

# Types, Mechanism, Sizing & Preparation of Liposomes

A Literature Review  
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# Outline

- ✓ Introduction
- ✓ Classification
- ✓ Mechanisms of Formation
- ✓ Methods of Preparation
- ✓ Therapeutic Applications
- ✓ Conclusion
- ✓ References

# Introduction

- Rational research in drug delivery began in 1950's.

- Need for Targeted/Controlled Drug Delivery:

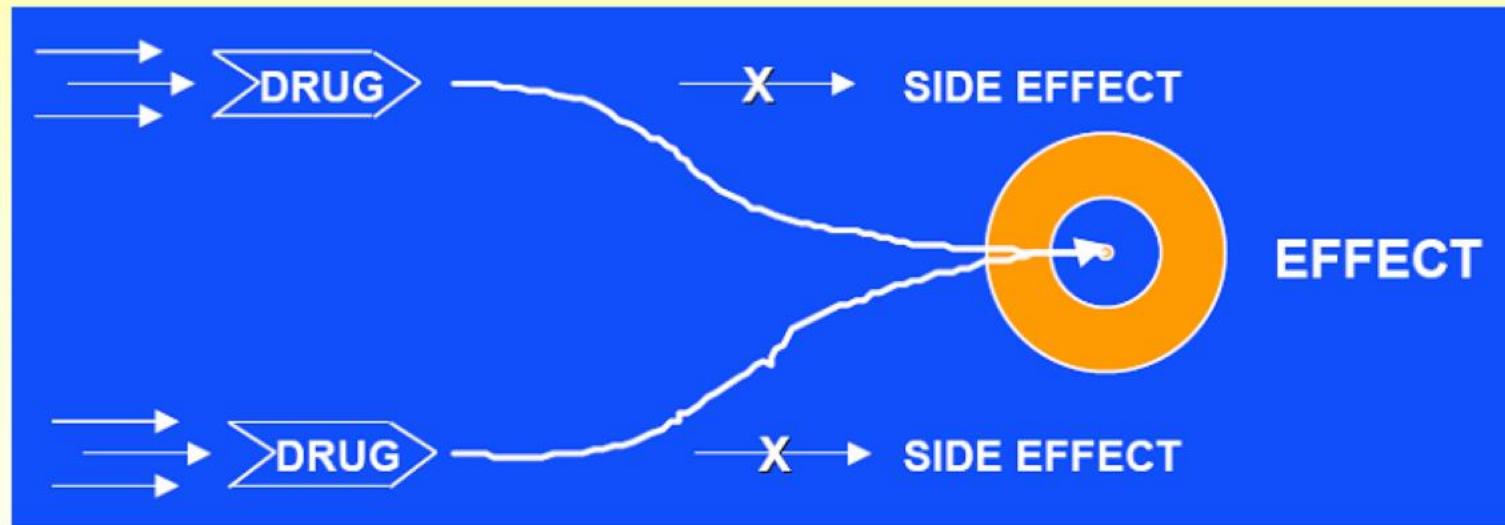
Delivery of the drug at a rate and/or a location dictated by the needs of the body or disease state over a specified period of time.

Temporal delivery: control over rate of drug release

Spatial delivery: control over location of drug release

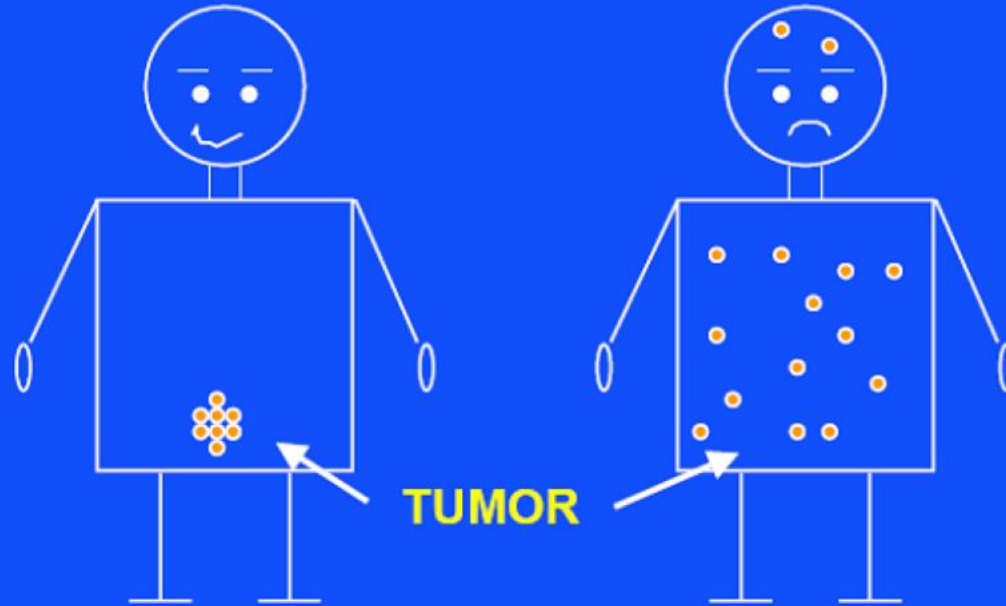
Combination of both of the above.

# The Magic Bullet



# Drug Targeting

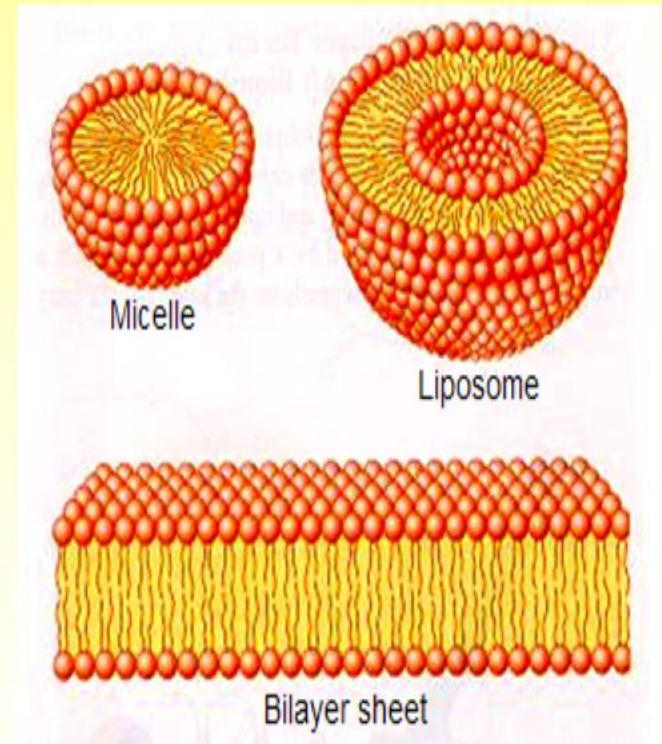
• = DRUG MOLECULE



**EXCLUSIVE DRUG DELIVERY TO SPECIFIC CELL TYPES**

# Liposomes

- Liposomes: A Novel Drug Delivery System.  
Liposomes were discovered in the early 1960's by Bangham and colleagues.
- Structurally, liposomes are concentric bilayered vesicles in which an aqueous volume is entirely enclosed by a membranous lipid bilayer mainly composed of natural or synthetic phospholipids
- Liposomes became the predominant drug delivery and targeting system.  
Ranging from tumour targeting, gene and anti-sense therapy, genetic vaccination, immuno-modulation, lung therapeutics, fungal infections, and some non- medical are like bio reactors, catalysts, ecology, skin care and topical cosmetic products.



# Advantages of Liposomes

1. Provides selective passive targeting to tumour tissues (liposomal doxorubicin).
2. Increased efficacy and therapeutic index.
3. Increased stability via encapsulation.
4. Reduction in toxicity of the encapsulated agent.
5. Improved pharmacokinetic effects (Reduced elimination, increased circulation life times).
6. Flexibility to couple with site-specific ligands to achieve active targeting.

# CLASSIFICATION OF LIPOSOMES

## Based on Structural Parameters

<b>TYPE</b>	<b>SPECIFICATION</b>
MLV	Multilamellar large vesicles->0.5 $\mu$ m
OLV	Oligolamellar vesicles-0.1-1 $\mu$ m
UV	Unilamellar vesicles (all size ranges)
SUV	Small Unilamellar Vesicles- 20-100 nm
MUV	Medium sized unilamellar vesicles
LUV	Large unilamellar vesicles->100nm
GUV	Giant unilamellar vesicles->1 $\mu$ m
MV	Multivesicular vesicles->1 $\mu$ m



# CLASSIFICATION OF LIPOSOMES

## Based on Method of Liposome Preparation

REV	Single or oligolamellar vesicles made by reverse- phase evaporation method.
MLV_REV	Multilamellar vesicles made by reverse- phase evaporation method.
SPLV	Stable plurilamellar vesicles
FATMLV	Frozen and thawed MLV
VET	Vesicles prepared by extrusion technique.
DRV	Dehydration – rehydration method.

# CLASSIFICATION OF LIPOSOMES

## Based upon Composition & Applications

Conventional liposomes(CL)

Neutral or negatively charged

Phospholipids and cholesterol.

Fusogenic liposomes (RSVE)

Reconstituted Sendai virus envelopes

pH sensitive liposomes

Phospholipid such as PE or DOPE

with either CHEMS or OA

Cationic liposomes

Cationic lipids with DOPE

Long circulatory stealth

Neutral high  $T_c^\circ$ , cholesterol and 5- 10%

liposomes (LCL)

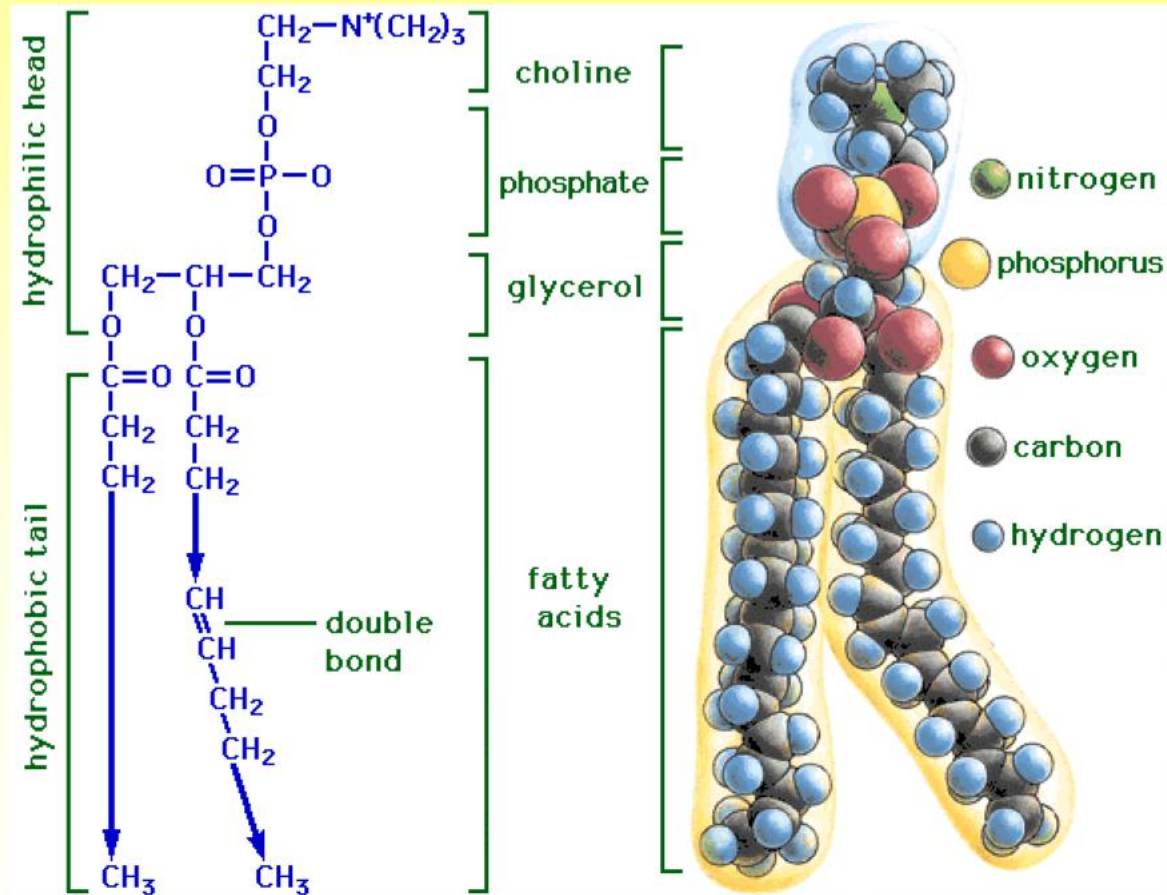
of PEG - DSPE or GM1

Immuno-liposomes

CL or LCL with attached monoclonal

antibody or recognition sequence

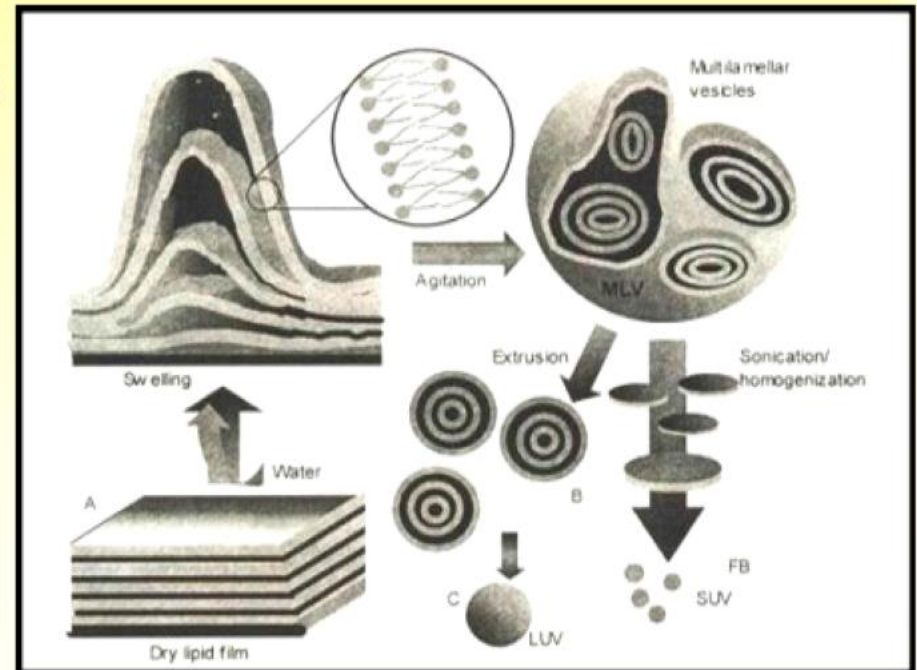
# Phospholipid Bilayer



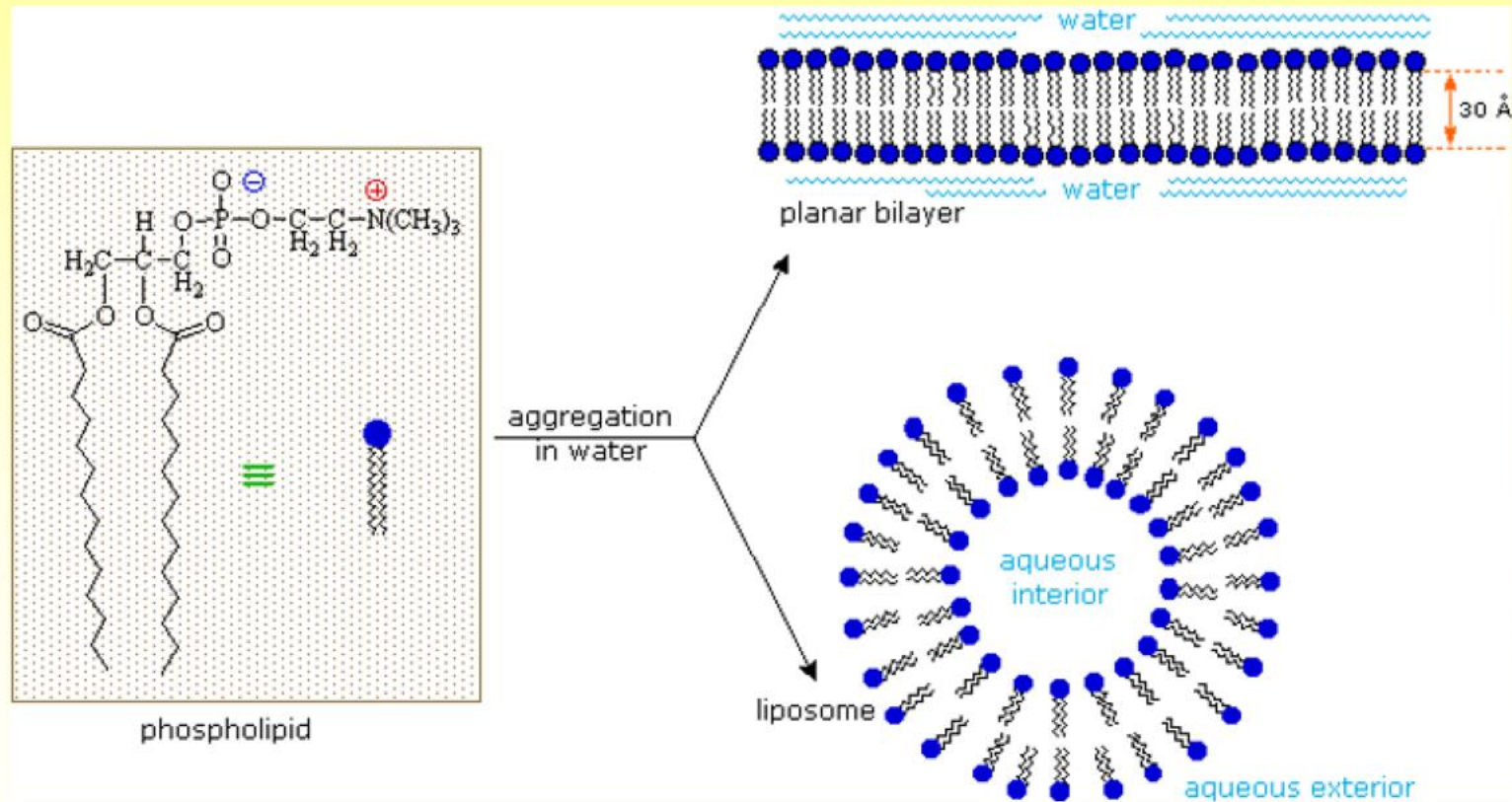
Structure of Typical Phospholipid within a Lipid Bilayer

# Mechanism of Liposomes Formation

- In aqueous medium the molecules in self assembled structures are oriented in such a way that the polar portion of the molecules remains in contact with the polar environment and at the same time shields the non polar part.
- Among the amphiphiles used in the drug delivery, viz. soaps detergents, polar lipids; the latter are often employed to form concentric bilayered structures.
- At high concentration of these polar lipids, liquid-crystalline phases is formed that upon dilution with an excess of water can be dispersed into relatively stable colloidal particles.



# Liposomes Formation



# Physicochemical Properties of Phospholipids

Phase transition of lipids:

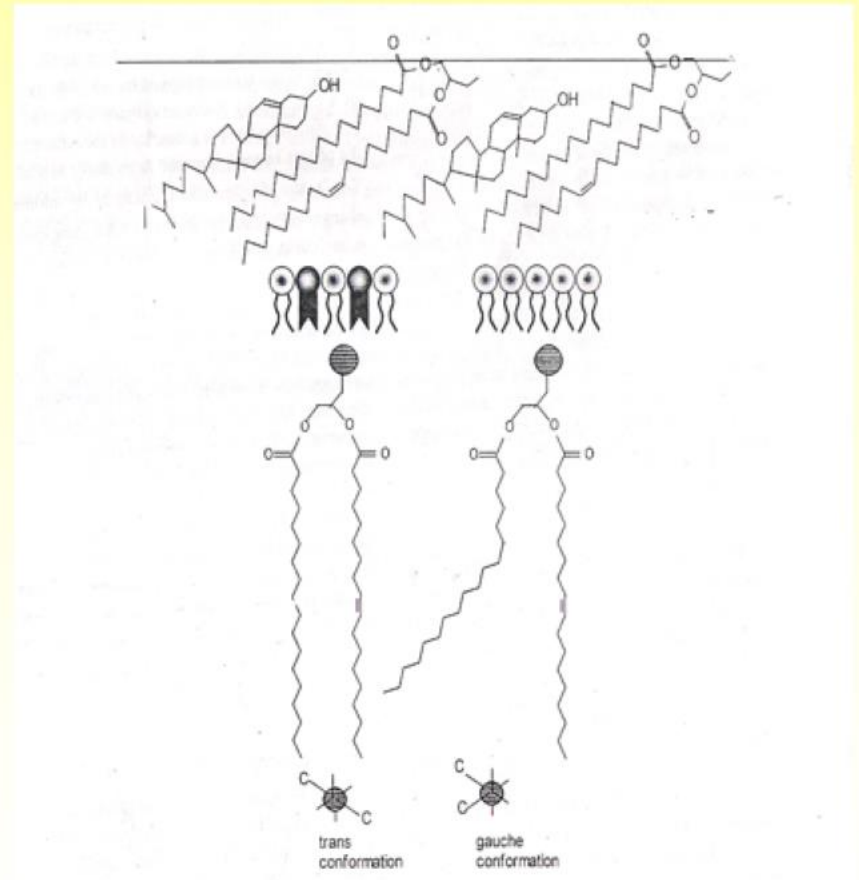
Relatively ordered state (gel phase) →→ Less ordered liquid crystal state (liquid phase)

Some of the physicochemical forces responsible for bilayer orientation are

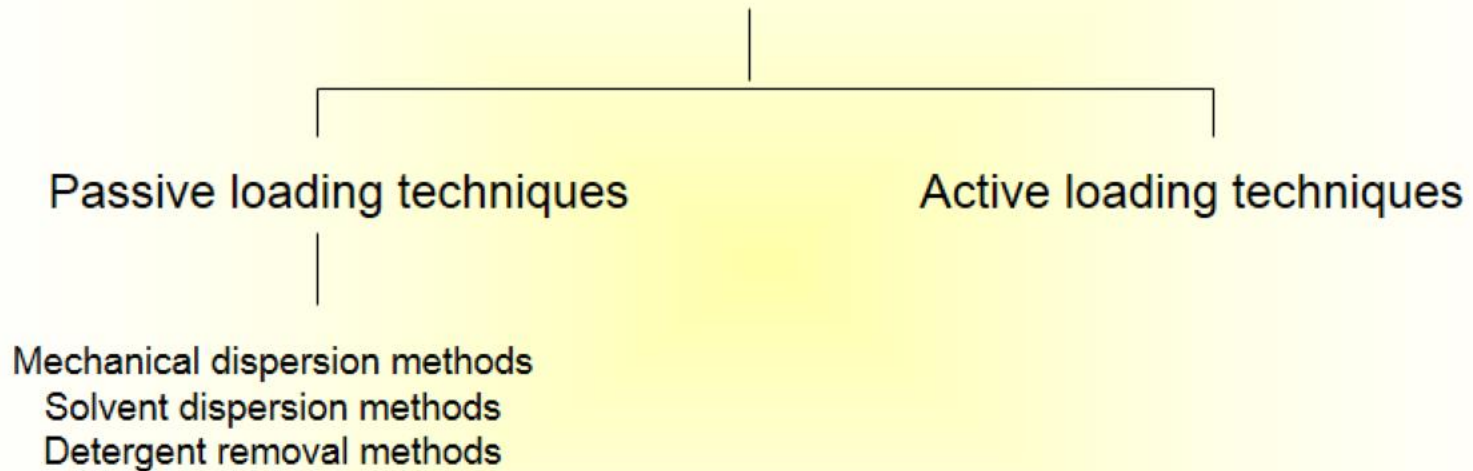
1. The large free energy difference between the aqueous and hydrophobic environment promotes the bilayer structures in order to achieve the lowest free energy level.
2. The driving force for bilayer configuration of liposomes is the hydrophobic interaction coupled with the amphiphilic nature of the principle phospholipid molecules.
3. Supra-molecular self-assemblages mediated through specific molecular geometry.

# Role of Cholesterol Bilayer Formation

- Acts as a fluidity buffer.
- After intercalation with phospholipid molecules alters the freedom of motion of carbon molecules in the acyl chain.
- Restricts the transformations of trans- to gauche- conformations.



# Method of Preparation of Liposomes





# Method of Preparation of Liposomes

## Passive Loading Techniques

- **Mechanical dispersion methods**

- Lipid film hydration by hand shaking, non-hand shaking or freeze drying
- Microemulsification
- Sonication
- French pressure cell
- Membrane extrusion
- Dried reconstituted vesicles
- Freeze-thawed liposomes

- **Solvent dispersion methods**

- Ethanol injection
- Ether injection
- Double emulsion vesicles
- Reverse phase evaporation vesicles
- Stable plurilamellar vesicles

- **Detergent removal methods**

- Dialysis
- Column chromatography
- Dilution
- Reconstituted Sendai Virus enveloped vesicles

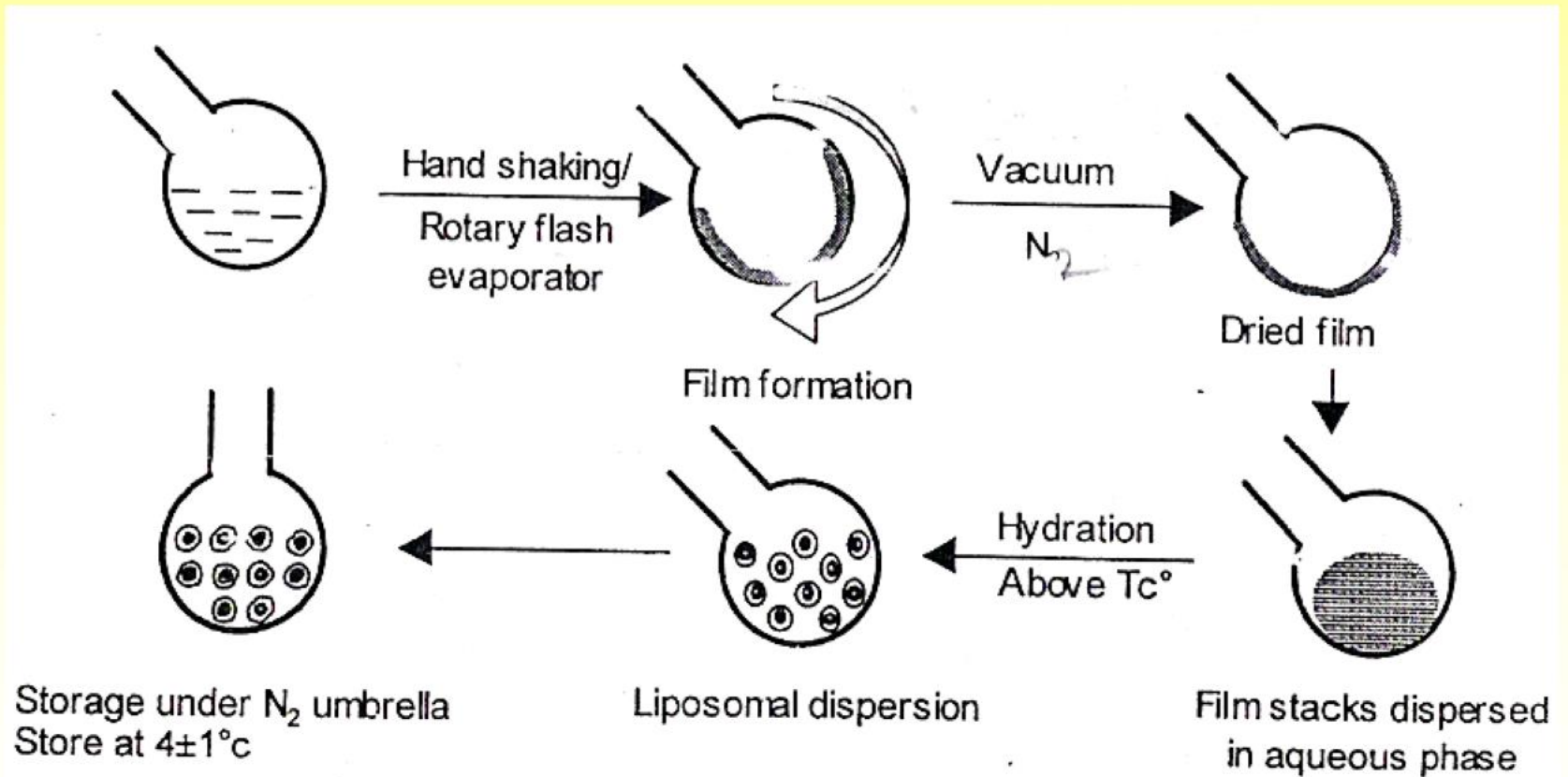
# Pro-Liposomes

- In order to increase the surface area of dried lipid film and to facilitate instantaneous hydration, the lipid is dried over a finely divided particulate support, such as powdered sodium chloride, or sorbitol or other polysacharrides.
- These dried lipid coated particulates are called pro-liposomes.
- Pro-liposomes form dispersion of MLV's on adding water into them, where support is rapidly dissolved and lipid film hydrates to form MLV's.
- The size of the carrier influences the size and heterogeneity of liposomes.
- This method also overcomes the stability problems of liposomes encountered during the storage of dispersion, dry or frozen form.
- It is ideally suited for preparations where the material incorporates into lipid membrane.
- The method is applicable in cases where 100% entrapment of components is not a requirement rather the stability is preferred.

# Mechanical Treatment of MLV's

- Multilamellar vesicles formed on hydration of dried lipids could be further engineered or modified for their size and other characteristics. A large number of methods are devised to reduce their size and to convert liposomes of the large size range into smaller homogenous vesicles. These include techniques such as micro-emulsification, extrusion, ultra-sonification and use of French pressure cell.
- A second set of methods is designed to increase the entrapment volume of hydrated lipids, and/or reduce the lamellarity of vesicles formed. These include procedures such as freeze drying, freeze thawing, or induction of vesiculation by ions or pH change.

# MECHANICAL DISPERSION METHODS

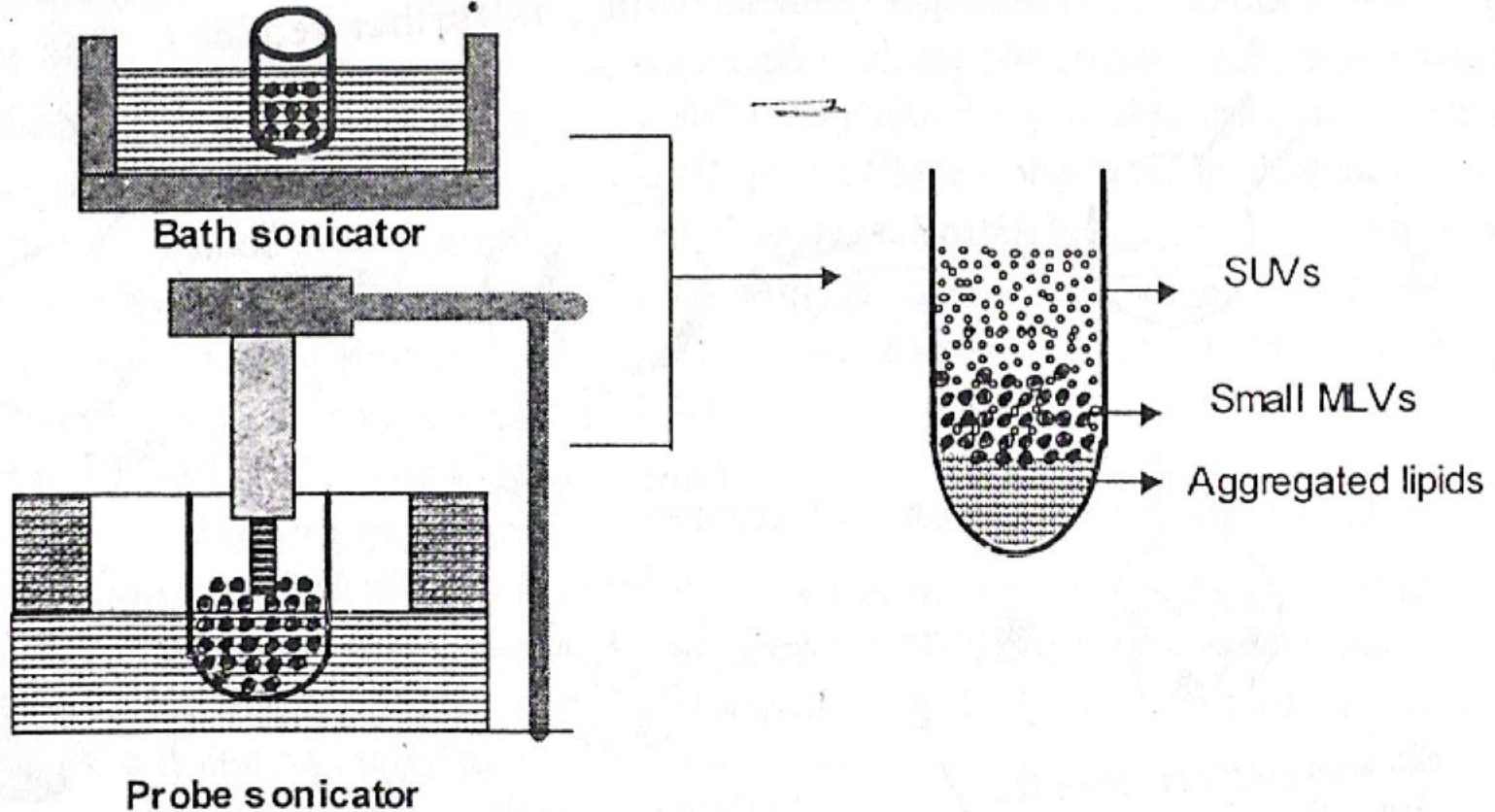


# FRENCH PRESSURE CELL LIPOSOMES



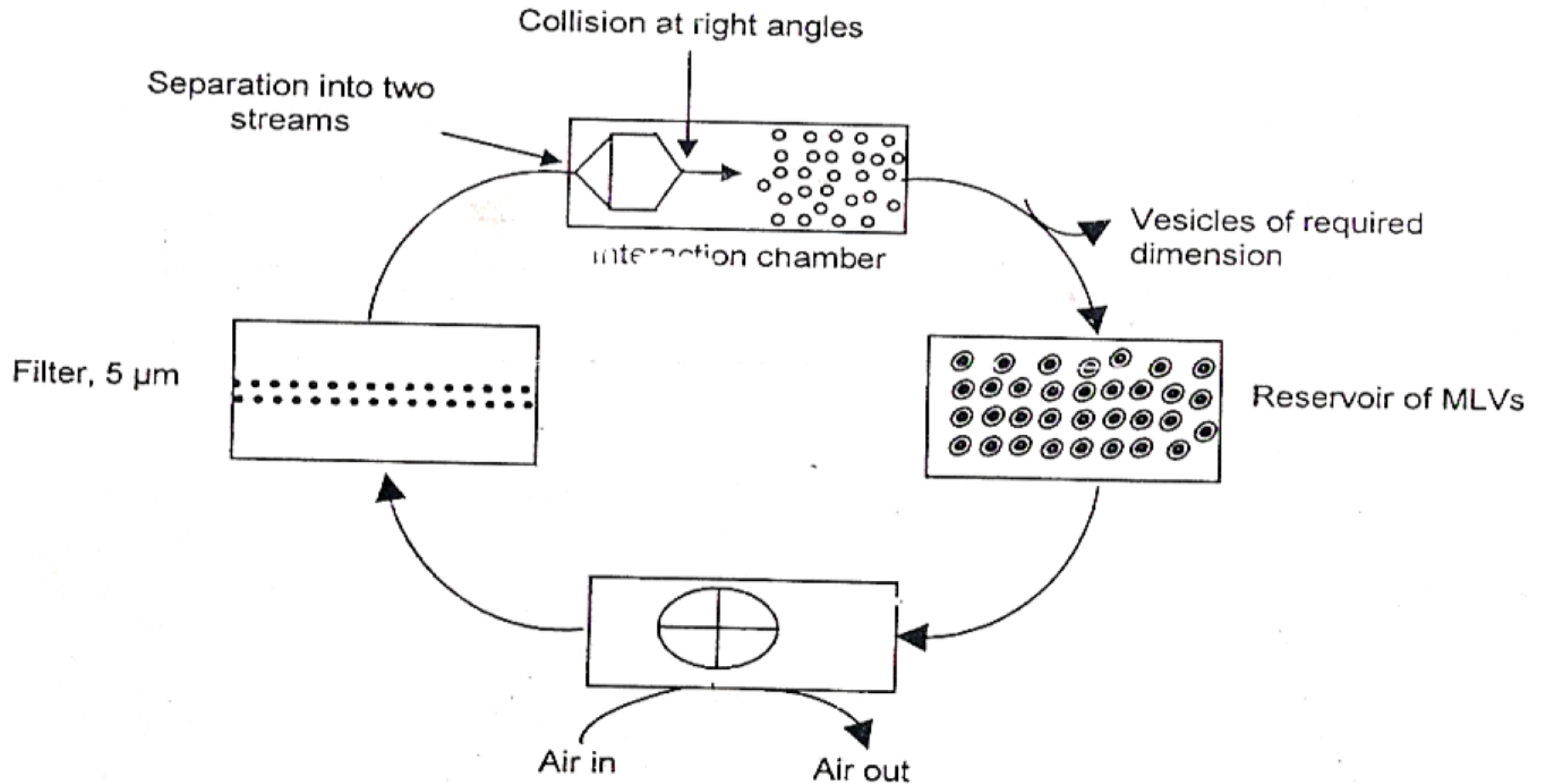
French Pressure Cell for Preparation of Uni/Oligo Lamellar Vesicles

# SONICATED UNILAMELLAR VESICLES (SUV's)

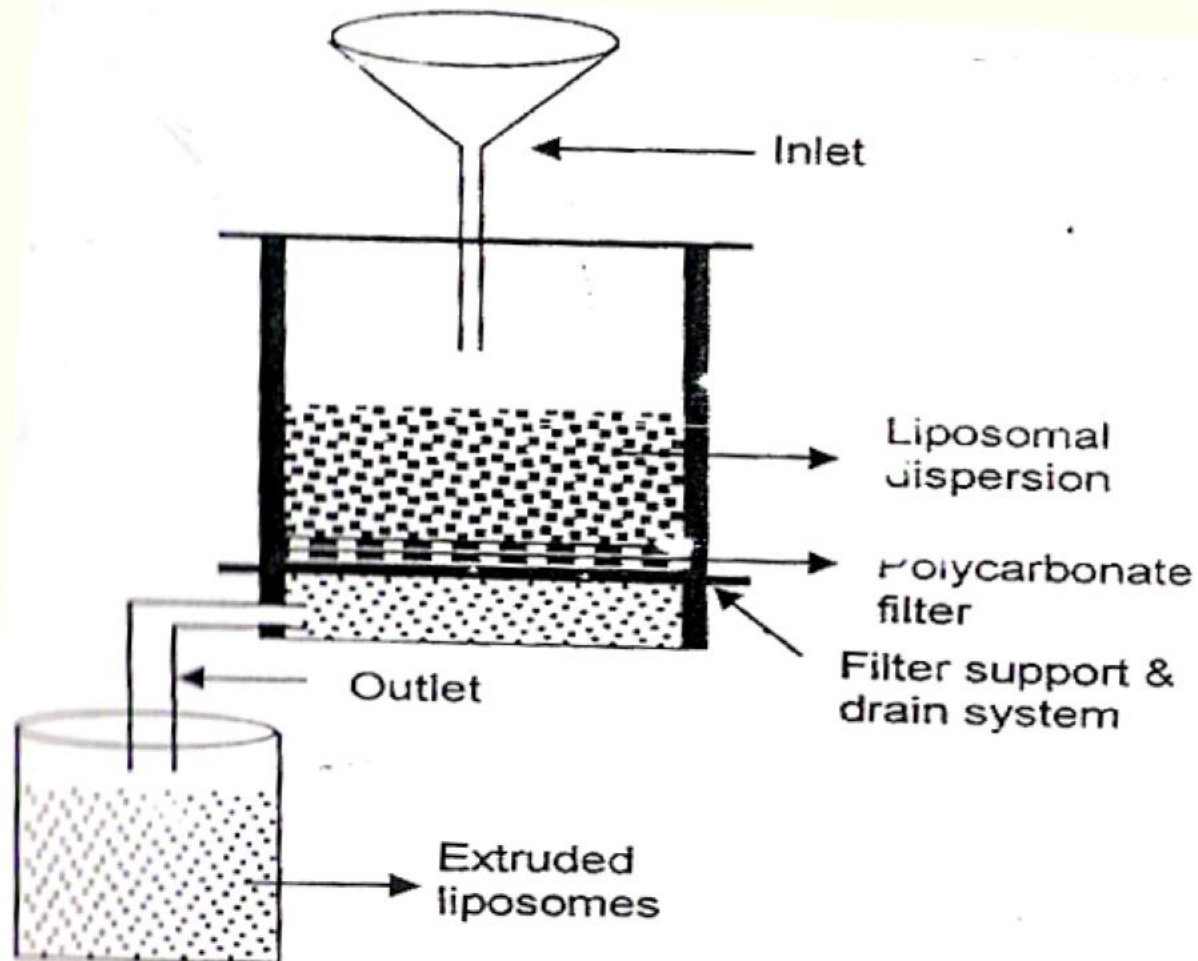


Preparation of SUV's from Bath/Probe Sonicator from MLV's

# USE OF MICROFLUIDIZER IN LIPOSOMES PREPARATION

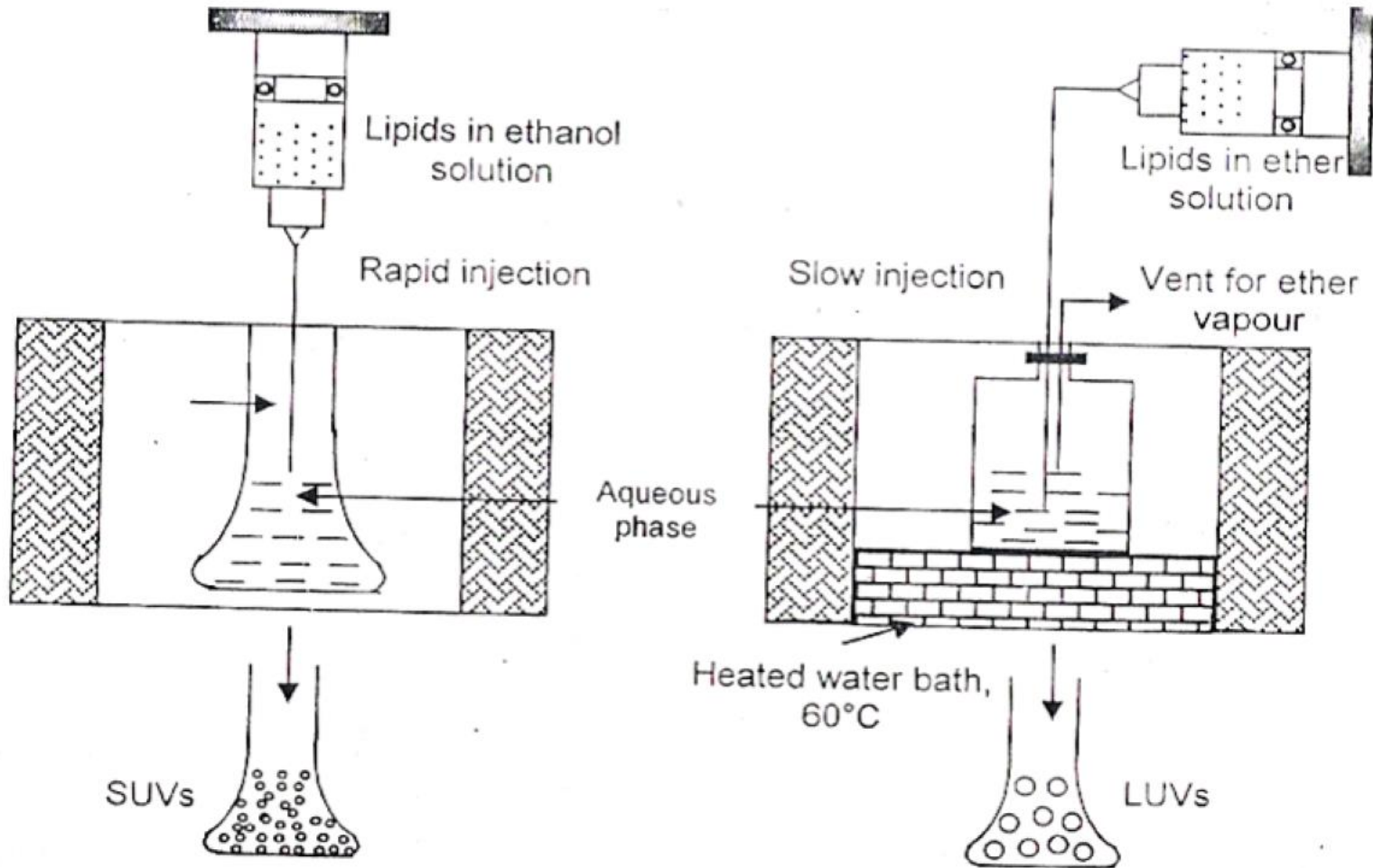


# VESICLES PREPARED BY EXTRUSION TECHNIQUES (VET's)

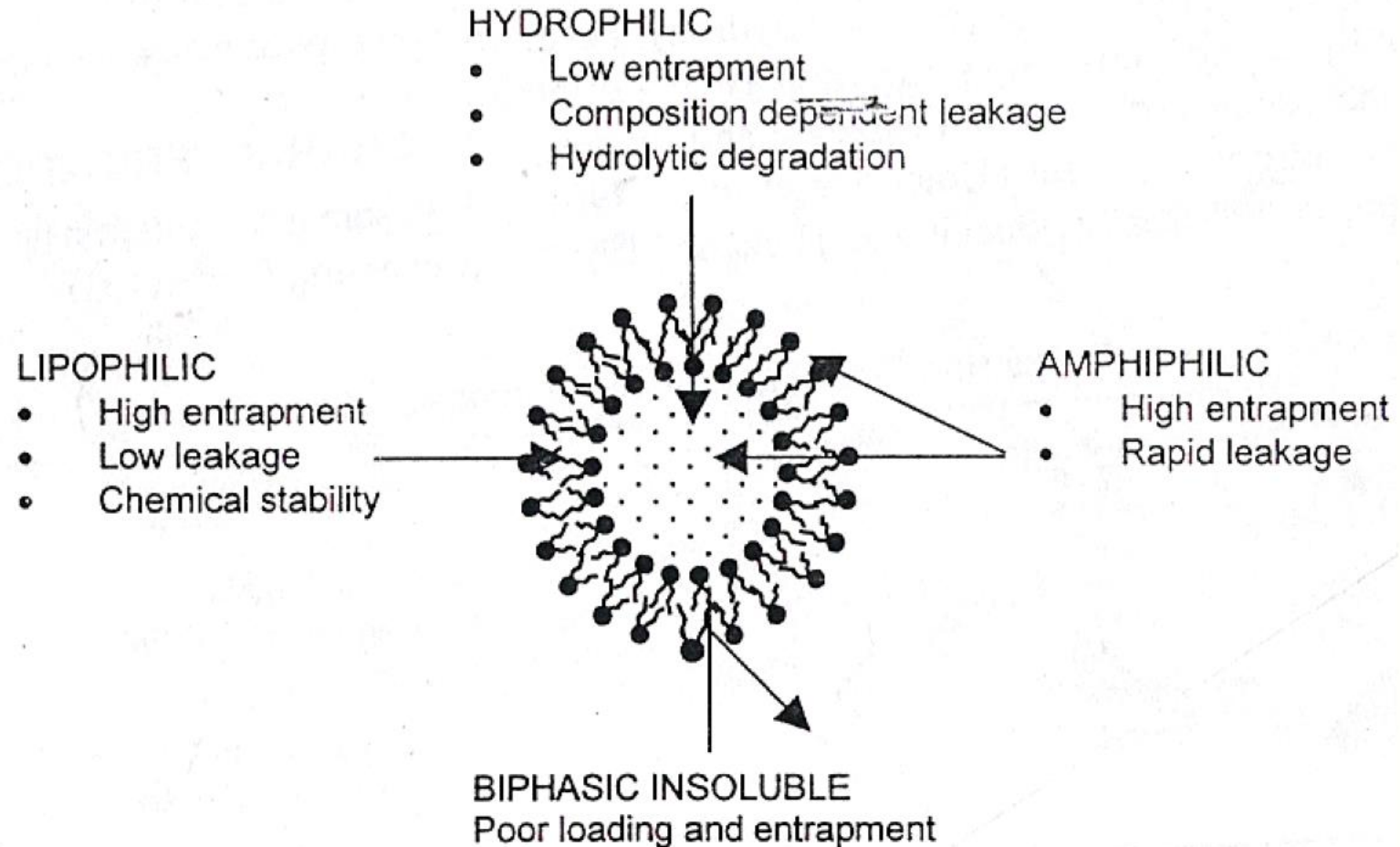




# ETHANOL & ETHER INJECTION



# MICROENCAPSULATION OR LOCUS OF DRUGS IN LIPOSOMES



# COMMERCIAL DEVELOPMENT AND SCALE UP

- Several liposome based products have been either approved or under the process of approval in the various parts of the world .
- However, scaling them up as a conventional market product still remains to be successfully implicated.

Problems generally encountered in the  
development of pharmaceutical liposomes:

1. Poor quality of the raw material mainly the phospholipids.
2. Poor characterization of the physicochemical properties of the liposomes.
3. Pay load is too low.
4. Shelf life is too short.
5. Scale up related problems.
6. Absence of any data on safety of these carrier systems on chronic use.

# THERAPEUTIC APPLICATION OF LIPOSOMES

1. LIPOSOMES AS DRUG DELIVERY VEHICLES
2. INCREASED THERAPEUTIC INDEX
3. LIPOSOMES AS A LYSOSOMOTROPIC CARRIER
4. LIPOSOMES IN MULTI-DRUG RESISTANCE
5. LIPOSOMES IN ANTIMICROBIAL, ANTIFUNGAL (LUNG THERAPEUTICS) & ANTIVIRAL (ANTI-HIV) THERAPY
6. AMPHOTERICIN B LIPOSOMES
7. LIPOSOMES AS A CARRIER OF IMMUNOMODULATORS
8. LIPOSOMES IN IMMUNO DIAGNOSIS
9. LIPOSOMAL VACCINES
10. LIPOSOMES AS RED CELL SUBSTITUTES AND ARTIFICIAL RBC'S
11. LIPOSOMES AS RADIOPHARMACEUTICAL & RADIODIAGNOSTIC CARRIERS

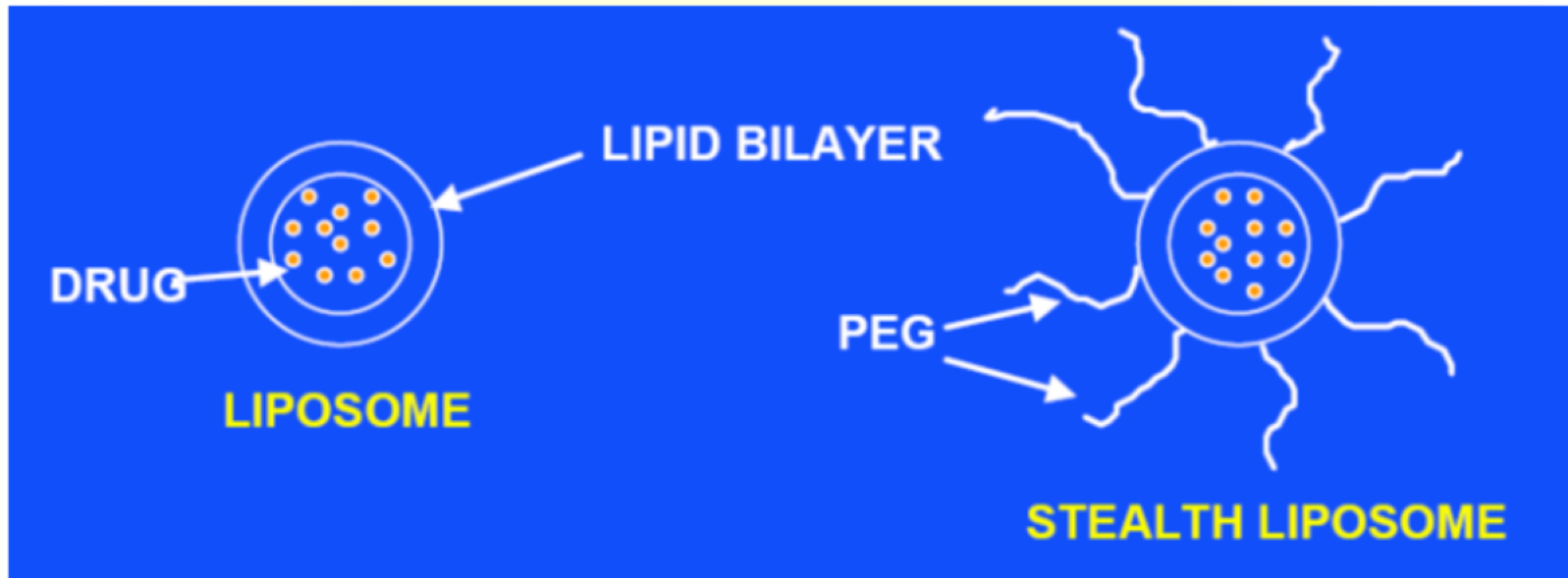
# THERAPEUTIC APPLICATION OF LIPOSOMES

## LIPOSOMES AS DRUG DELIVERY VEHICLES:

1. Enhanced drug solubilization (amphotericin B, minoxidil, paclitaxel, cyclosporin),
2. Protection of sensitive drug molecules (cytosine arabinose, ribozymes)
3. Enhanced intracellular uptake (anticancer, antiviral and antimicrobial drugs).
4. Altered pharmacokinetics and biodistribution (prolonged or sustained release of drugs with short circulatory half-lives).
5. Passive macrophage targeting and RES-accumulation
6. Surface engineered versions of liposomes

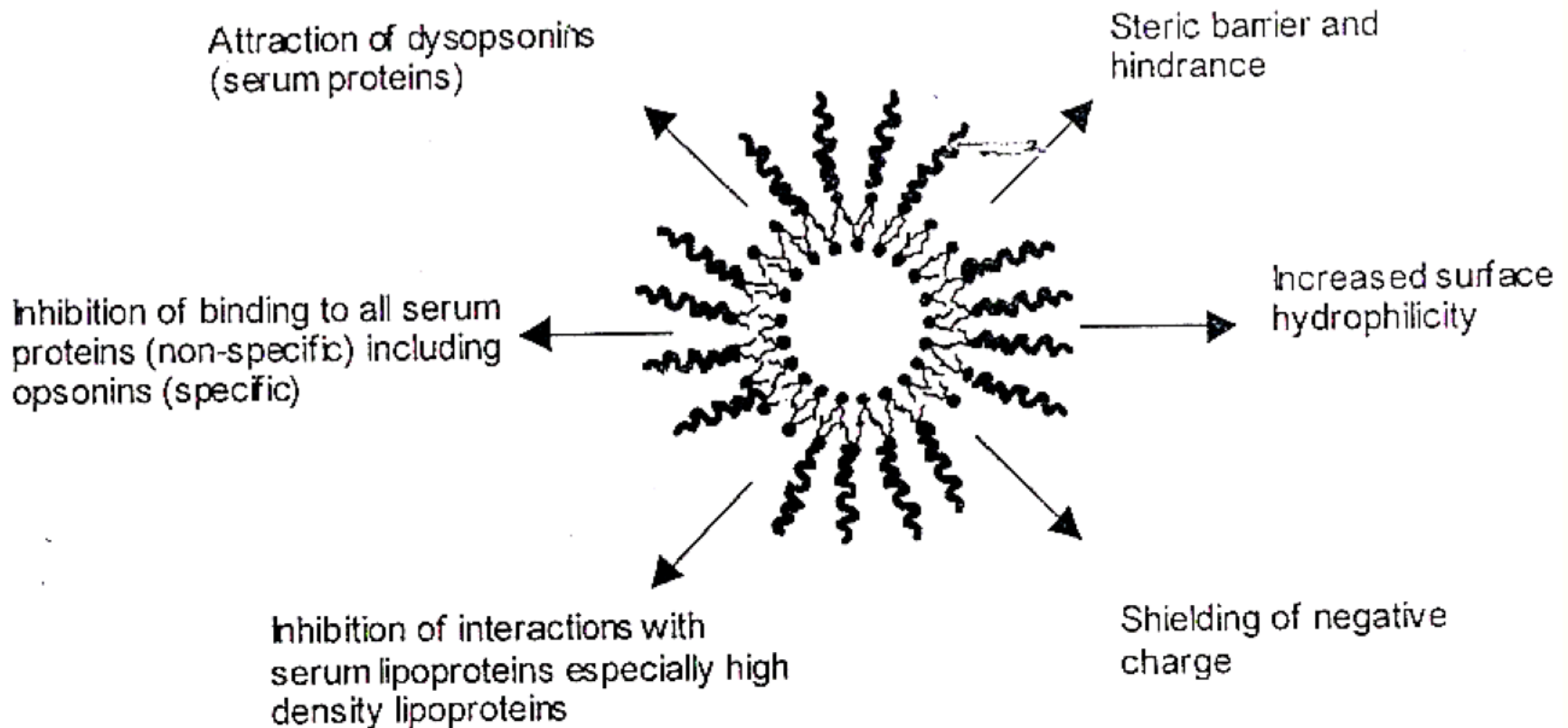
# THERAPEUTIC APPLICATION OF LIPOSOMES

## STEALTH LIPOSOMES:



# THERAPEUTIC APPLICATION OF LIPOSOMES

## PROPOSED MECHANISMS FOR STEALTH LIPOSOMES:



# THERAPEUTIC APPLICATION OF LIPOSOMES

## INCREASED THERAPEUTIC INDEX:

- Liposomes comprised of lipids that are relatively non toxic, non immunogenic, biocompatible and biodegradable lipids can deliver the drug systemically with an increased therapeutic index and minimized toxicity index.
- Similarly, site avoidance and selective delivery can be appreciated for drugs that usually have a narrow therapeutic index and that can be highly toxic to normal tissues.



# THERAPEUTIC APPLICATION OF LIPOSOMES

## LIPOSOMES AS A LYSOSOMOTROPIC CARRIER:

- Liposomes have been used as lysosomotropic carriers to en route the enzyme and supplement it therapeutically in enzyme deficiency diseases like Gaucher's disease ( $\beta$ -glucosidase deficiency) or Pompe's disease ( $\alpha$ -glucosidase deficiency).
- A variety of lysosomal enzymes can be entrapped in liposomes and can be delivered to patients suffering from lysosomal storage disorders.
- Liposomes have also been used in the treatment of metal poisoning, since the liver is the major site of heavy metal and iron accumulation.

# THERAPEUTIC APPLICATION OF LIPOSOMES

## LIPOSOMES IN MULTI-DRUG RESISTANCE

Multi-drug resistance is often associated with the over-expression of some drug-efflux pumps, known as P-glycoprotein pump (PGP) and multi-drug resistance associated protein pump (MRP).

Several mechanisms are proposed through which liposomes avoid multi-drug resistance of encapsulated drugs.

1. Negatively charged phospholipids (phosphatidylserine or cardiolipin) used in the liposomal formulations may directly regulate the P-glycoprotein transporter.
2. Liposomes may provide sustained and high levels of drug to resistant cells over long period of time.
3. The lysosomal localization of the drug protects it from the action of the P-glycoprotein, by avoiding immediate contact with P-glycoprotein transporter located at the plasma membrane.

# THERAPEUTIC APPLICATION OF LIPOSOMES

## **LIPOSOMES IN ANTIMICROBIAL, ANTIFUNGAL (LUNG THERAPEUTICS) AND ANTIVIRAL (ANTI-HIV) THERAPY:**

- Due to their intrinsic passive vectorization to RES-predominant organs, liposomes offer enormous potentials and opportunities for targeted drug delivery to intracellular pathogens like leishmaniasis, candidiasis, aspergillosis, histoplasmosis, cryptococosis ,giardiasis, malaria and tuberculosis.

# THERAPEUTIC APPLICATION OF LIPOSOMES

## AMPHOTERICIN B LIPOSOMES:

- Recent advances has focused on the improvement of its therapeutic index, through reduction of Amp B toxicity by its incorporation in lipid carriers.
- Several lipid formulations of Amp B are available in the market and include Fungizone (Bristol Myers- squibb, Woerden, The Netherlands), AmBisome (Nexstar, Boulder, CO, USA), ABELECT (The liposome company , Princeton, NJ, USA) and AMPHOTEC (Sequss Pharmaceuticals, Merlo, CA, USA)
- The rationale approach to the problem requires the drugs should be targeted to the macrophages in such a way that the interaction of the free drug with normal target tissues could be minimized.

# THERAPEUTIC APPLICATION OF LIPOSOMES

## LIPOSOMES AS A CARRIER OF IMMUNOMODULATORS

- The main purpose is to activate macrophages and render them tumouricidal. If macrophages are activated to their tumouricidal state, they become an ideal modality for the treatment of metastatic diseases that are resistant to other forms of therapy.
- Tumouricidal macrophages acquire the ability to recognize and destroy neoplastic cells both invitro and in vivo, while leaving normal cels unharmed by a non-immunological mechanism that requires cell-to-cell contact.
- The problem of rapid clearance of systemically administered cytokines and macrophage activators (as immunomodulators) and pathogenicity of microorganisms and their products can be overcome by encapsulating activating agents into liposomes composed of phospholipids.

# THERAPEUTIC APPLICATION OF LIPOSOMES

## LIPOSOMES AS RED CELL SUBSTITUTES AND ARTIFICIAL RBC'S

- Liposome encapsulated haemoglobin products are being investigated as artificial RBC'S (oxygen carrying rbc substitutes).
- Tsuchida reported that the aggregation and fusion of haemoglobin vesicles (Hb vesicles) and the leakage on long term storage can be prevented by using either polymerised phospholipids or by introduction of oligosachharide type of glycolipid in the bilayer membrane.
- Sterically stabilized liposome bearing haemoglobin are even better as they manifest less toxicity, less platelet activation and aggregation and less haemostatic generation.

# THERAPEUTIC APPLICATION OF LIPOSOMES

## LIPOSOMES AS RADIOPHARMACEUTICAL & RADIODIAGNOSTIC CARRIERS

- Liposomes loaded with the appropriate contrast agents have shown to be suitable for all imaging modalities. The imaging modalities are
  - $\gamma$ -scintigraphy
  - Magnetic resonance
  - Computed tomography
  - Ultra sound imaging or ultra sonography
- Liposomes (conventional and targeted including immunoliposomes and stealth liposomes) are used in different imaging modalities to locate the sites specifically. Liposomal uptake by RES, which is useful strategy in localization of contrast agents in RES rich organs like liver, spleen and bone marrow, is not useful for localization to non RES organs.

# Conclusions

- ❖ Liposomes over the years have been investigated as the major drug delivery systems due to their flexibility to be tailored for varied desirable purposes.
- ❖ The flexibility in their behaviour can be exploited for the drug delivery through any route of administration and for any drug or material irrespective of its physicochemical properties.
- ❖ The uses of liposomes in the delivery of drugs and genes to tumour sites are promising and may serve as a handle for focus of future research.



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# Suggestions

Please.....!!!!!!!!!!!!!!!!!!!!!!

**Thanking You  
One & All.....!!!!!!**