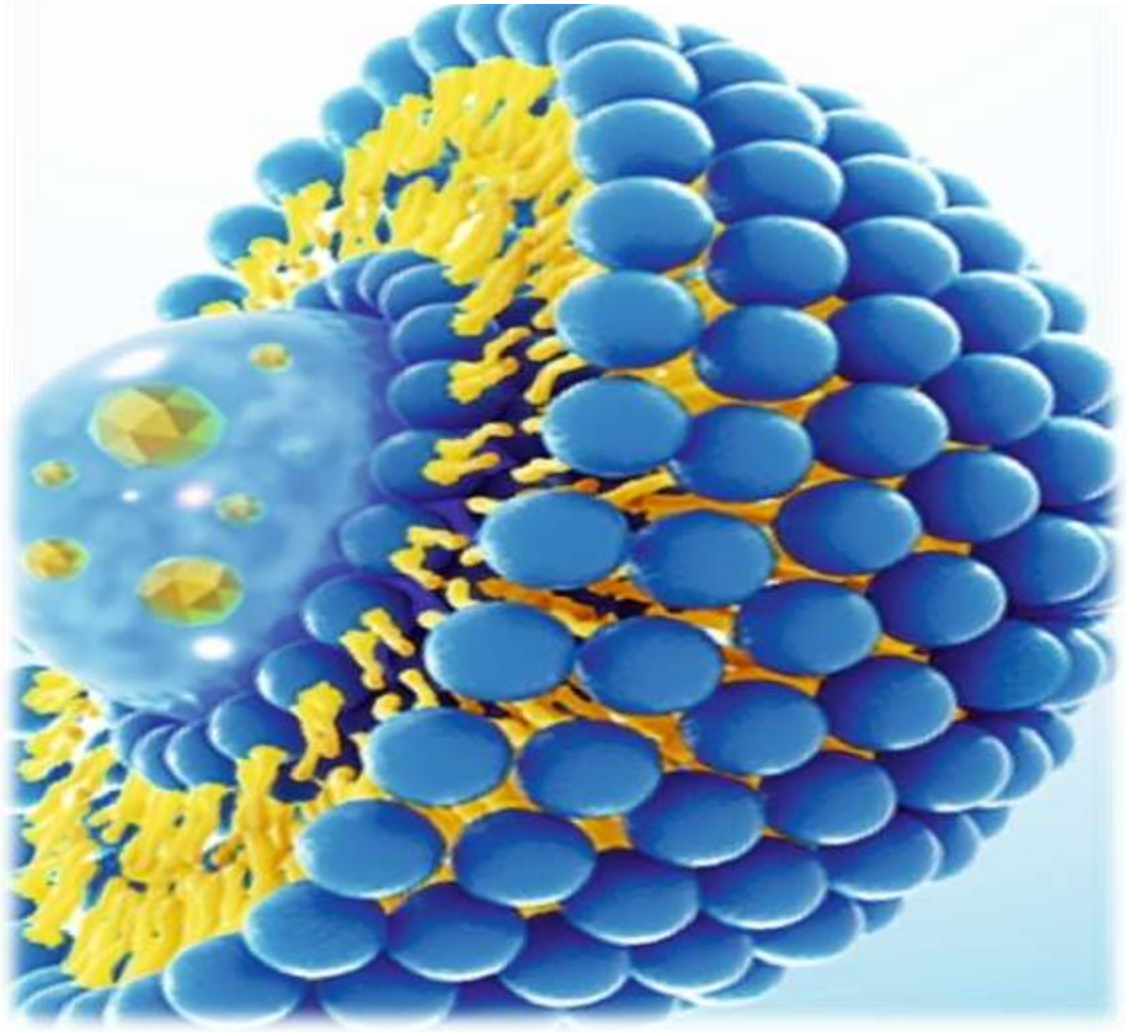


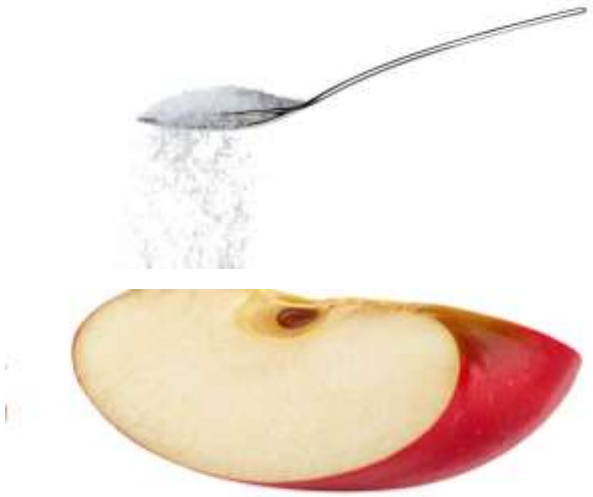
Liposomal Product



Liposomal Iron

Liposomes & Liposomal Technology: An Overview

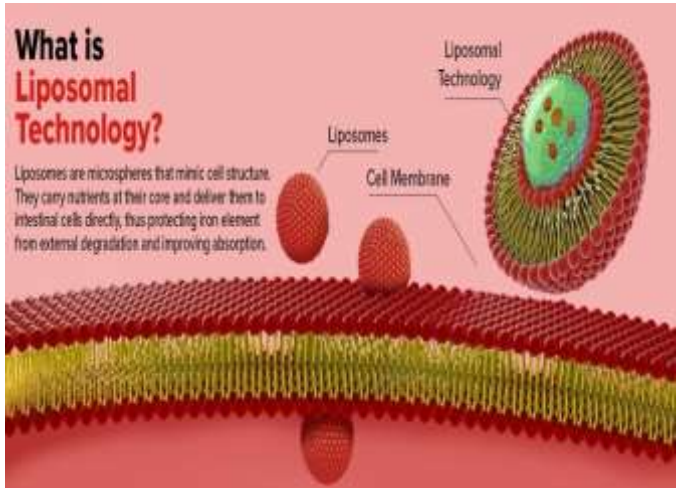
A cut apple (a mimic to cell surface) and salt (a mimic to liposomal mineral)



The apple is absorbing salt

Salt is slowly absorbed inside the apple

Schematic comparison between Liposome and cell wall

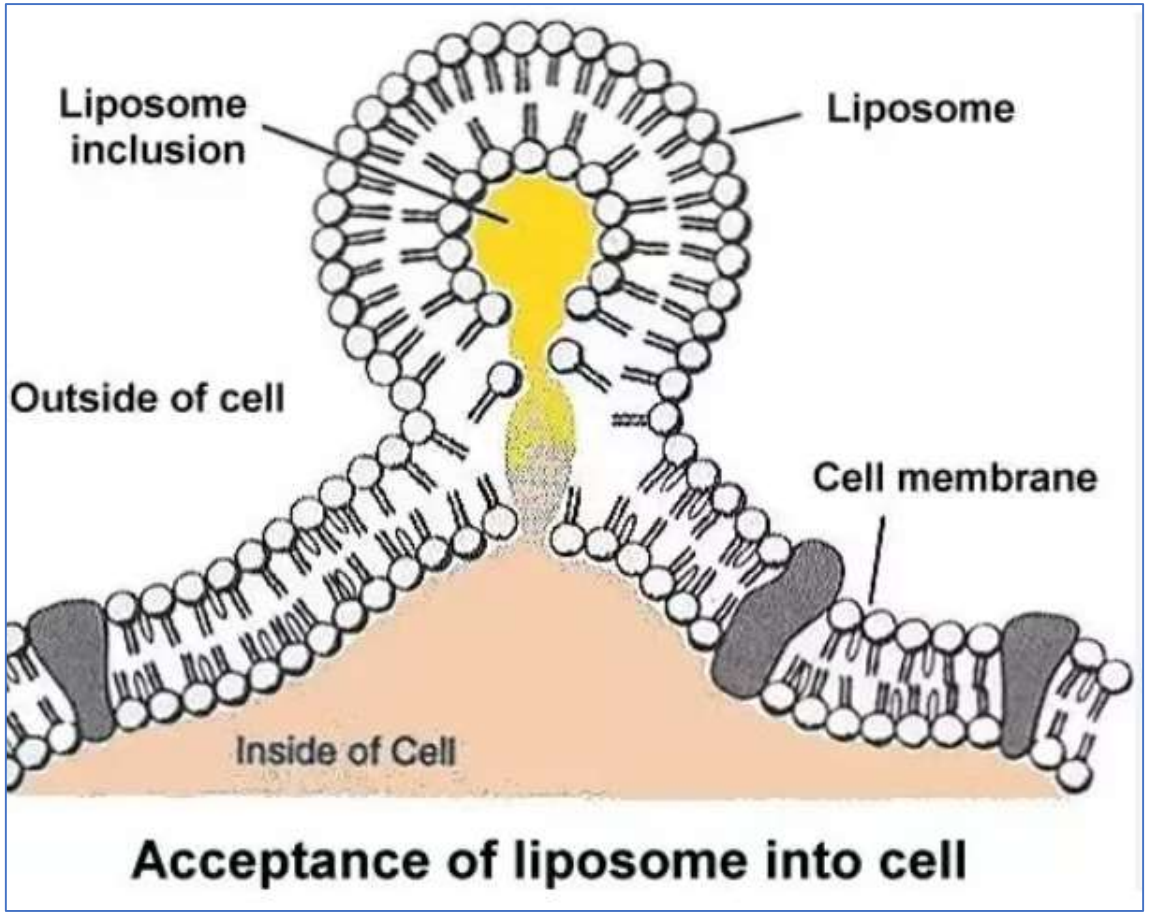


- Liposomes are primarily created from phospholipids such as Phosphatidyl choline (Lecithin) .
- Liposomes are aqueous dispersions of amphiphilic lipids and have low water solubility. They are organized as a bilayer sheet that encloses an internal aqueous compartment and are known as lipid bilayer vesicles.
- The amphiphilic lipids comprise a hydrated head group at the water interface of the bilayer attached to a hydrophobic group that forms the interior of the bilayer by association with the hydrophobic group of lipids from the opposite leaflet of the bilayer.

Reference: Monteiro N, Martins A, Reis RL, Neves NM. 2014 Liposomes in tissue engineering and regenerative medicine. J. R. Soc. Interface 11: 20140459. http://dx.doi.org/10.1098/rsif.2014.0459

Liposomes & Liposomal Technology: An Overview

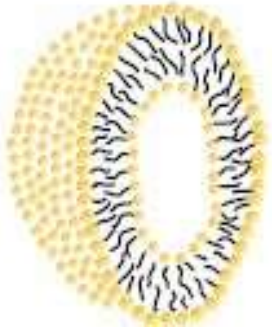
Inside cell, liposome gradually dissolves similar to turpentine oil mixing in oil paint



Acceptance of liposome into cell

LIPOSOME

Longitudinal cut of a liposoma



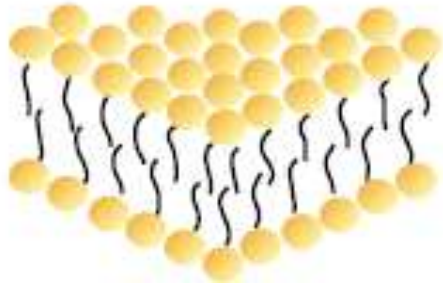
External medium

Phospholipid molecule

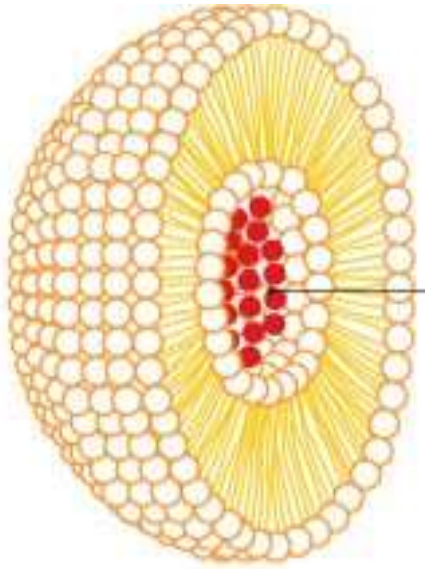
Hydrophilic polar head

Hydrophobic nonpolar tails

Distribution of phospholipid molecules in aqueous medium



Lipidic blayer



Liposomal Iron

CHARACTERISTICS COMPARISON-LIPOSOMAL IRON VS CONVENTIONAL IRON

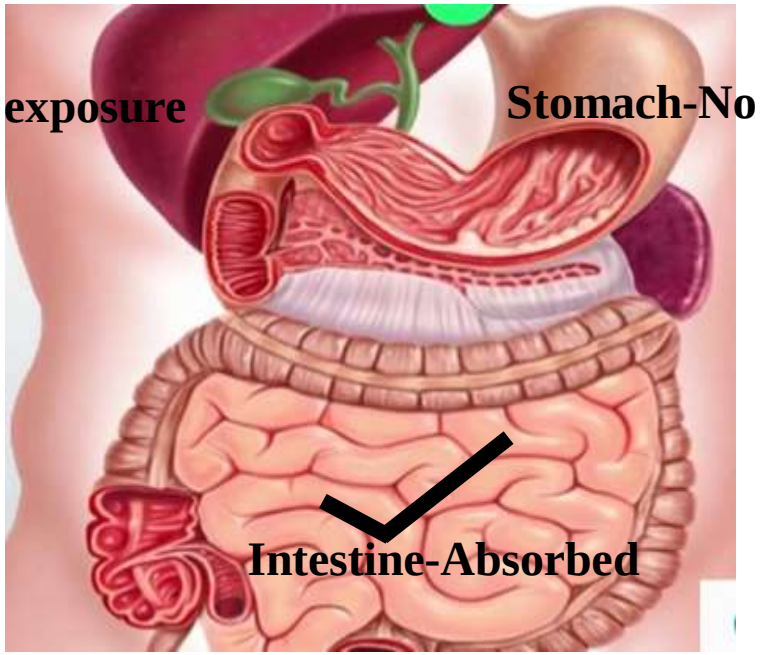
Characteristics	Liposomal iron 	Conventional iron 
Phospholipid bilayer	Present	Absent
Effect of gastric acidity	None	Present
Oxidation of iron	No	Yes
Targeted iron delivery	Yes	No
Absorption of iron	**30%-35%**	10%
Absorption via intestinal M cells	**YES**	No
Iron dose	30 mg (low)	100 mg (high)
Food effect	No	Yes
Oxidative damage to intestinal epithelium	No	Yes
Gastrointestinal side effects	Minimal/absent	Yes
Metallic taste	No	Yes
Chelation with other metals	No	Yes

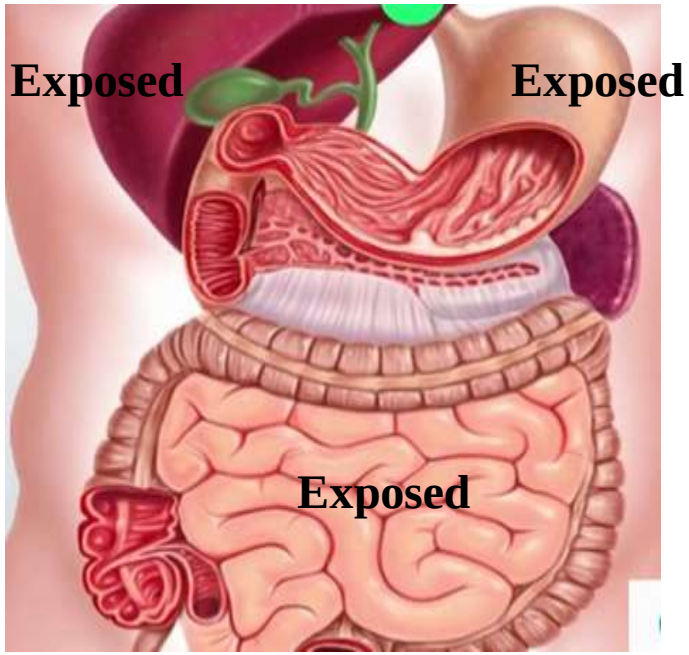
ABSORPTION

- Liposomal iron absorption is significatly different from free iron intestinal absorption.
- This product **bypasses stomach and liver**, and gets **directly absorbed**.
- It involves a sophisticated technology that uses liposomes as a carrier, where **iron without coming in contact to Gastro intestinal mucosa** gets directly absorbed in the intestine.

Liposomal

Normal





NORMAL IRON ABSORPTION

Dietary Iron Intake

Stomach

Gastric acid helps convert dietary iron into absorbable form .

Duodenum (Small Intestine)

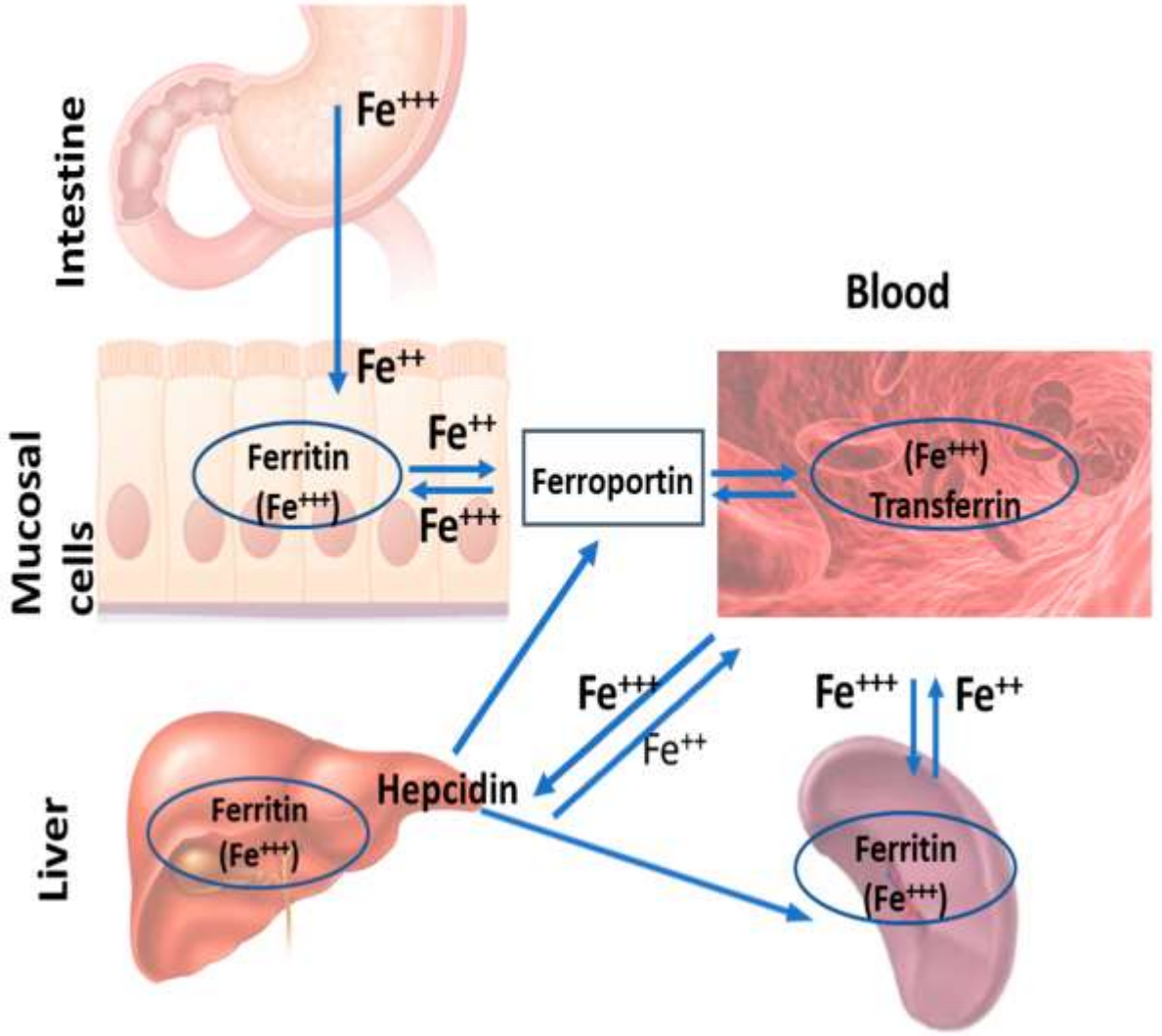
Absorption is facilitated by a protein located on the surface of enterocytes (cells lining the small intestine).

Transport

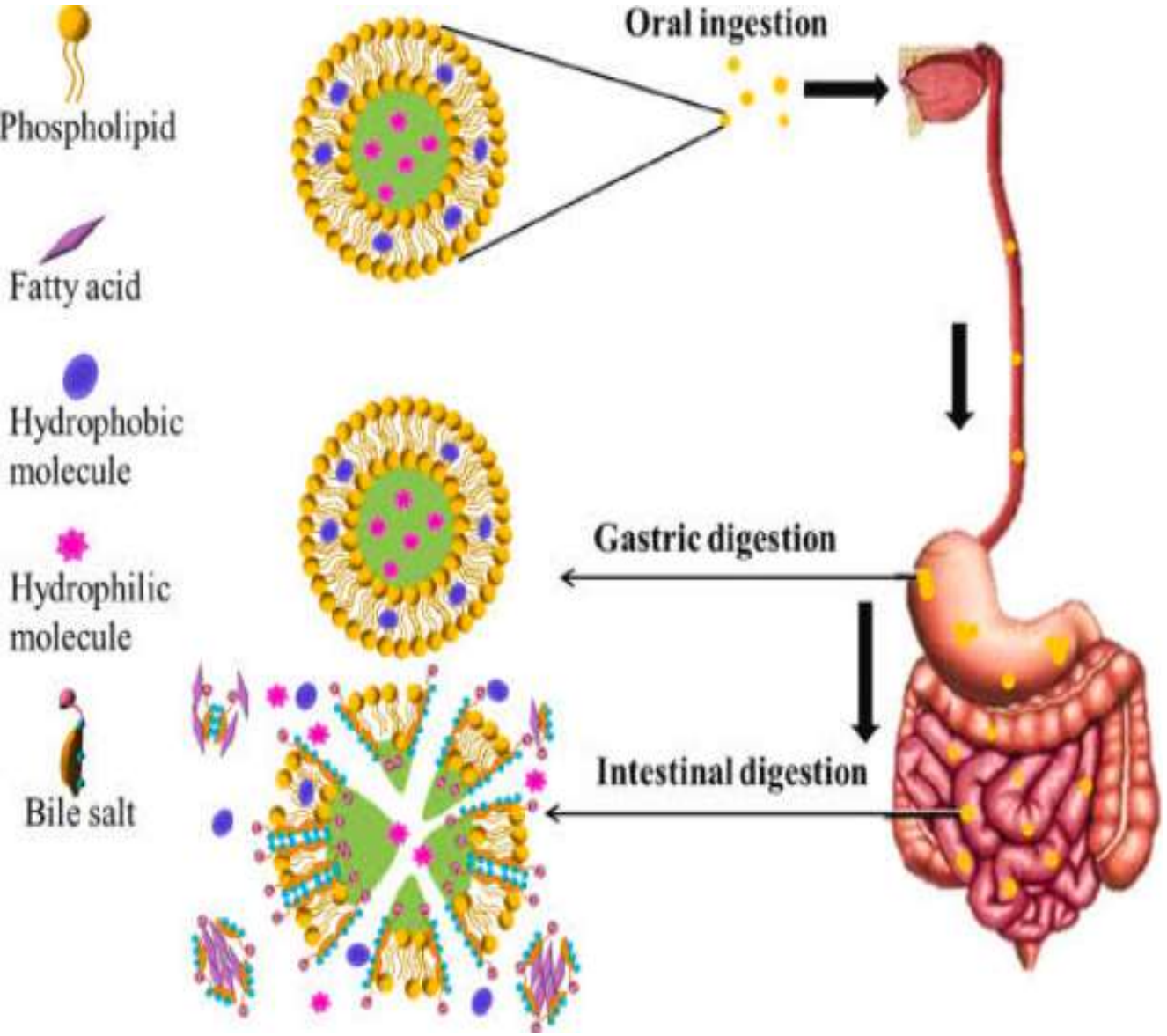
Bloodstream

Iron is released into the bloodstream and binds to a transport protein called transferrin, which delivers it to various tissues and organs.

Utilization Iron is used by the body for various functions (Production of hemoglobin enzymes, other essential processes)



LIPOSOMAL IRON ABSORPTION



Liposomal Iron Supplement

Liposomal iron supplements are consumed orally.

Stomach

Resistant to the acidic environment of the stomach, protecting the encapsulated iron from degradation and oxidation.

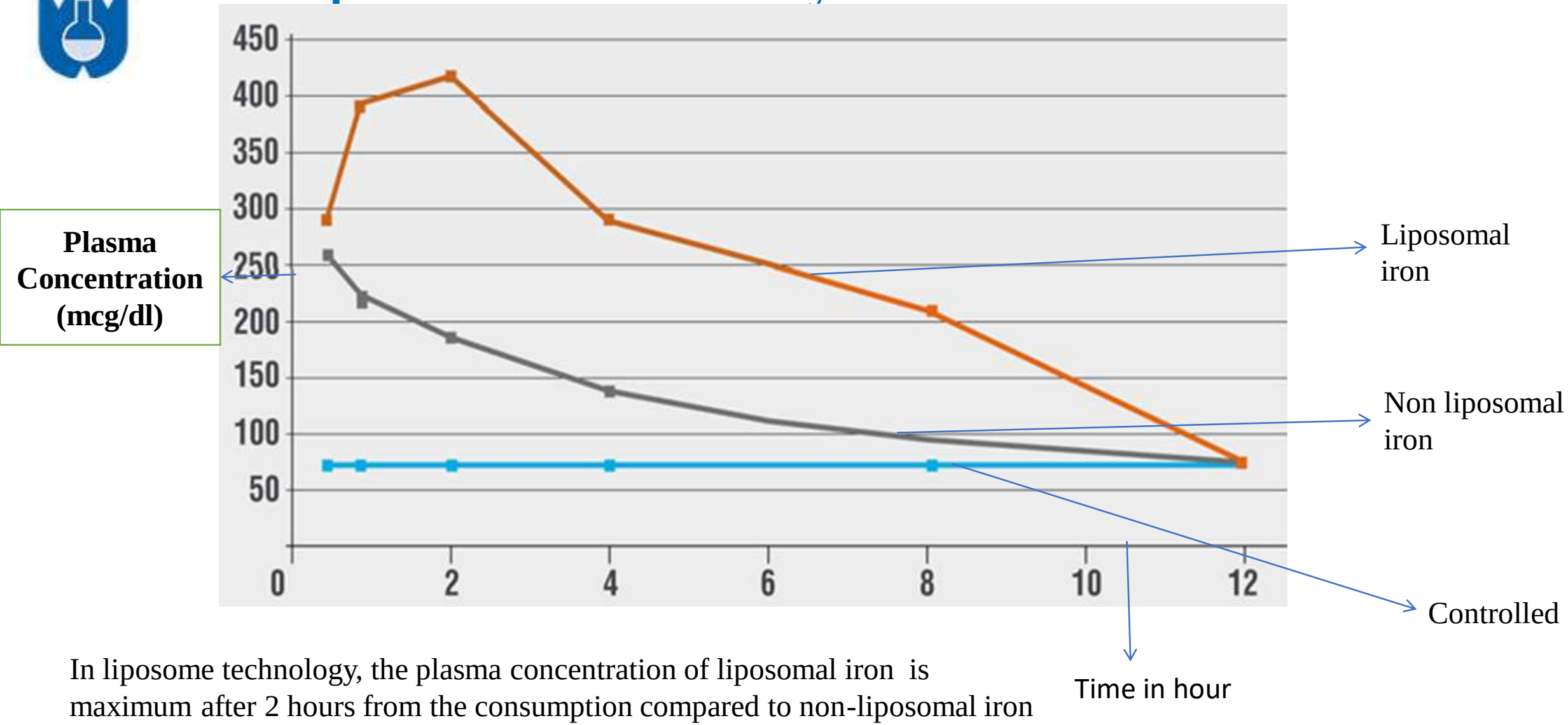
Duodenum (Small Intestine)

- Liposomes containing iron reach the small intestine, specifically the duodenum.
- In the duodenum, liposomes release iron into the intestinal lumen.

Efficient Absorption: Liposomal iron is absorbed more efficiently compared to traditional iron supplements because it bypasses gastric digestion

Bloodstream: The absorbed liposomal iron is released into the bloodstream and can be utilized by the body, similar to iron obtained from a normal diet.

Absorption and Bioavailability



In liposome technology, the plasma concentration of liposomal iron is maximum after 2 hours from the consumption compared to non-liposomal iron which guarantees greater bioavailability of the element for all metabolic processes

UNIQUE FEATURES OF LIPOSOMAL IRON

- **Higher bioavailability** compared to traditional iron supplements.
- **Better absorption** than traditional iron so low dose is required for absorption.
- Liposomal Iron can be given in **low dose** as **the absorption is significantly higher(30-35%)** compare to conventional iron (**absorption 10%).**
- Since it is Liposomal Iron ,it can be given with other nutrients ,hence no contraindication compare to conventional iron.
- **No digestive tract irritation** (even at high dose).
- Liposomal iron is almost devoid of side effects like-Gastric irritation, Nausea ,Constipation etc.
- Iron supplements can interfere with the absorption of other nutrients like calcium, zinc, and magnesium. Liposomal iron have a reduced impact on the absorption of these nutrients, making it a more **versatile option**.
- Iron can promote oxidative stress in the body, which can be harmful. Liposomal delivery may help mitigate this risk by **minimizing the release of free iron ions that contribute to oxidative damage**.

Different Forms of Liposomal Iron (Fe – 78-90mg/g)

Normal / Regular

Soluble

DC Granules

Effervescence Granules

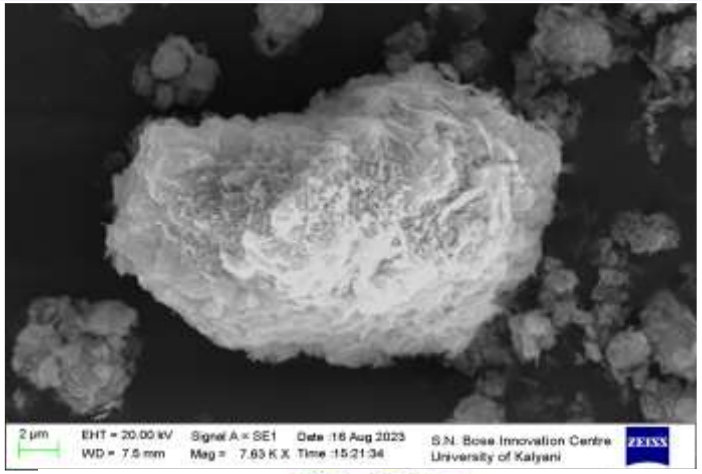
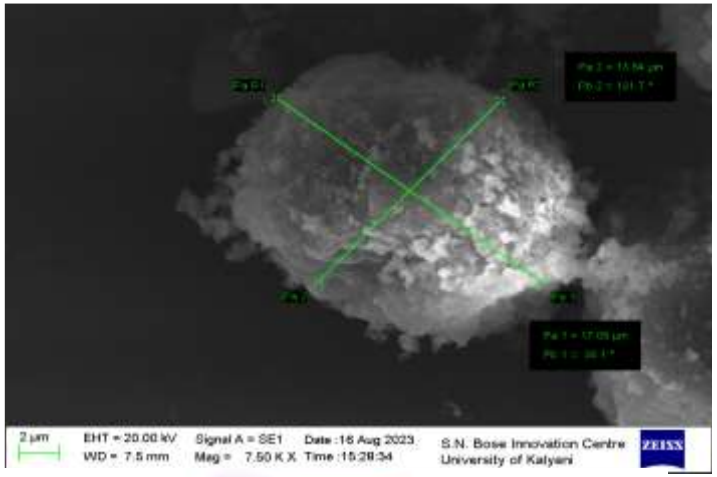
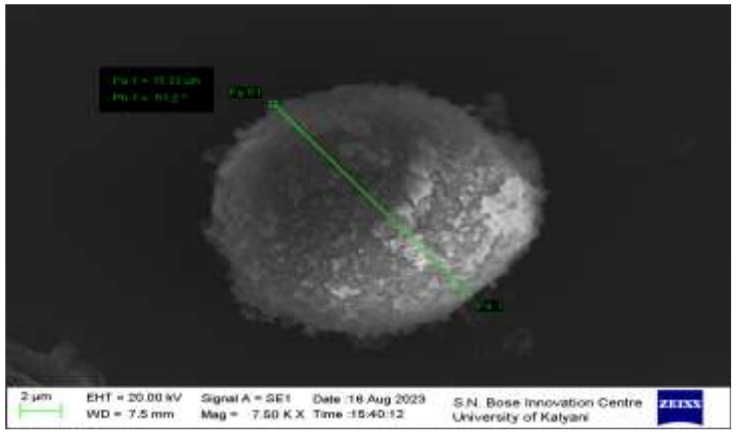
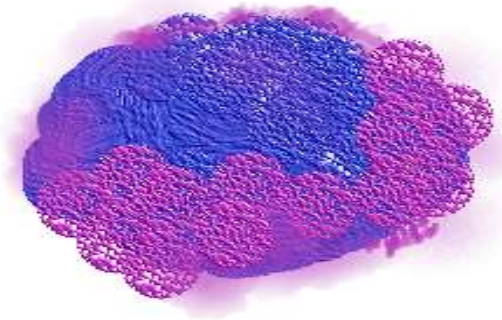
Sublingual Tablet

Suspension

LIPOSOME CHARACTERISATION AS PER GUIDANCE FOR INDUSTRY

- Morphology of the liposome- Available
- Surface characteristics of the liposomes, as applicable- Available
- Net charge, typically measured as zeta potential of the liposomes- Available
- Parameters of the contained drug (drug encapsulation efficiency, liposome drug loading)- Available
- Particle size- Available
- Leakage rate of drug from the liposomes throughout shelf life- Available
- Liposome integrity changes (e.g., drug release, drug encapsulation efficiency, liposome drug loading, size) in response to changes in factors such as salt concentration, pH, temperature, or addition of other excipients, as applicable- Available
- Liposome structure supported by spectroscopic or other analytical method(s)- Available

Morphology ,Surface Characteristics & Spectroscopic view of Liposomes

	Raw Material (Insoluble grade Ferric Pyrophosphate)	2% Soy Lecithin Coating	10% Soy Lecithin Coating
SEM Magnification on Single Particle view. 7500X magnification view			
2D representation			
	4 µm to 7 µm	12 µm to 13 µm	15 µm to 16 µm

Inference	Raw Material (Insoluble grade Ferric Pyrophosphate)	2% Soy Lecithin Coating	10% Soy Lecithin Coating
Particle size	Particle size was observed in a range of 4 µm to 7 µm	Particle size was observed in a range of 12 µm to 13 µm	Uniform Particle size was observed in a range of 15 µm to 16 µm
Particle shape	Uncoated Crystals	Few Spherical particles, less uniform consistency	Large number of uniform spherical particles, more homogeneous consistency
Encapsulation	Not Applicable	Fewer particles observed to be encapsulated, increase in particle size indicates coating is happening	More particles observed to be encapsulated, increase in particle size is indicative of coating. Random particles were checked for its size and observed a uniform ~15 µm particle size through out the batch

Reference: Drug Design, Development and Therapy 2016:10

Zeta Potential data (ICH parameter no: 3)

Batch	pH 7.0
10% Coating	-31.97

Acceptance criteria: ± 30

Inference:

1. The characterization of the surface charges property of microspheres is determined by measuring zeta potential.
2. The higher value of zeta potential implies a greater colloidal stability and results in inhibiting the aggregation of liposomal formulation.
3. It is reported that microspheres with a zeta potential above (+/-) 30 mV show stabilization in suspension.
4. Zeta potential value for our products at pH 7.0 represents a stable Liposomal Product.

Reference: Olga Popovska et al / Int. J. Pharm. Phytopharmacol. Res. 2013; 3 (3): 182-189

Encapsulation efficiency of Liposomal iron

Product	Encapsulation efficiency	Elemental Iron Assay
Liposomal Ferric Pyrophosphate	89.01%	8.12%

Acceptance criteria: 7.8% to 9.0 % of Elemental iron

Acceptance criteria: NLT 85% Encapsulation efficiency

Ref: USP Food Chemical Codex

Particle Size study (ICH parameter no: 06)

S.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
8	**Mesh#325**	**5.22**	**1.34**	**74.35**

Inference:

1. Within a certain range of particle size, encapsulation efficiency decreased with the reducing of the particle size.
2. It was shown that particle size had little influences on encapsulation efficiency when microspheres were in the size range of 18 - 200 mesh, but when the particle size was 325 mesh the encapsulation efficiency tend to decrease.
3. That means irrespective of particle size, Encapsulation efficiency is more than 85% within 18-200 mesh(1000 micron - 75 micron sieve size).

Reference: X. FU, Q. PING, & Y. GAO, 2005, Effects of formulation factors on encapsulation efficiency and release behaviour in vitro of huperzine A-PLGA microspheres. Journal of Microencapsulation 22(7): 705–714

Leakage rate of drug from the liposomes (shelf life period)

Accelerated Stability Data

Stability Condition: Accelerated Stability Study

Storage /Test Conditions
Temperature:40°C ± 2°C75%
Related Humidity: 75% ± 5%

Test Result: 6 month
Acceptance criteria: 7.8% to 9.0 % of Elemental iron
Acceptance criteria: NLT 85% Encapsulation efficiency

Liposomal Ferric Pyrophosphate

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

Integrity

Liposomal Ferric Pyrophosphate insoluble is coated first with an inert material. This creates a protective layer over the API, which inhibits any further interaction with other ingredients.

Further Lecithin coating is performed to prepare the Liposomal Iron.

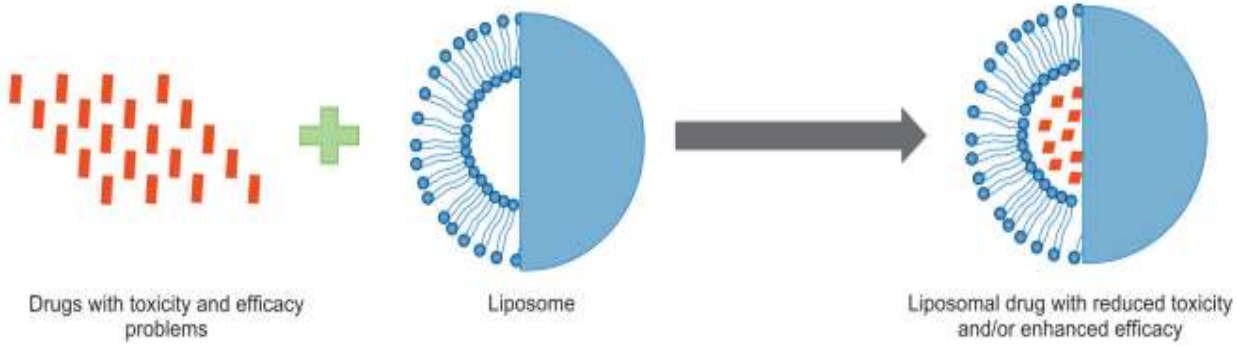
Reference: Liu, P.; Chen, G.; Zhang, J. A Review of Liposomes as a Drug Delivery System: Current Status of Approved Products, Regulatory Environments, and Future

Liposome integrity changes (e.g., drug release, **drug encapsulation efficiency**, liposome drug loading, size) in response to changes in factors such as salt concentration, **pH**, temperature, or addition of other excipients, as applicable

Drug Loading Capacity

Drug Loading	**0.71 mg per mg of Liposomal Product**

% Drug loading (mg/mg) = (Mass of FePP in LiFePP / Mass of LiFeP recovered) x 100



Formulation of drugs in liposomes

Reference: Acta Poloniae Pharmaceutica ñ Drug Research, Vol. 67 No. 3 pp. 283ñ290, 2010

Ref: USP Food Chemical Codex

References

- Slide 3- Maladkar, M., Sankar, S., & Yadav, A. (2020). A novel approach for iron deficiency anaemia with liposomal iron: concept to clinic. Journal of Biosciences and Medicines, 8(09), 27.
- Slide 4- Hussain, U., Zia, K., Iqbal, R., Saeed, M., & Ashraf, N. (2019). Efficacy of a novel food supplement (Ferfer®) containing microencapsulated Iron in liposomal form in female Iron deficiency anemia. Cureus, 11(5).
- Slide 5- Pisani, A., Riccio, E., Sabbatini, M., Andreucci, M., Del Rio, A., & Visciano, B. (2015). Effect of oral liposomal iron versus intravenous iron for treatment of iron deficiency anaemia in CKD individualss: a randomized trial. Nephrology dialysis transplantation, 30(4), 645-652.
- Slide 6- Hussain, U., Zia, K., Iqbal, R., Saeed, M., & Ashraf, N. (2019). Efficacy of a novel food supplement (Ferfer®) containing microencapsulated Iron in liposomal form in female Iron deficiency anemia. Cureus, 11(5).
- Slide 7- Xu, Z., Liu, S., Wang, H., Gao, G., Yu, P., & Chang, Y. (2014). Encapsulation of iron in liposomes significantly improved the efficiency of iron supplementation in strenuously exercised rats. Biological Trace Element Research, 162, 181-188.
- Slide 8- Baomiao, D., Xiangzhou, Y., Li, L., & Hualin, Y. (2017). Evaluation of iron transport from ferrous glycinate liposomes using Caco-2 cell model. African health sciences, 17(3), 933-941.
- Slide 9- Parisi, F., Fusè, F., Brunetti, M., Mazzocco, M., Berti, C., & Cetin, I. (2017). Effects of different regimens of iron supplementation on iron status and pregnancy outcomes in a cohort of healthy pregnant women: a randomized control trial. The Journal of Maternal-Fetal & Neonatal Medicine, 30, 1787-1792.

THANK YOU