



LipsoBio



Liposomal Vitamin C

Dr. Shiv Ph.D.

(Dr H N Shivaprasad)

Global Director – Business Development
Botanic Healthcare Pvt Ltd, INDIA

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TEM and Cryo TEM analysis

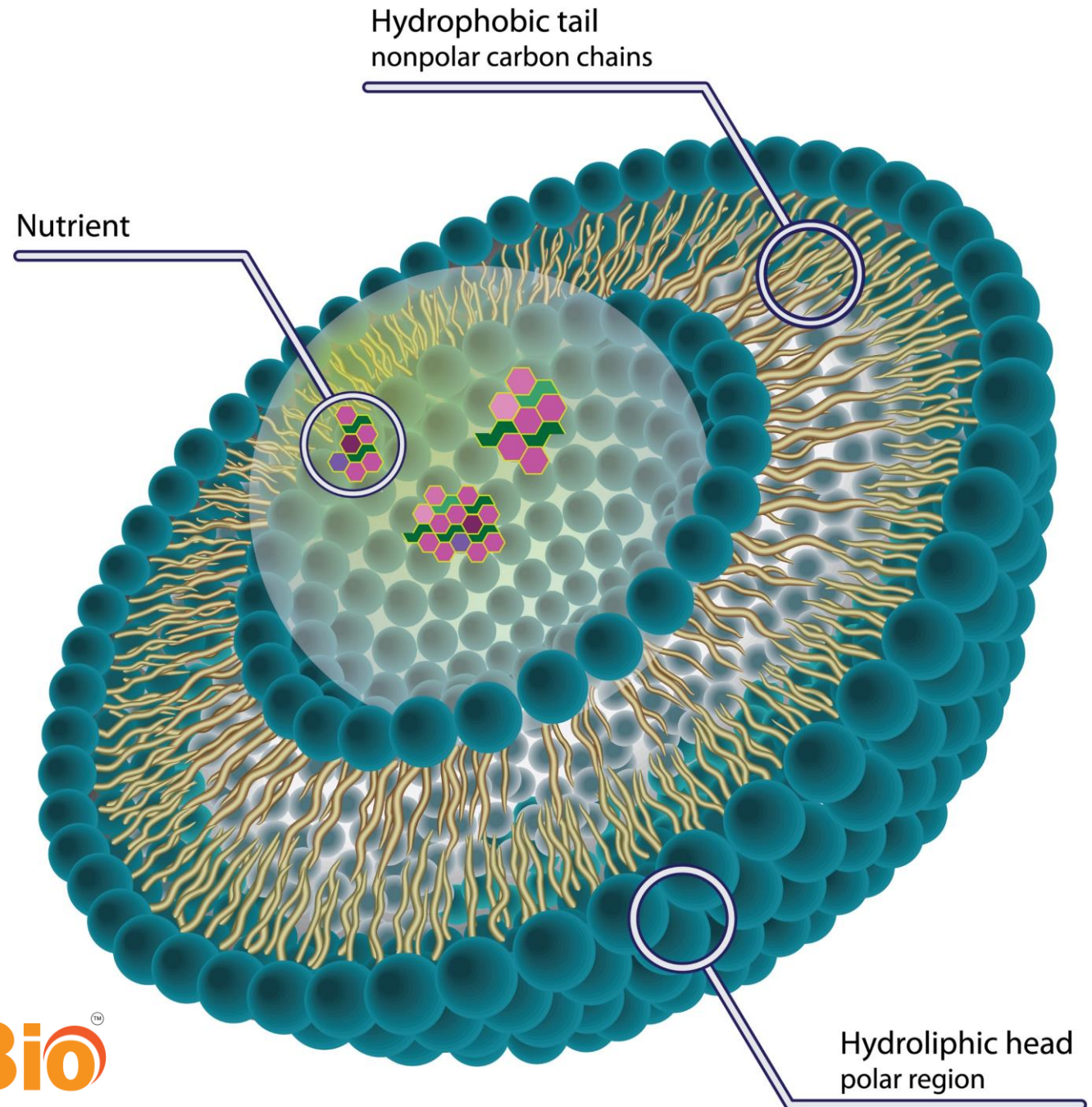
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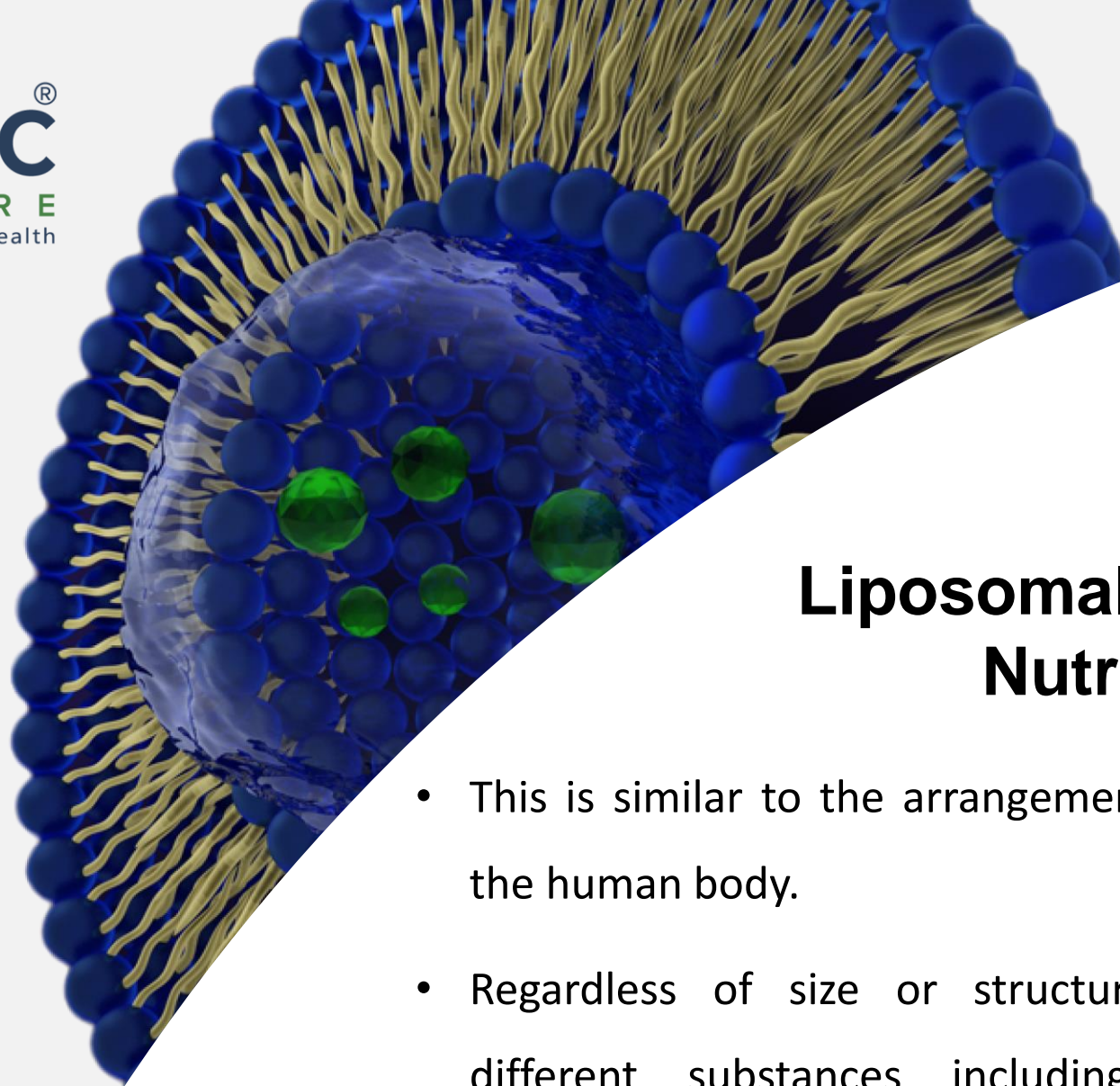
**Botanical Healthcare Capabilities
in Liposomal Technique**



Liposomes

Liposomes are lipid vesicles made of phospholipids linked together to form a double membrane





Liposomal Botanicals and Nutraceuticals

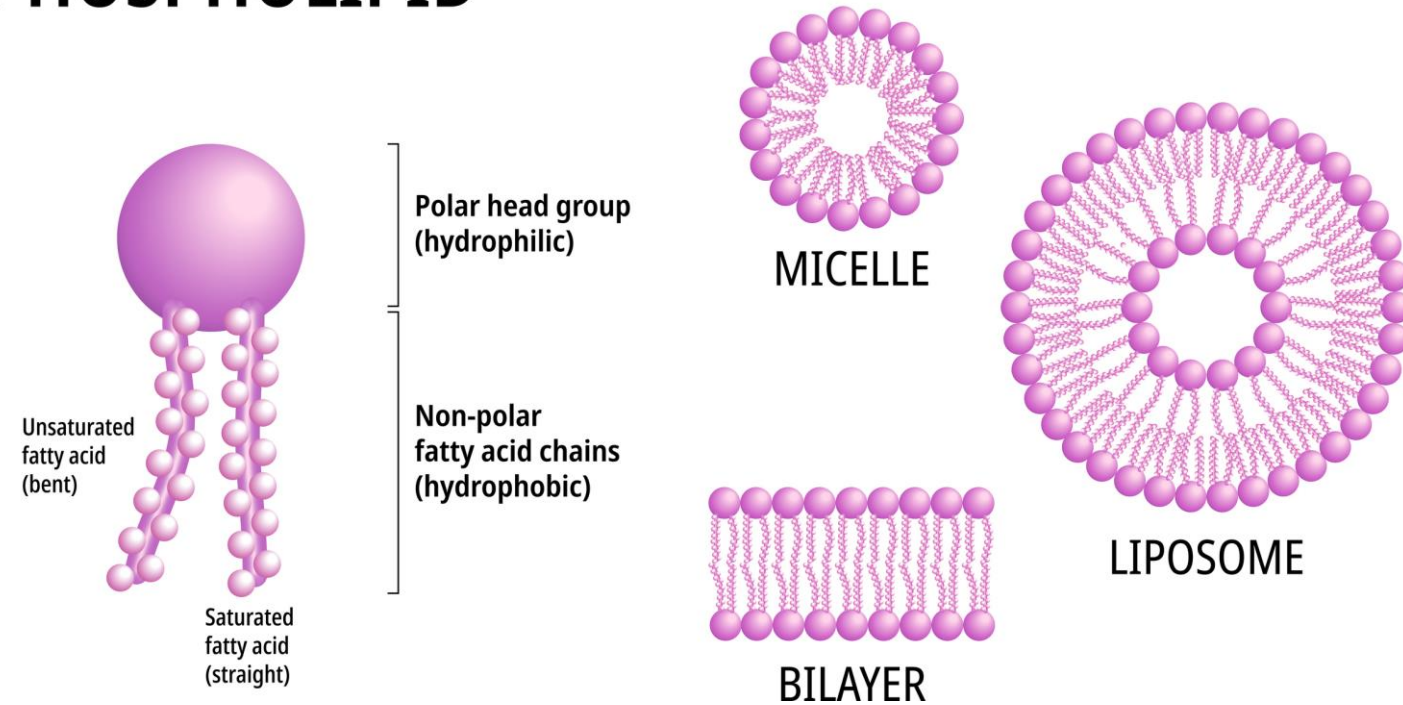
- This is similar to the arrangement in the cell membranes of the human body.
- Regardless of size or structure, liposomes can enclose different substances including botanical extracts and nutraceutical ingredients.

Liposomes

Liposomes brought a very interesting proposition into lipid deliveries: the phospholipids arranged into spherical cell membrane–like lipid bilayers with their water-loving “heads” toward the aqueous medium and their fatty “tails” tucked toward each other.

These structures created pockets of entrapped water along with water soluble compounds and the potential to protect them from hostile digestive conditions and potentially facilitating gastrointestinal (GI) uptake.

PHOSPHOLIPID



Advantages

Advantages of Liposomal Delivery

- High bioavailability and absorption compared with other oral forms of supplements.
- Micronized encapsulation protects against the harsh environment of the GI tract and increases transmucosal (oral) uptake and absorption.
- Increased intracellular delivery.
- Can hold both hydrophilic and hydrophobic compounds

Liposomes composition

The most frequently used phospholipids for liposomes preparation are

- phosphatidylcholines (PC),
- phosphatidylethanolamines (PE),
- phosphatidylserines (PS) and
- phosphatidylglycerols (PG).

Liposomes preparation and characterization



Conventional methods:

- Film hydration method
 - Reverse phase evaporation method
 - Solvent injection method
 - Detergent removal method
 - Heating method
1. Microfluidic channel method
 2. Supercritical fluidic method
 3. Post-preparation treatments
 - Freeze-thawing
 - Freeze-drying



Novel preparation methods:

Liposomal Vitamin C from Botanic Healthcare

- Vitamin C is a water-soluble vitamin known chemically as L-ascorbic acid. Vitamin C supplements are the most commonly used supplement in the world. Vitamin C plays a critical role in supporting the immune system and in helping to protect the joints and arteries from the damaging effects of free radicals, through its actions as a powerful antioxidant
- The majority of Vitamin C products are either desiccated tablets or effervescent products. While these products generally contain 1g of Vitamin C, the body only absorbs about 7-8% of the total amount. This means that for every gram of Vitamin C that is ingested, only 70-80mg is absorbed, and plasma Vitamin C levels stay relatively low.
- Liposomal Vitamin C provides a well-tolerated, easily absorbed and highly bioavailable form of Vitamin C without any unpleasant side-effects.

Liposomal Vitamin C from Botanic Healthcare

- While numerous clinical studies back the benefits of taking vitamin C, traditional oral pills and powders provide limited absorption. This is because, when traditional vitamin C is taken, this nutrient has to contend with stomach acid and enzymes before finally reaching your small intestine, where there is actually very little vitamin C leftover for absorption into cells.
- This is why liposomal vitamin C is beneficial. Because of its unique encapsulation technology, liposomal vitamin C is simply more bioavailable than other forms of vitamin C, making it the superior option.

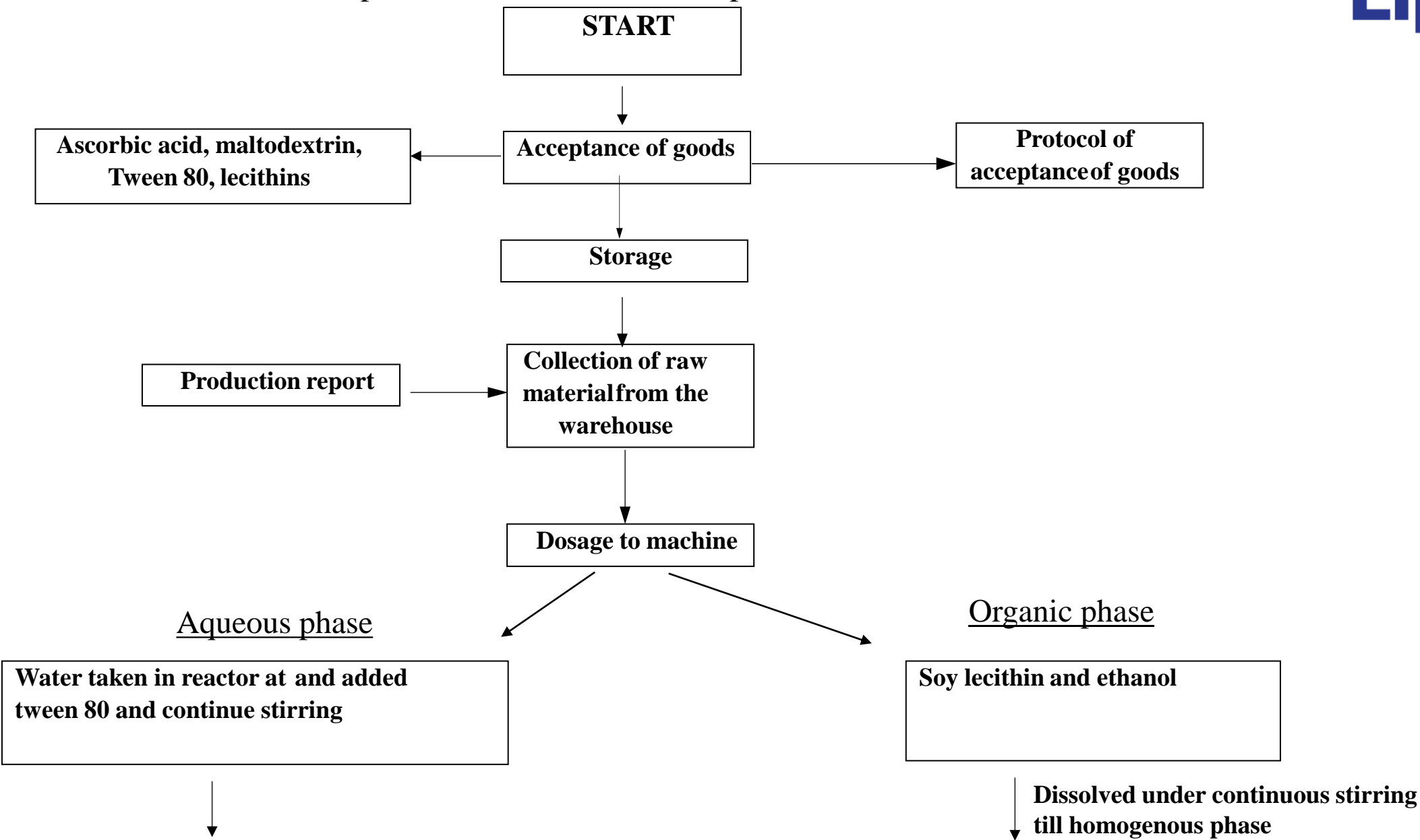
Stability of LipsoBio Liposomal Vitamin C

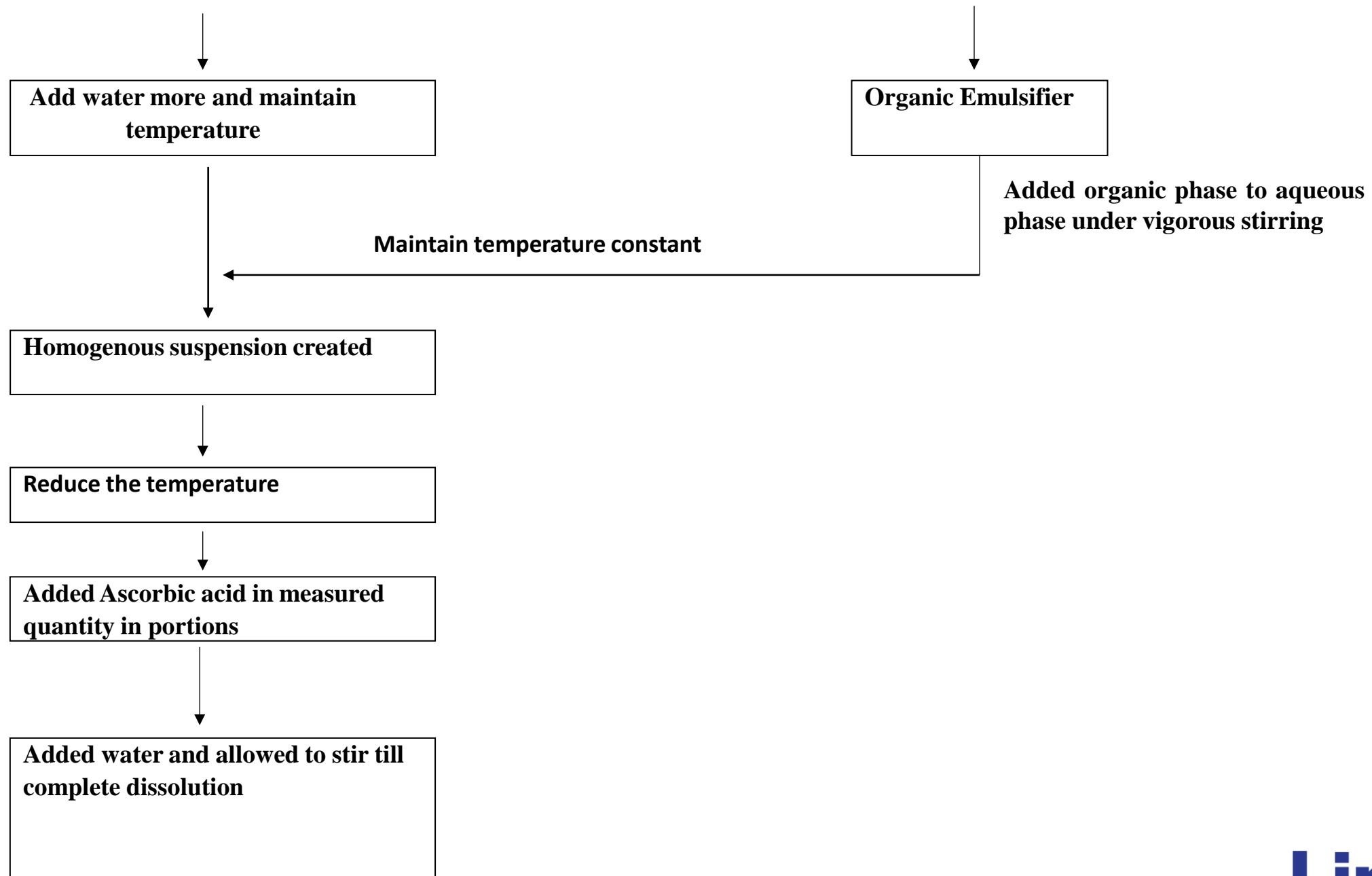
Stability of stored liposomes under different climatic conditions.

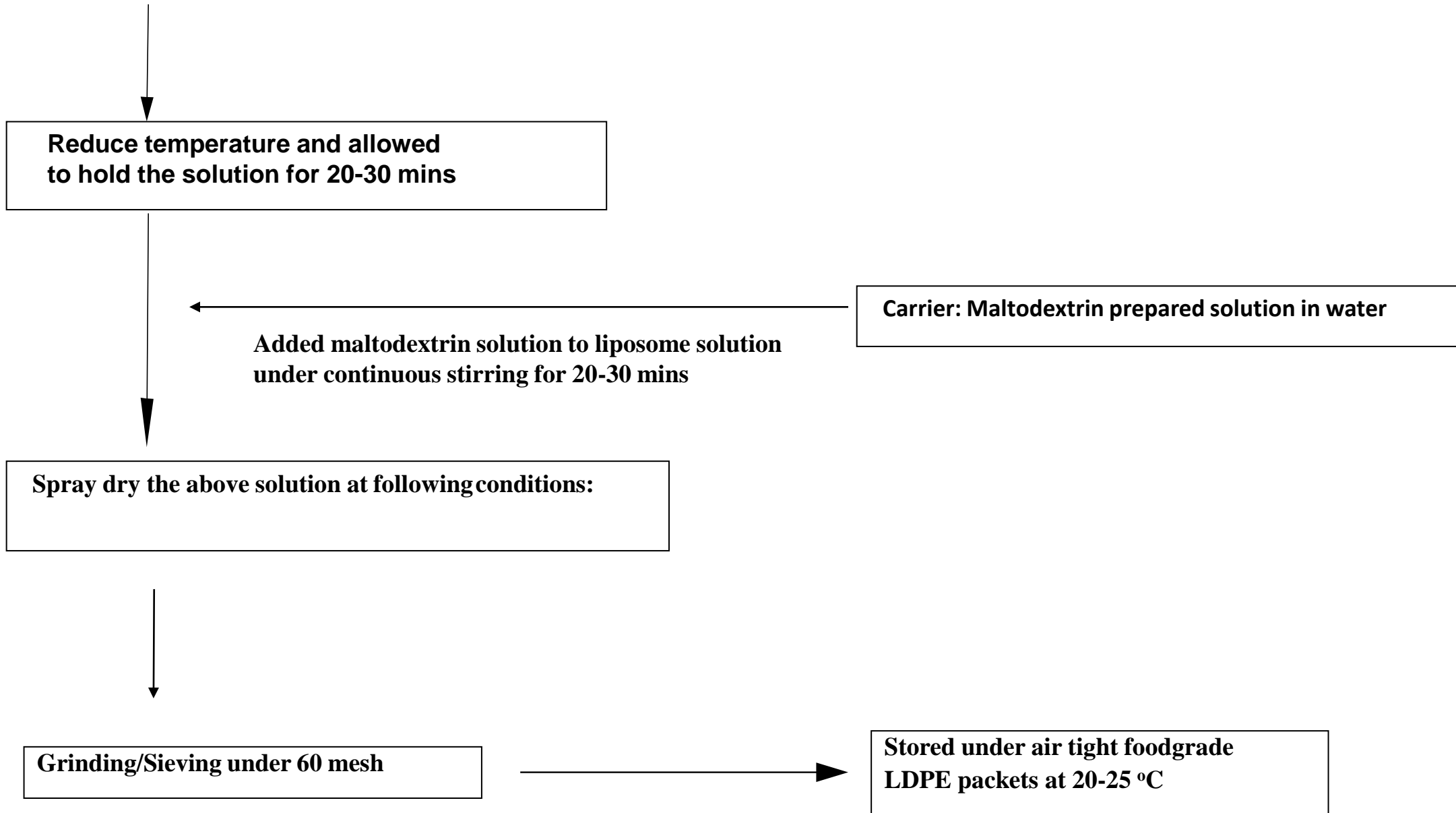
- refractive index,
- average vesicle diameter,
- zeta potential,
- pH,
- content,
- encapsulation efficiency (EE%),
- morphology,
- stability and
- antioxidant activity.

Climatic conditions (4 °C, 25 °C and 40 °C) for 30 days.

Liposomal Vitamin C 70% Preparation flowchart



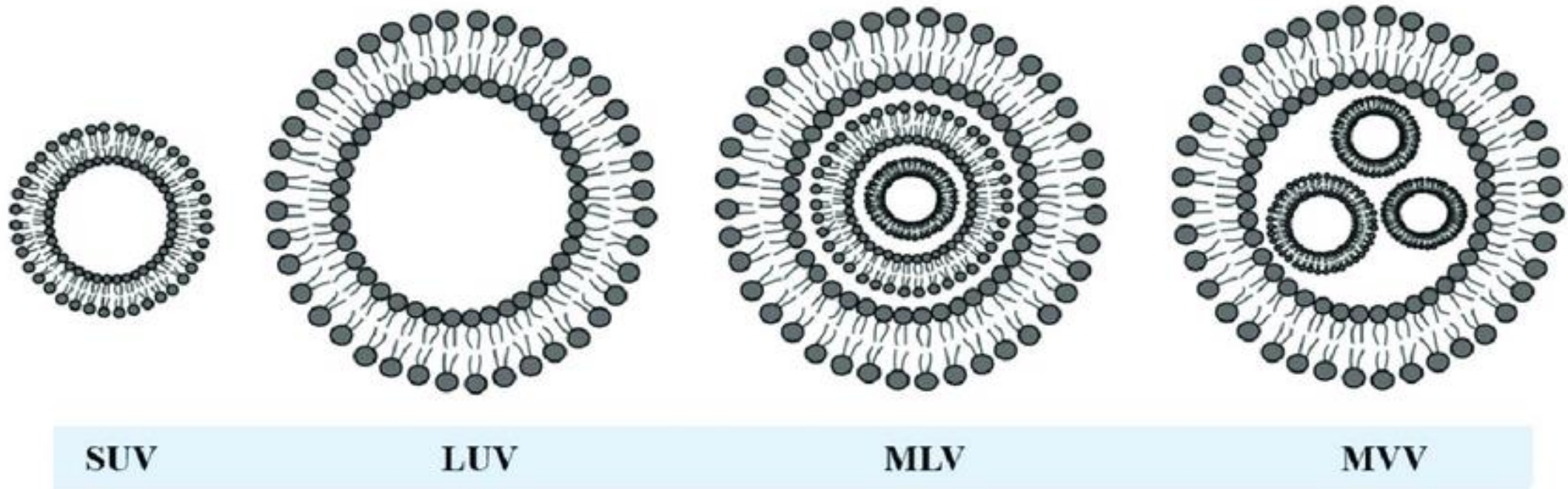




Liposomal Vitamin C & Transmission Electron Microscopy (TEM)

Morphological analysis of liposomes was performed by transmission electron microscopy (TEM). TEM is an important method for the characterization of size and shape of nanoparticles as it can directly visualize single particles

Although subject to artifacts, negative staining TEM can also be a useful method to image liposomes, as it is faster and simpler than cryo-EM, and requires less advanced equipment. When it comes to TEM, we can understand the particle size and shape, where with cryo-TEM along with the above lamellarity can also be evaluated.



According to the liposomes structures, they are classified into four categories based on size and number of bilayers: small unilamellar vesicles (SUV), large unilamellar vesicles (LUV), multilamellar vesicle (MLV), and multivesicular vesicles (MVV).

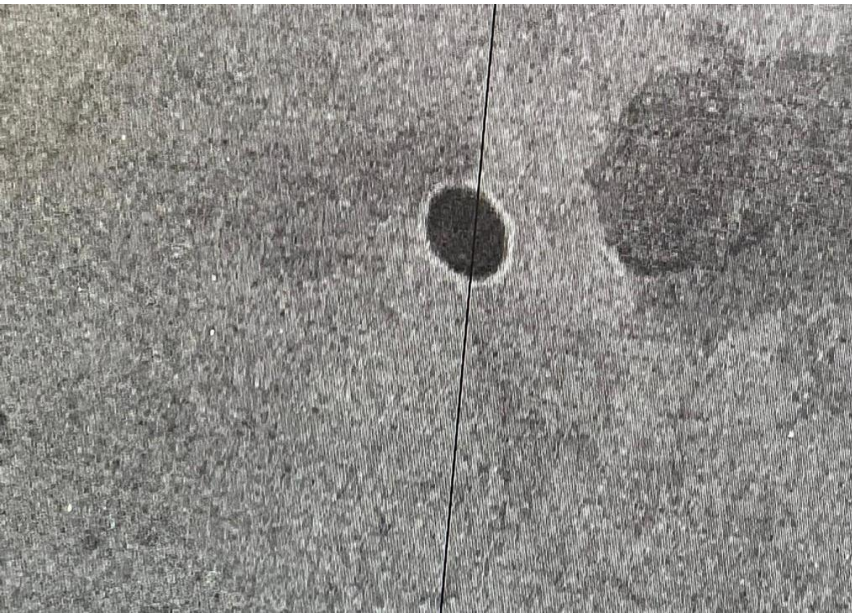
TEM Analysis

TRANSMISSION ELECTRON MICROSCOPY

Botanic Healthcare Samples
were analyzed in TEM

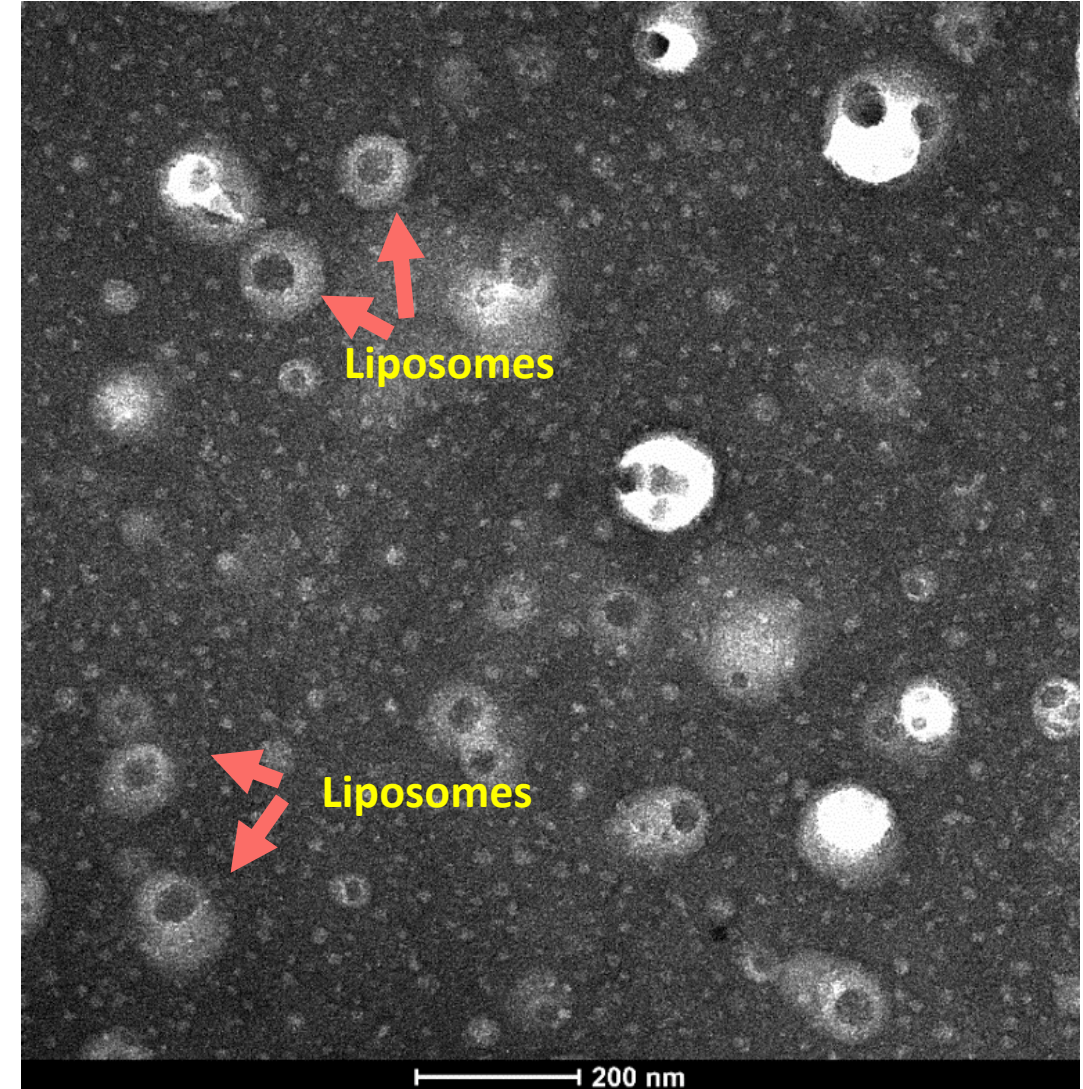
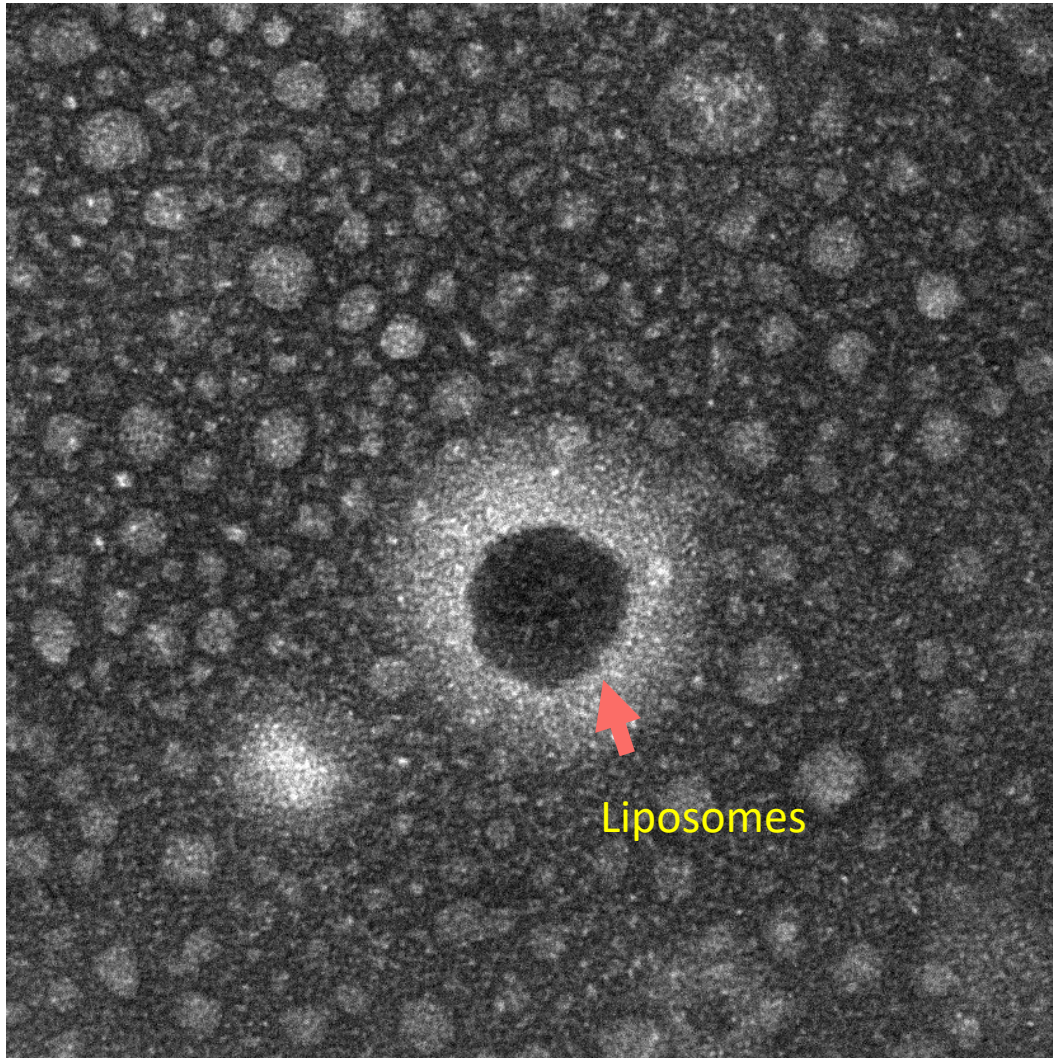


Sample Preparation GRID



Commercial lot evaluation in TEM

Morphology by TEM



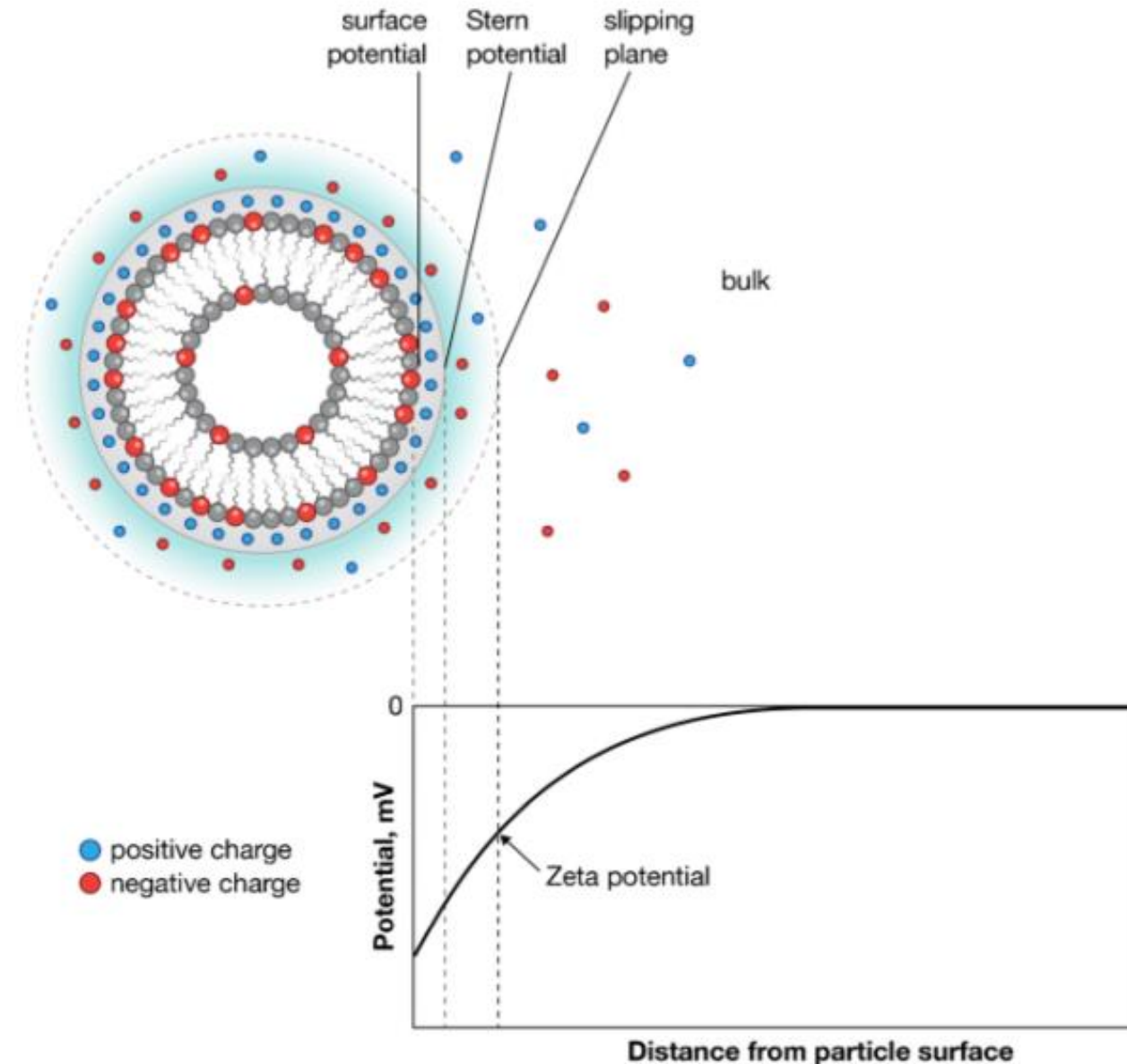
Morphology by TEM

- The TEM imaging results show that the liposomes were spherical in size
- Mean particle size of the liposomes, which was anticipated to be near 200 nm for liposomal vitamin C
- It is generally recognized that the size of liposomes has a significant role on tissue cell targeting
- Images of liposomal vitamin C indicate that the liposomes were bilayer (unilamellar) vesicles, where the average sizes ranged between 100 nm to 200 nm.

Size and Zeta Potential

- The particle size and zeta potential of the Lipsobio Vitamin C were measured using Dynamic Light Scattering Spectrometer 'Malvern' Zetasizer Nano ZS System

The sample size distribution was obtained by measurement of scattered light by particles (dynamic light scattering, DLS) illuminated with a laser beam. Zeta potential was established by an electrophoretic light scattering method based on the Doppler effect. Zeta potential charge values were derived from triplicate measurements, where each consisted of a minimum of ten individual runs.



The zeta potential of a particle is the overall charge that a particle acquires in a particular medium, it has been defined as the potential at the hydrodynamic shear boundary. Larger zeta potentials predict a more stable dispersion, which means that all the particles in suspension will tend to repel each other thus preventing aggregation. Normally, particle suspensions with zeta potentials $> +30$ mV or < -30 mV are considered stable. Measurement of liposomes zeta potentials can provide insight about their stability, circulation times, protein interactions, particle cell permeability, and biocompatibility.

Liposomal Glutathione

Liposomal Glutathione: Glutathione is the master antioxidant in the human body. Structurally, glutathione is a tripeptide made up of **cysteine, glycine and glutamic acids**. There are two forms of glutathione as oxidized and reduced forms. Reduced form is the active form and potent antioxidant. The problem with reduced glutathione is it gets depleted by stomach acids. Hence, its absorption is pretty low. Therefore, alternative forms such as liposomal glutathione is the best solution. Liposomal glutathione is an active form of glutathione. The encapsulation process protects the glutathione molecule from stomach acids. It also increases the absorption by up to 80%.

Cryo - TEM

- In comparison to staining, cryo-TEM images the sample in its most native state. The sample is made to undergo vitrification in a thin layer of solvent and imaged at very low temperatures, such that the medium neither changes phase nor evaporates in the high vacuum. The thin layer is made by applying and subsequently blotting a drop of sample on carbon coated grid. Vitrification of water is achieved by very rapid cooling, usually by plunging the sample into liquid ethane cooled to its melting point.
- This technique allows for the analysis of liposomes in their most native state, and is a valuable tool to determine the size, shape, internal structure, and lamellarity of liposomes.

Cryo - TEM

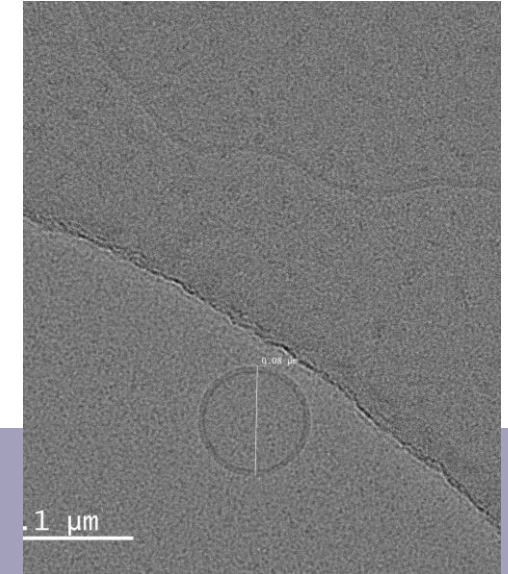
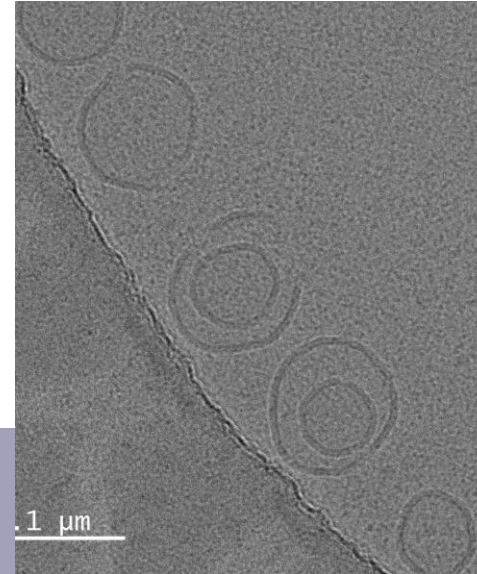
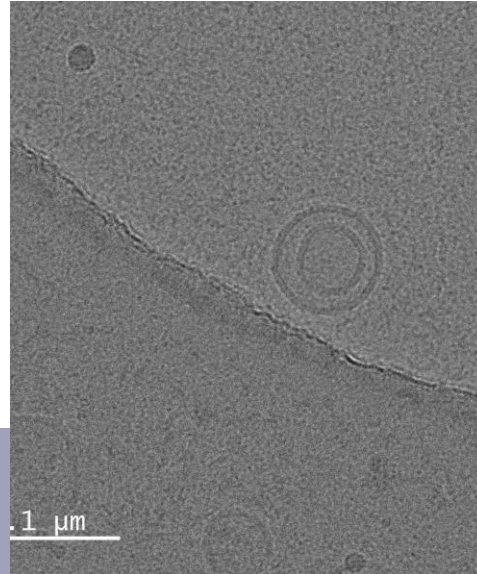
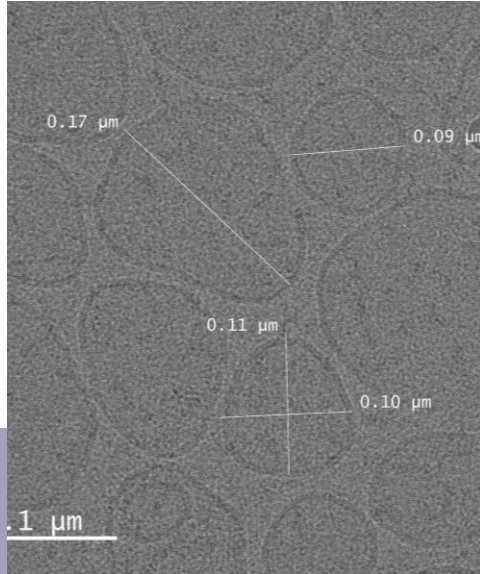
- The major advantage of rapidly freezing liposome samples is minimizing the formation of ice crystals and preserving proteins or other materials. Cryo-TEM is the most useful form of microscopy currently available to study liposomes, as it avoids issues with chemical fixation, dehydration, cutting and staining — all of which can affect the morphology of vesicles.

Cryo – TEM Images from Indian Institute of Science, Bangalore, INDIA

Sample : Liposomal Glutathione
Batch : BHC/LNB/022/136A

Indian Institute of Science, Bangalore

ADVANCED CENTER FOR CRYO-ELECTRON MICROSCOPY FACILITY



Liposomal Multivitamin and Minerals

Multivitamin/mineral (MVM) supplements: A particularly notable development influencing the physical form of products and potentially altering the bioavailability of nutrients within MVMs is the introduction of liposomal delivery mechanisms, in which nutrients are packaged in liposomes to promote enhanced absorption and bioavailability. Several investigations support the ability of liposomal packaging to improve vitamin absorption. However, few studies have examined the influence of liposomal packaging on mineral absorption, particularly within the context of an MVM product.



High Speed Homogenizer





Controlled Spray Drying



- Liposomal Vitamin D3 powder
- Liposomal Vitamin E
- Liposomal Vitamin C
- Liposomal Mix for Kids

- Liposomal Mg powder
- Liposomal Zn powder (Citrate)
- Liposomal iron Fe (bisglycinate)

Liposomal based vitamins and minerals



- Boswellia extract
- Curcumin extract
- Salacia extract
- Lutein extract
- Milk thistle extract



An aerial photograph of the Botanic Healthcare Processing Facility. The facility consists of several large, modern industrial buildings with white walls and blue and green vertical stripes. The roofs of the buildings are covered with solar panels. A tall, slender smokestack or chimney is visible in the center of the facility. The buildings are surrounded by lush green trees and landscaping. A paved road runs along the bottom left of the facility, and a gravel area is visible on the right. The entire facility is enclosed by a white fence. The background shows a vast green field under a clear sky.

Botanic Healthcare Processing Facility





BHC Certifications



GMP Certified by
TUV SUD South Asia



HACCP Certified by
TUV SUD South Asia



GMP Registered
Dietary Supplements



“ Get in Touch with us

INDIA

Botanic Healthcare Pvt. Ltd.

Corporate Office / R&D Center
Plot No. 16/1/12 & 13, TSIIIC IDA,
Nacharam, Mallapur Road,
Hyderabad, Telangana 500076, INDIA
Phone: + 91 4035027800
Email : info@botanichealthcare.net
Branch offices: Bangalore | Noida

USA

Botanic Healthcare LLC

55 Crestview Dr Clinton
New Jersey 08809 USA
Cell: +1 908 399 5452
Phone: +1 908 617 9152
Email : bdm@botanichealthcare.net

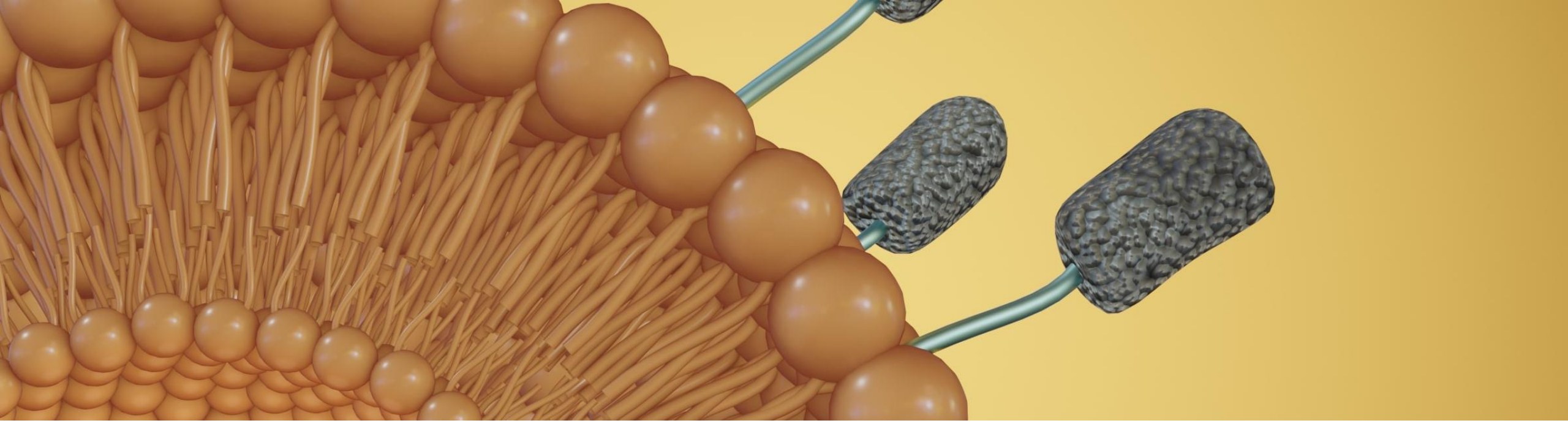
NEW ZEALAND

Botanic Healthcare (NZ) Ltd.

1/243 Cambridge terrace, Christchurch
Central city, 8013 New Zealand
Cell: +64-211 551 894
Email : info@botanichealthcare.net



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Thanks