NIOSOMES, AQUASOMES, PHYTOSOMES AND ELECTROSOMES.

CONTENTS

NIOSOMES

DEFINITION

- Niosome are **non-ionic surfactant vesicles** obtained on hydration of synthetic nonionic surfactants with or without incorporation of cholesterol or their lipids.
- They are **structurally similar to liposomes in having a bilayer** however, the materials used to prepare niosomes make them **more stable** and thus niosomes offer many more advantages over liposomes.
- The sizes of niosomes are microscopic and lie in nanometric scale.
- The particle size ranges from **10nm-100nm.**

STRUCTURE OF NIOSOME

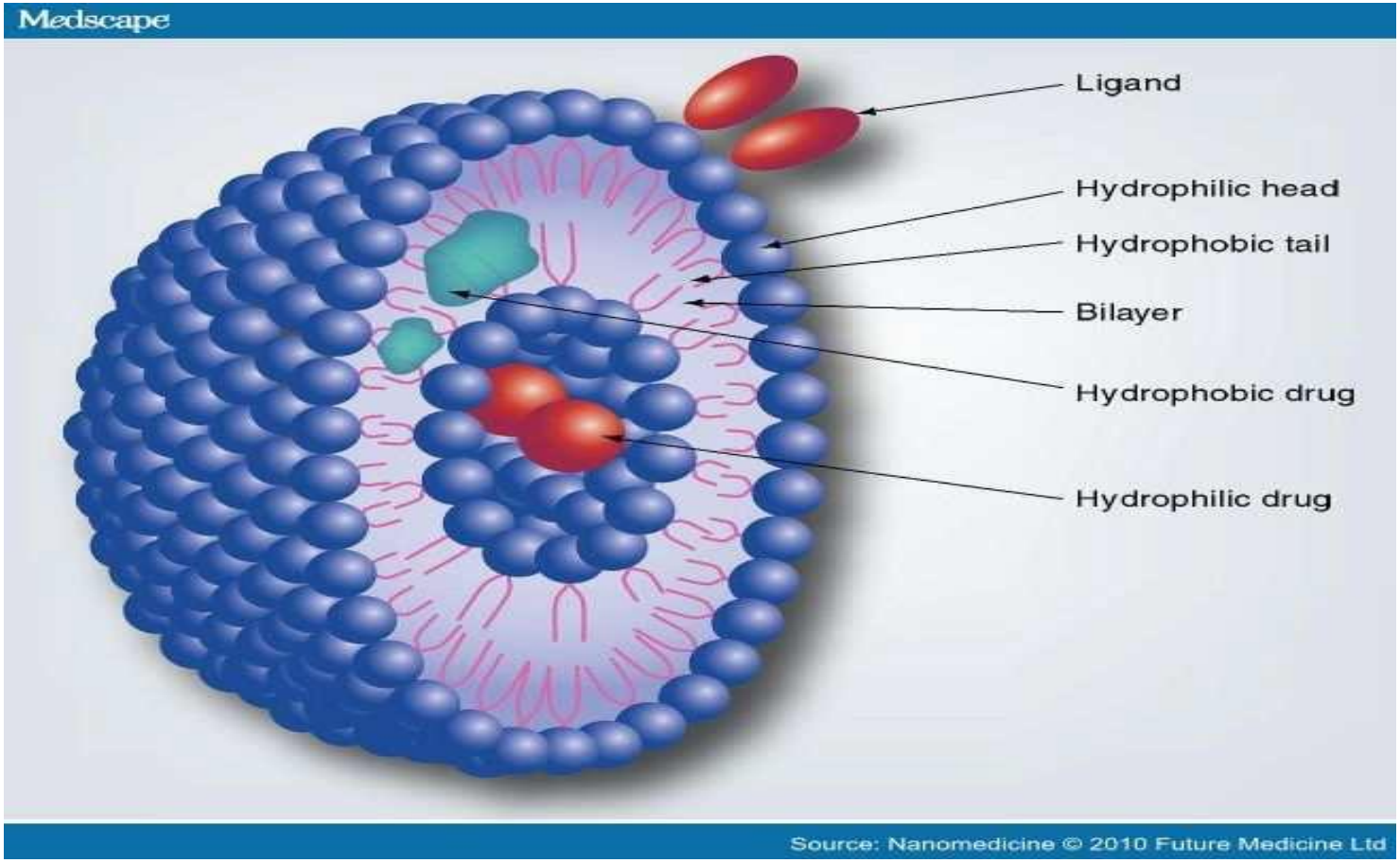

Figure-1 Structure of Niosomes

ADVANTAGES

1. The vesicle suspension being water based offers greater **Patient Compliance** over oil based systems.
2. Since the structure of the Niosome offers place to accommodate **hydrophilic, lipophilic as well as amphiphilic drug moieties,** they can be used for a variety of drugs.
3. The characteristics such as **size, lamellarity** etc. of the vesicle can be varied depending on the requirement.
4. The vesicles can act as a **Depot** to release the drug slowly and offer a controlled release.
5. Due to their ability to entrap both hydrophobic and hydrophilic drugs, niosomes are reported as ideal carriers for the delivery of drugs such as doxorubicin, vaccines, insulin, siRNA and so on.

6. For nano-vesicle-based delivery systems, niosomes can be used as an alternative to liposomes and polymersomes for **chemical drug delivery**.
7. They can also provide a way for the **co-delivery** of two different kinds of drugs to achieve the desired therapeutic effects. As with liposomes and polymersomes, niosomes have some advantages such as biocompatibility, low toxicity, biodegradability, etc.
8. Niosomes may serve as good carriers for the delivery of various **Protein And Peptide Drugs,** and also show good performance in vaccine formulation and application.
9. Niosomes have been widely used as **Oligonucleotide Carriers** for the treatment of many kinds of diseases in reported studies. They can be used for the **Delivery Of Gene Materials** due to some advantages such as good chemical and physical stability, relatively smaller sizes, etc.

DISADVANTAGES

- Physical instability
- Aggregation
- Fusion
- Leaking of entrapped drug
- Hydrolysis of encapsulated drugs which limiting the shelf life of the dispersion.

COMPOSITIONS

- The major components used for the preparation of Niosomes are,

1. Non-ionic surfactants
2. Cholesterol
3. Drug
4. Ionic amphiphiles

1. **Non ionic surfactant-** are the main ingredient, rather than phospholipid. Non-ionic surfactants used in the niosomes are amphipathic, including terpenoids, polysorbates, Spans, alkyl oxyethylenes etc.
2. **Cholestrol-** The proper amount of cholesterol is added to the niosomes to achieve the most stable formulation due to its interaction with non-ionic surfactants . It plays the role of regulating the structure and flexibility of the membrane as a dependable buffer.
3. **Drug-** Both hydrophilic and hydrophobic drugs, can be encapsulated in the niosomes.
4. **Ionic amphiphiles** -used in the niosomes for three purposes: loading drugs, increasing the efficacy and enhancing stability

PREPARATION METHODS OF NIOSOMES

A. Ether injection method

B. Hand shaking method (thin film hydration technique)

C. Sonication Method

D. Micro fluidization method

E. Multiple membrane extrusion method

F. Reverse phase evaporation technique (REV)

G. Trans membranes pH gradient (inside acidic) Drug upake Process: or remote loading technique

A. Formation of Niosomes from Proniosomes

A. Ether injection method

Preparation steps:

Surfactant is dissolved in diethyl ether

↓

Then injected in warm water maintained at 60°C through a 14

gauze needle

↓

Ether is vaporized to form single layered Niosomes.

Methods of Preparation

- Ether injection:-
  - Surfactant: cholesterol solution in ether
  - 14 gauge needle
  - Size: 50 – 1000 µm

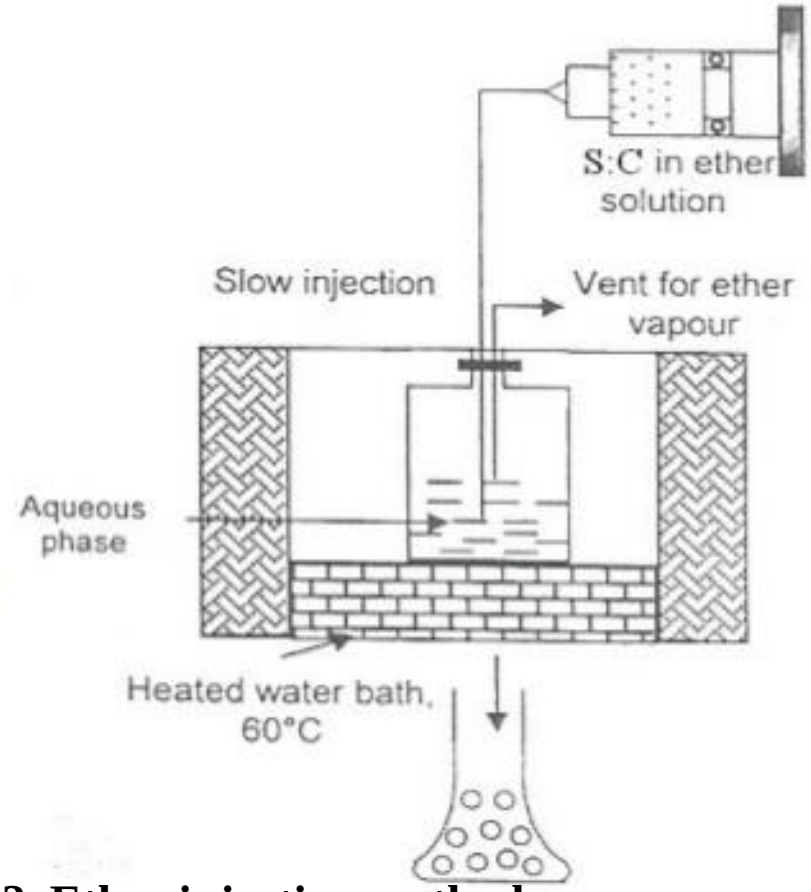

Figure 2. Ether injection method

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B. Hand shaking method (thin film hydration technique)

Preparation steps:

Surfactant + cholesterol + solvent

↓

Remove organic solvent at Room temperature

↓

Thin layer formed on the Walls of flask

↓

Film can be rehydrated to form multilamellar Niosomes.

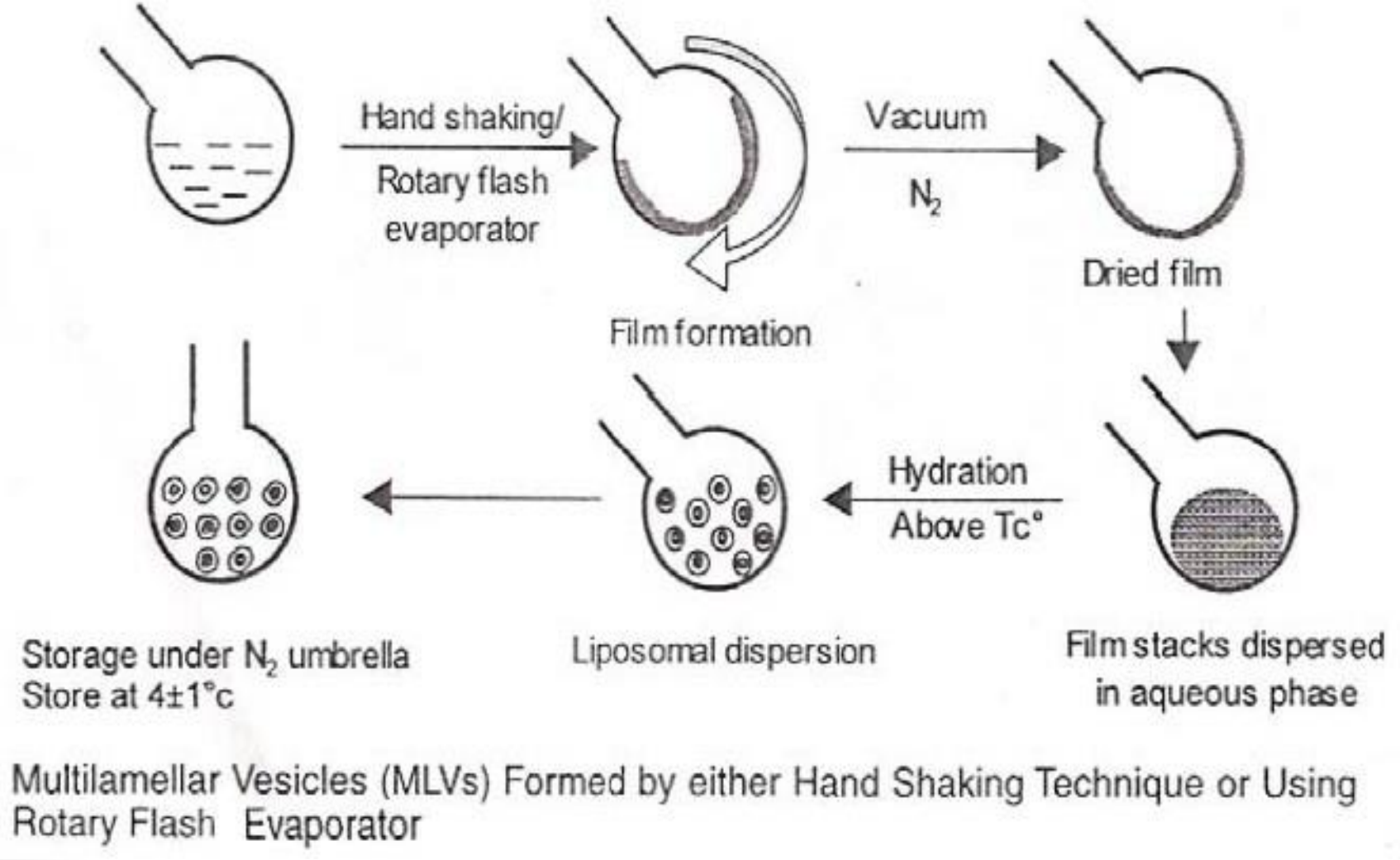


Figure 3. Hand shaking method

C. Sonication Method

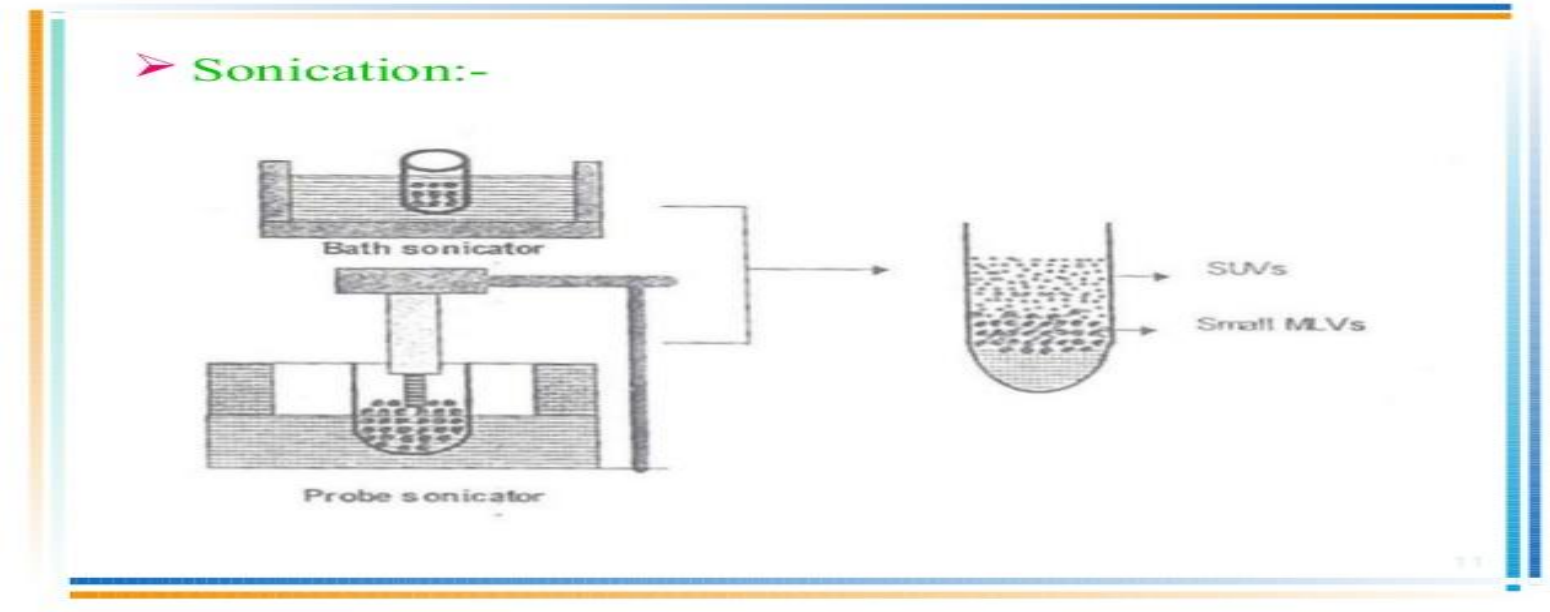
Preparation steps

Drug in buffer + surfactant/cholesterol in 10 ml

↓

Above mixture is sonicated for 3 min at 60°C using titanium

probe yielding niosomes.



- This is also a suitable way to control the particle sizes of the niosomes.
- Sonication can decrease the diameters of niosomes with narrow size distribution.
- But probe sonication involves the use of high levels of energy, and may cause a sudden increase of temperature and the shedding of titanium

D. Micro fluidization Method

- It is a new method for formulation of niosome it is based on jet principle
- i.e., by mixing two kinds of fluids such as alcohol and water in microchannels

.

Preparation steps

Two kinds of fluid in ultra high speed jets inside interaction chamber

↓

Impingement of thin layer of Liquid in micro channels

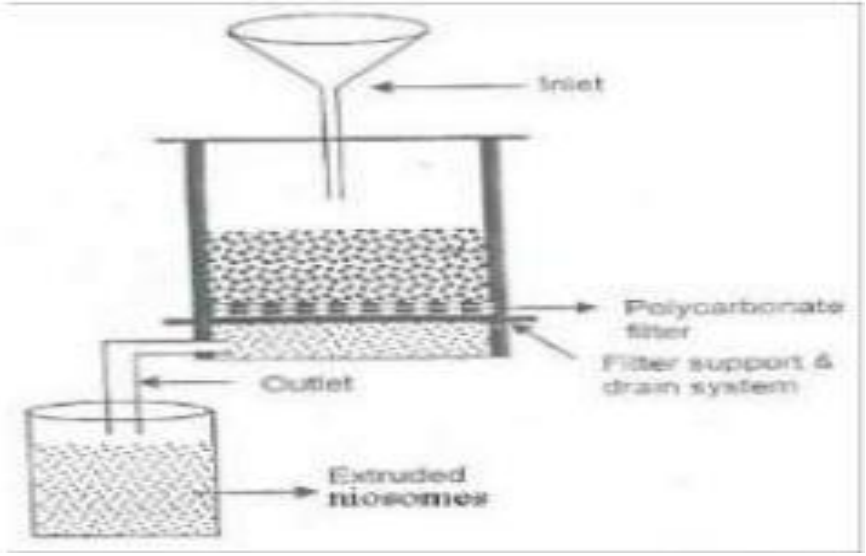
↓

Formation of uniform Niosomes

- Niosomes can be formulated with the desired particle sizes and size distribution by optimizing the parameters, such as mixing conditions, surfactants and other materials

E. Multiple membrane extrusion method

- Mixture of surfactant, cholesterol and diethyl phosphate in chloroform is made into thin film by evaporation.
- The film is hydrated with aqueous drug solution.
- Resultant suspension is extruded through polycarbonate membranes which are placed in series upto 8 passages



F. Reverse Phase Evaporation Technique (REV)

Cholesterol + surfactant dissolved in ether + chloroform

↓

Sonicated at 5°c and again Sonicated after adding PBS

↓

Drug in aqueous phase is added to above mixture

↓

Viscous Niosomes suspension is diluted with PBS

↓

Organic phase is removed at 40°C at low pressure

↓

Heated on a water bath for 60°C for 10 mins to yield Niosomes.

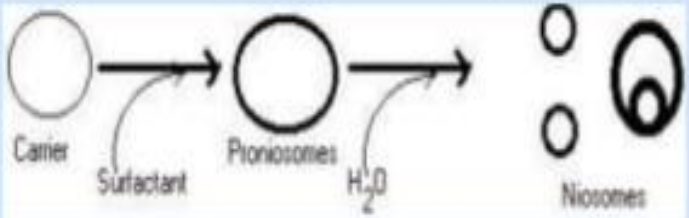
FORMATION OF NIOSOMES FROM PRONIOSOMES

- Proniosomes, also called dry niosomes, are dry-form formulations of the non-ionic surfactant vesicles which can be converted into niosomes after hydration in a short time, and are now widely used in the formulation of niosomes due to their good stability.
- Proniosomes consist of a water-soluble carrier coated with non-ionic surfactants, and are easily hydrated into niosomes before usage.
- Possesses several advantages such as good physical and chemical stability for long-term storage, convenience for transportation, and ease to scale up.

Formation of niosomes from proniosomes

Water soluble carrier such as sorbitol is coated with surfactant.

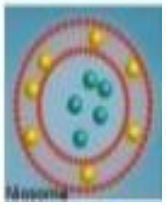
This preparation is termed "Proniosomes".



The result of the coating process is a dry formulation in which each water-soluble particle is covered with a thin film of dry surfactant.

The Niosomes are recognized by the addition of aqueous phase at T > Tm and brief agitation.

T=Temperature.
Tm = mean phase transition temperature



CONCLUSION

Niosomes may function as a good nano-vesicle delivery platform and provide a promising method for the delivery of chemical drugs, protein drugs and gene materials for the purpose of disease prevention and treatment.

Compared with liposomes, they have some advantages, such as good chemical and physical stability, low cost and easy formulation.

More work need to be undertaken in the field to yield more information for niosome development.

AQUASOMES

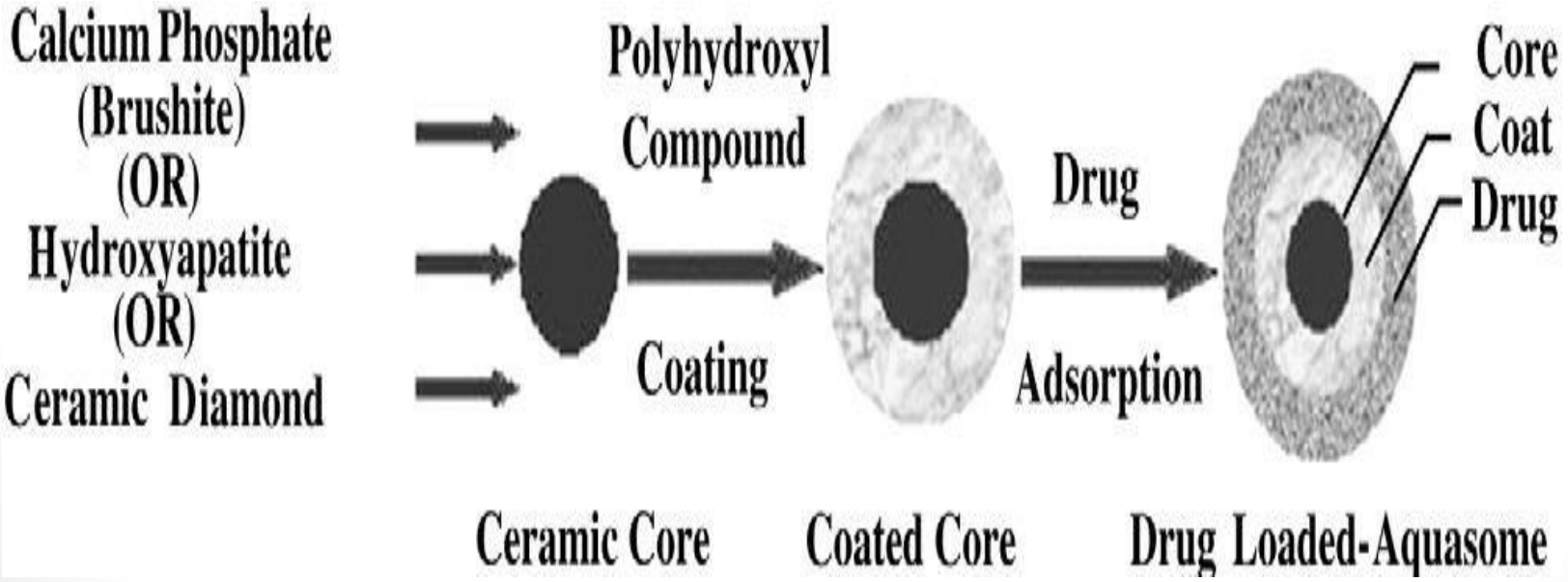
DEFINITION

- Aquasomes are nanoparticulate carrier system but instead of being simple nanoparticle these are **three layered self assembled structures.**
- This three layered system contains a **Core** coated with **Polyhydroxy oligomer** upon which **Biochemically active molecules** are adsorbed.
- **Ceramics** are mainly used as core material because of high degree of order and structural regularity.
- Polyhydroxy oligomer coating provides water like environment & protect biochemically active molecule from dehydration.
- Particle size lower than 1000 nm.

METHOD OF PREPARATION OF AQUASOMES

3steps.

- I - Formation of an inorganic core
- II - Coating of the core with polyhydroxy oligomer
- III- Loading of the drug of choice to this assembly



I. Formation of an inorganic core

Core preparation

- Preparation technique of core depends on the type of core to be used.
- Generally nanocrystalline tin oxide, carbon ceramic (diamond), calcium phosphate, hydroxyapatite are used as core. Among these materials nanocrystalline calcium phosphate and hydroxyapatite are widely used as core material for aquasomes.

Types:

a) Synthesis of nanocrystalline tin oxide core ceramic

b) Self assembled nano crystalline brushite (calcium phosphate dihydrate)

c) Nanocrystalline carbon ceramic, diamond particles

a)Synthesis of nanocrystalline tin oxide core ceramic

- It can be synthesized by direct current reactive magnetron sputtering.
- It is a high rate vacuum coating technique that allows the deposition of many types of materials including metals ceramic onto as many types of substrate materials by the use of specially formed magnetic applied to a diode sputtering target.
- here, a 3 inches diameter target of high purity tin is sputtered in a high pressure gas mixture of argon and oxygen.
- The ultrafine particles formed in the gas phase are then collected on copper tubes cooled to 77K with flowing nitrogen.

b)Self assembled nanocrystalline brushite (calcium phosphate dihydrate)

- These can be prepared by colloidal precipitation and sonication by reacting solution of disodium hydrogen phosphate and calcium chloride.

c) Nanocrystalline carbon ceramic, diamond particles

- These can also be used for the core synthesis after ultra cleansing and sonication.

- The equation for the reaction is as follows:

$2\ Na_2HPO4 + 3\ CaCl_2 + H_2O \rightarrow Ca_3(PO4)_2 + 4\ NaCl + 2\ H_2 + Cl_2 + (O)$

II. Coating of the core with Polyhydroxy Oligomer

- In the second step, ceramic cores are coated with carbohydrate (Polyhydroxyl Oligomer).
- The coating is carried out by addition of carbohydrate into an aqueous dispersion of the core under Sonication.
- These are then subjected to Lyophilization to promote an irreversible adsorption of carbohydrate onto the ceramic surface.
- The unadsorbed carbohydrate is removed by centrifugation.
- The commonly used coating materials are **Cellobiose, citrate, pyridoxal-5- phosphate, Trehalose and sucrose**.
- Core to coat ratio of 1:4 or 1:5 caused formation of spherical coated particles.

III. Loading of the drug of choice to this assembly

- The final stage involves the loading of drug to the coated particles by adsorption.
- For that, a solution of known concentration of drug is prepared in suitable pH buffer, and coated particles are dispersed into it.
- The dispersion is then either incubated at low temperature for drug loading or lyophilized after some time so as to obtain the drug-loaded formulation (i.e., aquasomes).
- The preparation thus obtained is then characterized using various techniques.

APPLICATIONS OF AQUASOMES

1. Insulin delivery
2. Oral delivery of acid labile enzyme
3. As oxygen carrier
4. Antigen delivery
5. Delivery of drug
6. For delivery of gene
7. For delivery of enzymes
8. Miscellaneous

1. Insulin Delivery

- Prepared aquasomes using a **calcium phosphate ceramic core** for the parenteral delivery of insulin.
- The core was coated with various disaccharides such as **cellobiose, trehalose, and pyridoxal-5-phosphate.**
- Subsequently the drug was loaded to these particles by adsorption method.
- **Prolonged reduction of blood glucose** was observed with all formulations except cellobiose-coated particles.
- Pyridoxal-5-phosphate coated particles were found to be more **effective in reducing blood glucose levels** than aquasomes coated with trehalose or cellobiose.

2. Oral delivery of acid labile enzyme

- The use of a nanosized ceramic core–based system for oral administration of the acid-labile enzyme **serratiopeptidase.**
- The nano core was prepared by colloidal precipitation under sonication at room temperature.
- The core was then **coated with chitosan** under constant stirring, after which the enzyme was adsorbed over it.
- The enzyme was protected by further encapsulating the enzyme-loaded core into alginate gel.
- These aquasomes were found to be **protecting the structural integrity of enzymes** so as to obtain a **better therapeutic effect**

3. As oxygen carrier

- Prepared hydroxyapatite ceramic cores by co-precipitation and self-precipitation.
- These cores were coated with various sugars including cellobiose, trehalose, maltose, and sucrose.
- Subsequently, hemoglobin was adsorbed over the coated ceramic core, and the percentage drug loading was estimated by the Benzidine method.
- The Oxygen carrying capacity of Aquasome formulation was found to be similar to that of fresh blood

4. Antigen delivery

- Vehicle for hepatitis B vaccine for effective immunization.
- Hydroxyapatite core was coated with cellobiose, and finally hepatitis B surface antigen was adsorbed over the coated core.
- The antigen-loading efficiency of plain hydroxyapatite core (without cellobiose coating) was found to be approximately 50%.
- whereas the coated core was observed to load approximately 21% antigen.

5. Delivery of drug

- Prepared Aquasomes loaded with **Indomethacin** through the formation of an inorganic core of calcium phosphate covered with a lactose film and further adsorption of Indomethacin as a low-solubility drug.

- SEM and TEM techniques confirmed the spherical shape of Aquasomes.

- However, results of drug(Indomethacin) release studies from these carriers are yet to be determined.

6. For delivery of gene

- Delivery system loaded with genetic material.
- Studies reveal that Aquasomes protect and maintain structural integrity of the gene segment.

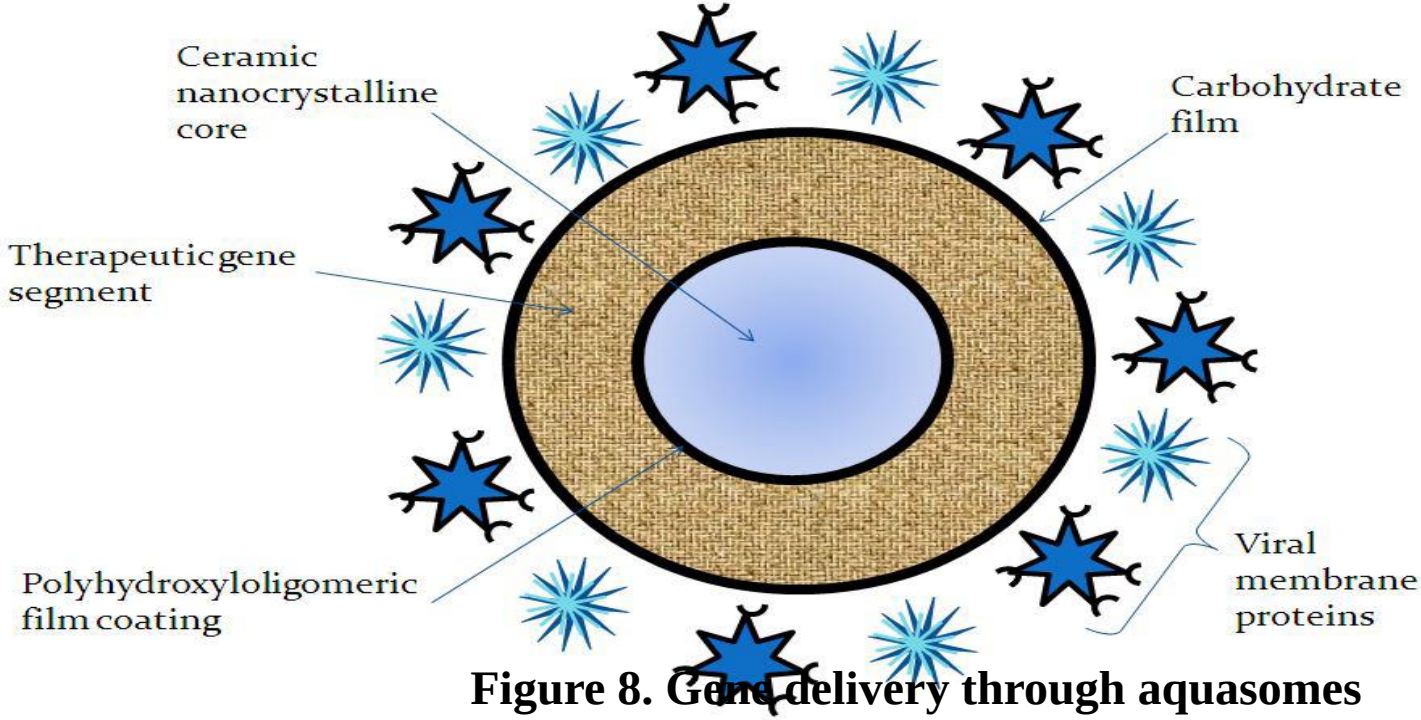


Figure 8. Gene delivery through aquasomes

7. For Delivery of Enzymes

- For delivery of enzymes like **DNAse and pigment/dyes** because enzymes activity fluctuates with molecular conformation and cosmetic properties of pigment are sensitive to molecular conformation.

- DNAse a therapeutic enzyme used in the **treatment of cystic fibrosis** was successfully immobilized on Aquasomes and targeted to the specific site and elicited significant therapeutic effect as desirable.

8. Miscellaneous

- Prepared spherical porous hydroxyapatite particles by spray-drying.
- These particles were tried as a carrier for the delivery of drugs such as **interferon α (IFN α), testosterone enanthate, and cyclosporine A.**
- The spherical porous hydroxyapatite particles were shown to be useful as a **biodegradable** and subcutaneously injectable drug carrier.
- The reinforcement of spherical porous hydroxyapatite particles was suggested to be very effective for sustained release of drugs

CONCLUSION

Various research works on aquasomes indicated that it can be used as successful nanoparticulate drug carrier. Research works suggested antigen, insulin, hemoglobin, vaccine can be delivered through aquasomes.

It helps in delivering conformationally sensitive molecule to the site of action. Also aquasomes helps in delivering protein molecule by preventing destructive denaturation.

Though it has many advantages to be used as drug carrier, extensive researches are yet required to study the effect on in-vivo system, to identify if it has any toxic effect in certain conditions and to prove its safety & efficacy in human body.

PHYTOSOMES

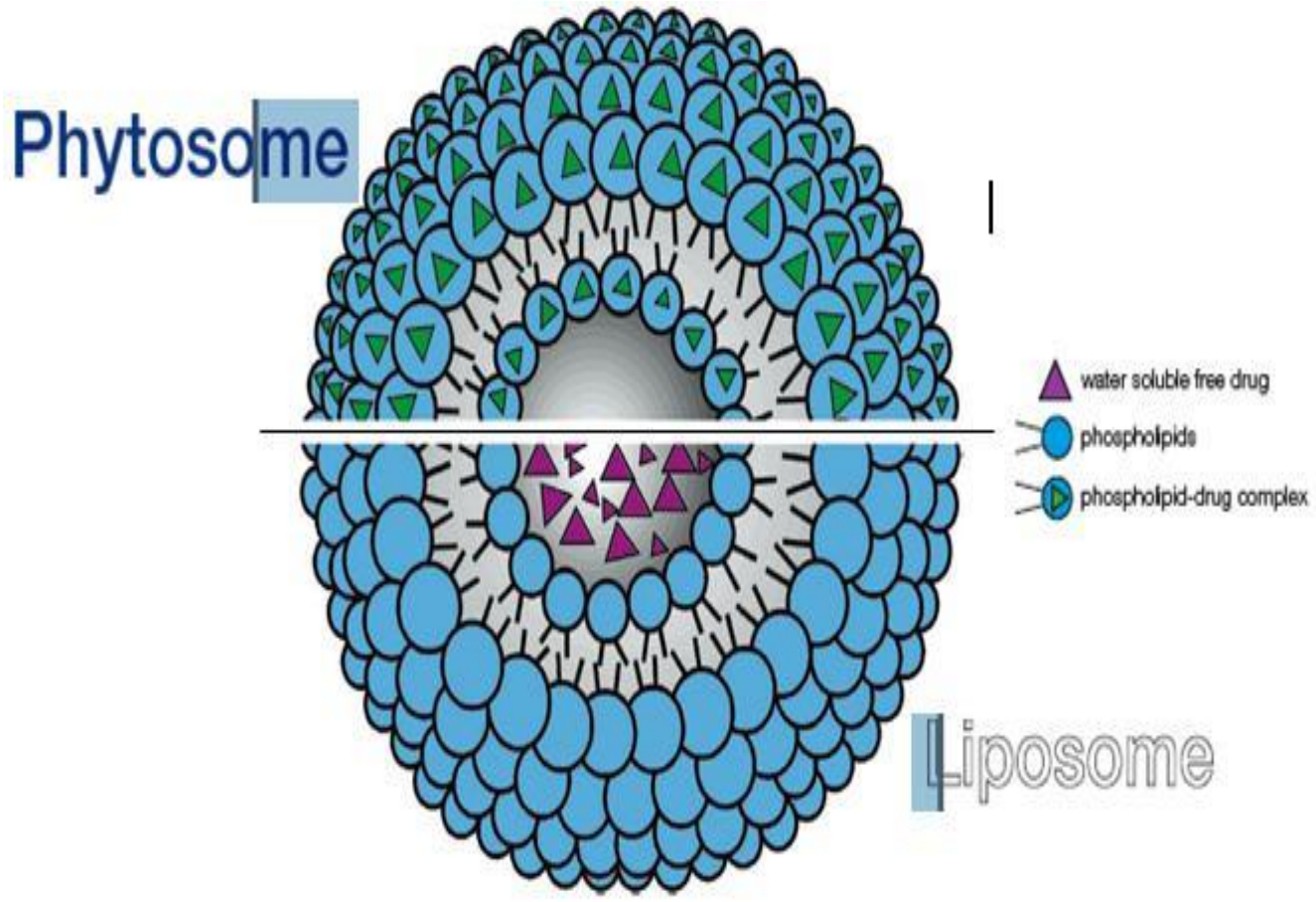
- The term ‘Phyto’ means **plant** while ‘Some’ means **cell-like**.
- Phytosome is a **vesicular drug delivery system** in which phytoconstituents of herbal extract surround and bound by lipids (one phyto-constituent molecule linked with at least one phospholipid molecule).
- Phytosome protect valuable component of herbal extract from destruction by digestive secretion and gut bacteria and because of which they shows better absorption which produces **better bioavailability and improved pharmacological and pharmacokinetic parameters than conventional herbal extract**.

MECHANISM OF PHYTOSOME TECHNOLOGY:

- Phytosomes results from the reaction of a stoichiometric amount of the phospholipid (phosphatidylcholine) with the standardized extract or polyphenolic constituents (like simple flavonoids) in an aprotic solvent.
- Phosphatidylcholine- bifunctional compound, the phosphatidyl moiety is lipophilic and the choline moiety is hydrophilic in nature.
- Choline head of the phosphatidylcholine molecule binds to these compounds while the lipid soluble phosphatidyl portion comprising the body which then envelopes the choline bound material.

PHYTOSOMES	LIPOSOMES
1. Active chemical constituents are anchored through chemical bonds to the polar head of the phospholipids.	1. Active principle is dissolved in the medium of the cavity or in layers of the membrane. No chemical bond is formed.
2. The phosphotidylcholine and the individual plant compound form a 1:1 or 2:1 complex depending on the substance.	2. Here hundreds and thousands of phosphotidylcholine molecules suround the water soluble molecule.
3. The phytosome is a unit of a few molecules bonded together.	3. liposome is an aggregate of many phospholipid molecules that can enclose other phytoactive molecules.

How does "Phytosome" differ from a "Liposome" ?



Main difference between liposome and phytosome

ADVANTAGES OF PHYTOSOME

- Phytosome is much better absorbed than liposome because drug is in complex form with lipid.
- Leakage of drug during storage does not occur in phytosome, because drug is bonded with lipid, however loss may occur due to some chemical degradation i.e. hydrolysis.
- Phosphatidylcholine used in preparation of phytosomes, besides acting as carrier also act as a hepatoprotective.
- The physiochemical stability of phytosome depends upon the physicochemical properties of drug-lipid complex.
- Application of phytpconstituent in form of phytosome improve their percutaneous absorption and as functional cosmetics.

PREPARATION OF PHYTOSOMES:

1. Active constituent of herbal extract+ Phospholipid is mixed in aprotic solvent for complex formation with constant stirring.
2. Complex is isolated with addition of non solvent Complex in drying form
3. Complex dissolve in organic solvent
4. Drying
5. Thin Film Formation
6. Hydration of thin film
7. Formation of phytosome complex (suspension)
8. Isolation by precipitation with non solvent (such as aliphatic hydrocarbons)
9. Drying (By lyophilization or spray drying)

APPLICATIONS OF PHYTOSOME

- The novel form of herbal products phytosomes are better absorbed than **conventional herbal extracts**.

- This was observed in SILIPHOSTM (Silybin phytosome). Silybin is chief component of Silymarin, valued for its **ability to protect and restore liver activities.**

- Phytosomes serve as a delivery system consisting of microscopic vesicles that improve the potential bioavailability, as can be observed in skin care or nutritional products.

- Phytosome are supposed to **increase the systemic bioavailability** of the hydrophilic phytoconstituents and there by **increases their therapeutic efficacy.**

- **Grape Seed Phytosome:** 50 to 100 mg Systemic antioxidant, Best choice for most people under age of fifty. Also specific for the eyes, lungs, diabetes, varicose veins, and protection against heart disease.

- **Green Tea Phytosome:** 50 to 100 mg Systemic antioxidant. Several studies have suggested that the flavonoids and caffeine in **green tea** can help elevate metabolic rate, increase **fat** oxidation and even improve insulin activity.

- **Ginkgo Biloba Phytosome:** 120 mg Best choice for most people over the age of 50. Protects brain and vascular lining

- **Hawthorn Phytosome:** 100 mg Best choice in heart disease or high blood pressure.

- **Leucoselect Phytosome:** 50-100 mg Best choice for antioxidant support, cardiovascular system.

- **Curcumin phytosome:** Powerful free radical scavenger, can help to support a balanced immune system response to normal metabolic stress, and can promote healthy joint mobility and flexibility.

CONCLUSION:

- Phytosomes are novel formulations which offer improved bioavailability of hydrophilic favonoids and other similar compounds through the skin or gastrointestinal tract.
- They have many distinctive advantages over other conventional herbal formulations. The formulation methodology for phytosome is simple and can be easily upgraded to a commercial scale.

ELECTROSOMES

BIOFUEL CELLS

Biofuel cells are electrochemical devices that use enzymatic reactions to catalyze the conversion of chemical energy to electricity in a fuel cell.

- They can be classified as **microbial fuel cells** (MFCs), which use living microorganisms or **enzymatic fuel cells**, which use purified enzymes.
- **Hybrid biofuel cells** combine the characteristics of both classes of biofuel cells.
- This concept was initially introduced by the use of redox enzymes surface-displayed on different microorganisms and in biofuel cells

ELECTROSOME

- Is a novel surface-display system based on the specific interaction between the cellulosomal scaffoldin protein and a cascade of redox enzymes that allows multiple electron release by fuel oxidation.
- The electrosome is composed of two compartments:

(i) **Hybrid Anode**, which consists of dockerin-containing enzymes attached specifically to cohesin sites in the scaffoldin to assemble an ethanol oxidation cascade, and (ii) **Hybrid Cathode**, which consists of a dockerin-containing oxygen-reducing enzyme attached in multiple copies to the cohesin-bearing scaffoldin

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THANK YOU