LIPOSOMES

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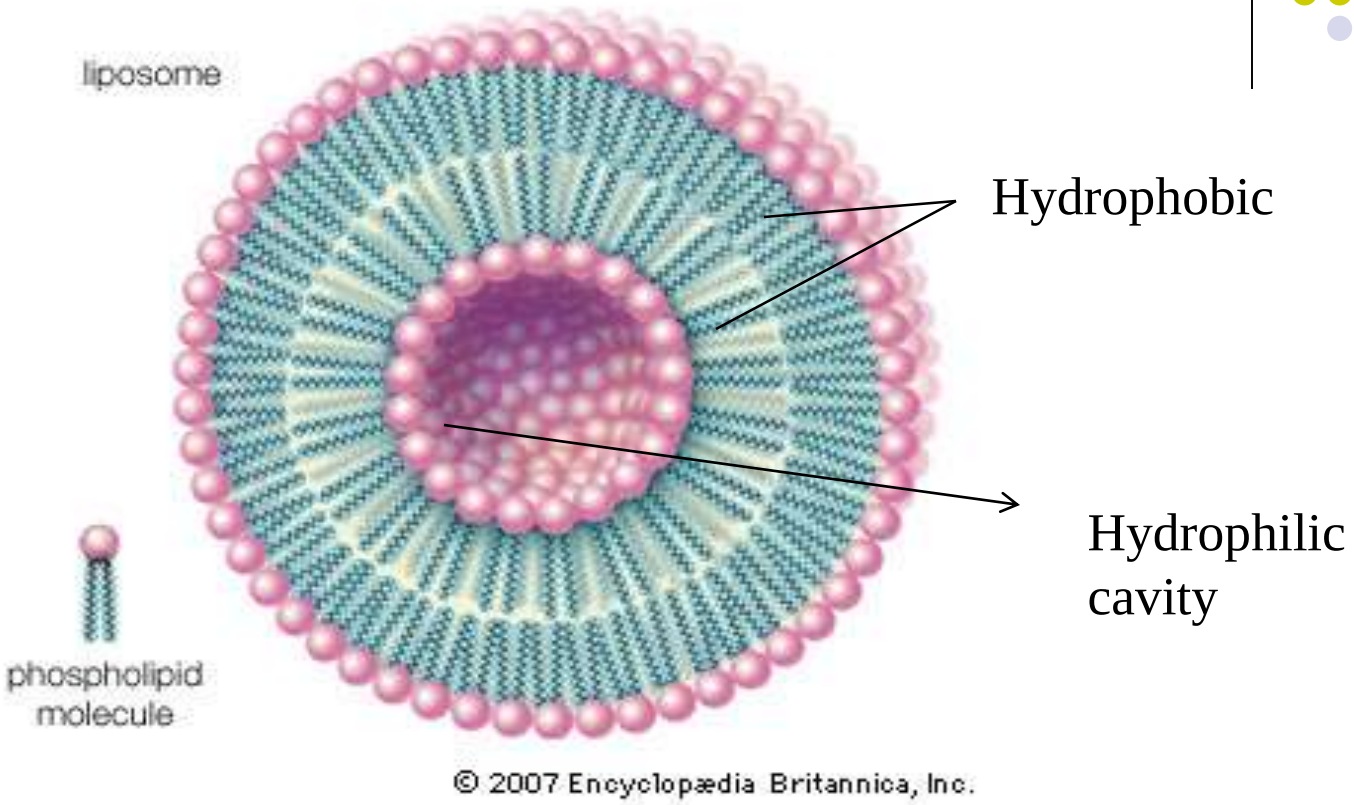
CHIPS

- Liposomes are simple microscopic vesicles in which an aqueous volume is entirely enclosed by a membrane composed of lipid molecule.
- Structurally, liposomes are concentric bilayered vesicles in which an aqueous volume is entirely enclosed by a membraneous lipid bilayer mainly composed of natural or synthetic phospholipids.


liposome
Hydrophobic
Hydrophilic cavity
phospholipid molecule
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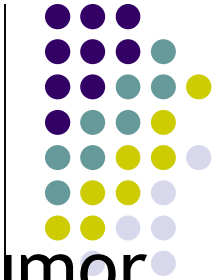
ADVANTAGES OF LIPOSOMES

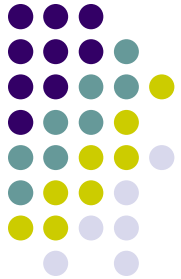


- Provides selective passive targeting to tumor tissues (liposomal doxorubicin)
- Increased efficacy and therapeutic index
- Reduction in toxicity of the encapsulated agent
- Site avoidance effect (avoids non-target tissues)
- Improved pharmacokinetic effects (reduced elimination increased circulation life times)
- Flexibility to couple with site specific ligands to achieve active targeting

DISADVANTAGES

- The development of liposomes at industrial level is difficult due to its physiological and physicochemical instability.
- They aggregate and fuse together upon prolonged storage disturbing the reproducibility.
- They are prone to degradation by oxidation and hydrolysis.

STRUCTURAL COMPONENTS OF LIPOSOMES

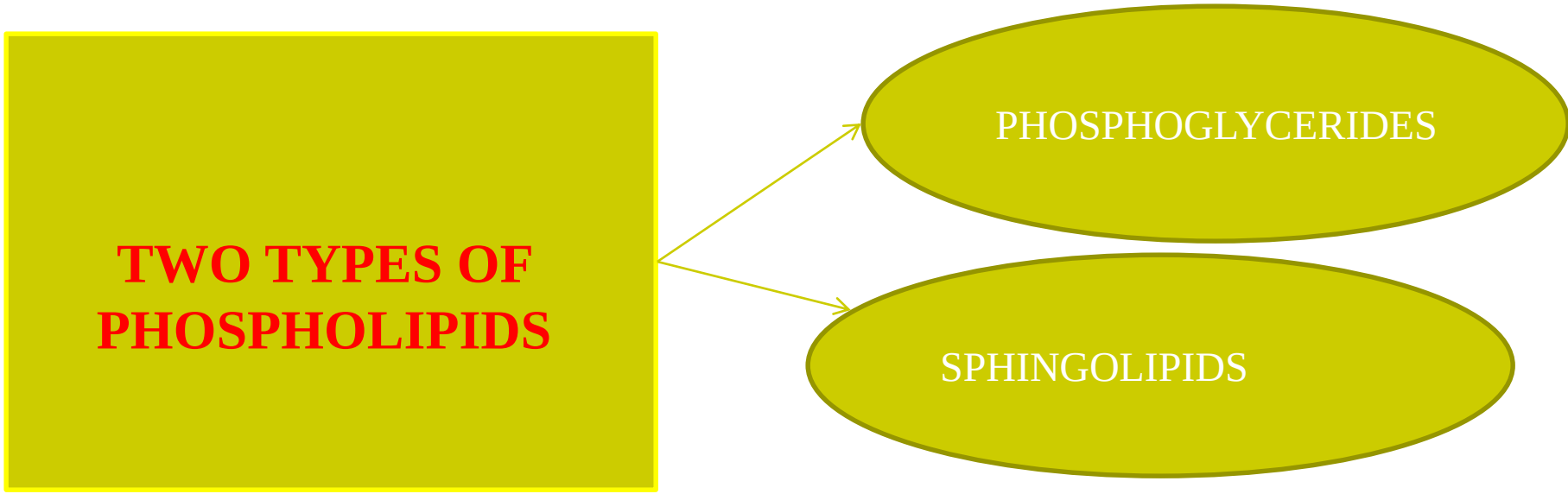


The main components of liposomes are

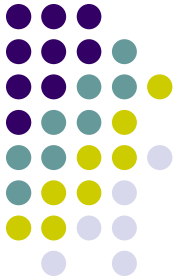
- PHOSPHOLIPIDS
- CHOLESTEROL

PHOSPHOLIPIDS

- Phospholipids are the major structural component of biological membranes such as the cell membrane.

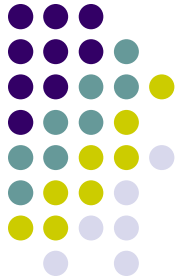


Some Other Commonly Used Phospholipids



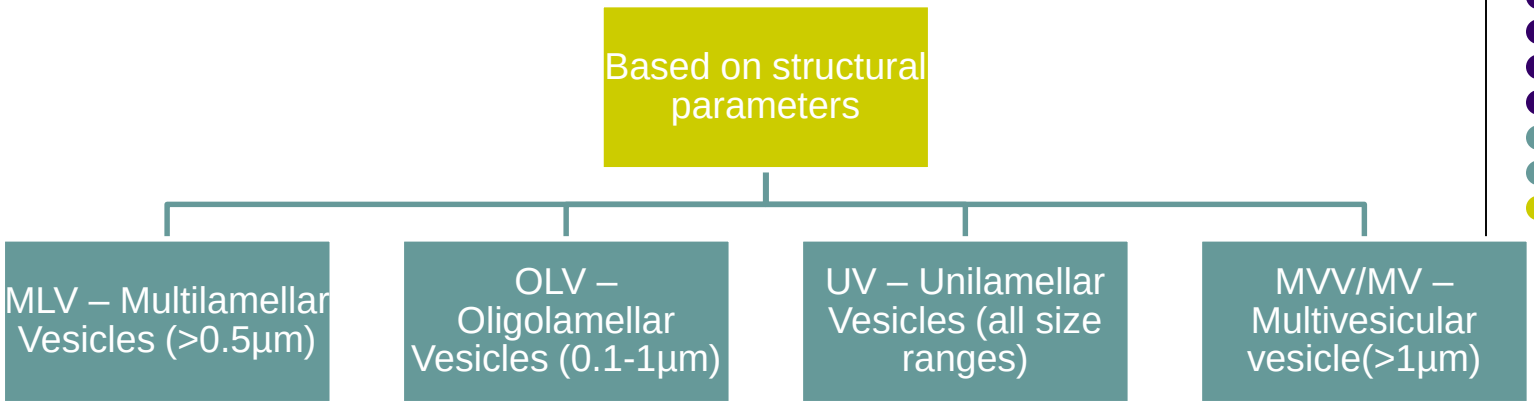
- Naturally occurring phospholipids:
  - -PC : Phosphatidylcholine
  - – PE : Phosphatidylethanolamine
  - – PS : Phosphatidylserine
- Synthetic phospholipids:
  - -DOPC : Dioleoylphosphatidylcholine
- – DSPC : Distearoylphosphatidylcholine

CHOLESTEROL



- Incorporation of sterols in liposome bilayer brings about major changes in the preparation of these membranes.
- Cholesterol by it self does not form a bilayer structure.

However, cholesterol acts as a fluidity buffer, i.e. below the phase transition temperature, it makes the membrane less ordered and slightly more permeable; while above the phase transition temperature it makes the membrane more ordered and stable.


Based on structural parameters
MLV – Multilamellar Vesicles (>0.5µm)
OLV – Oligolamellar Vesicles (0.1-1µm)
UV – Unilamellar Vesicles (all size ranges)
MVV/MV – Multivesicular vesicle(>1µm)

CLASSIFICATION :

- **Based on Structural Parameters:**

  a. **Multi-laminar vesicles (MLV):** made up of series of concentric bi-layer of lipid enclosing a small internal volume with size range > 0.5um.

  b. **Oligolamelar vesicles (OLV):** constitutes 2 to 10 bi layer of lipids surrounding a large internal volume with size range of 0.1 – 1um.

c. **Unilamellar vesicle (ULV):** single layer of lipids. Based on the size of the single layer they are further divide into the following types with in ULV as

- Small unilaminar vesicle: size of 20 to 40 nm
- Medium unilaminar vesicle: size of 40 to 80 nm
- Large unilaminar vesicle: size of 100 to 1000 nm
- Gaint unilaminar vesicle: size of more than 1000 nm

d. **Multivesicular Vesicle (MV):** constitutes for multiple vesicles and size range >1um.

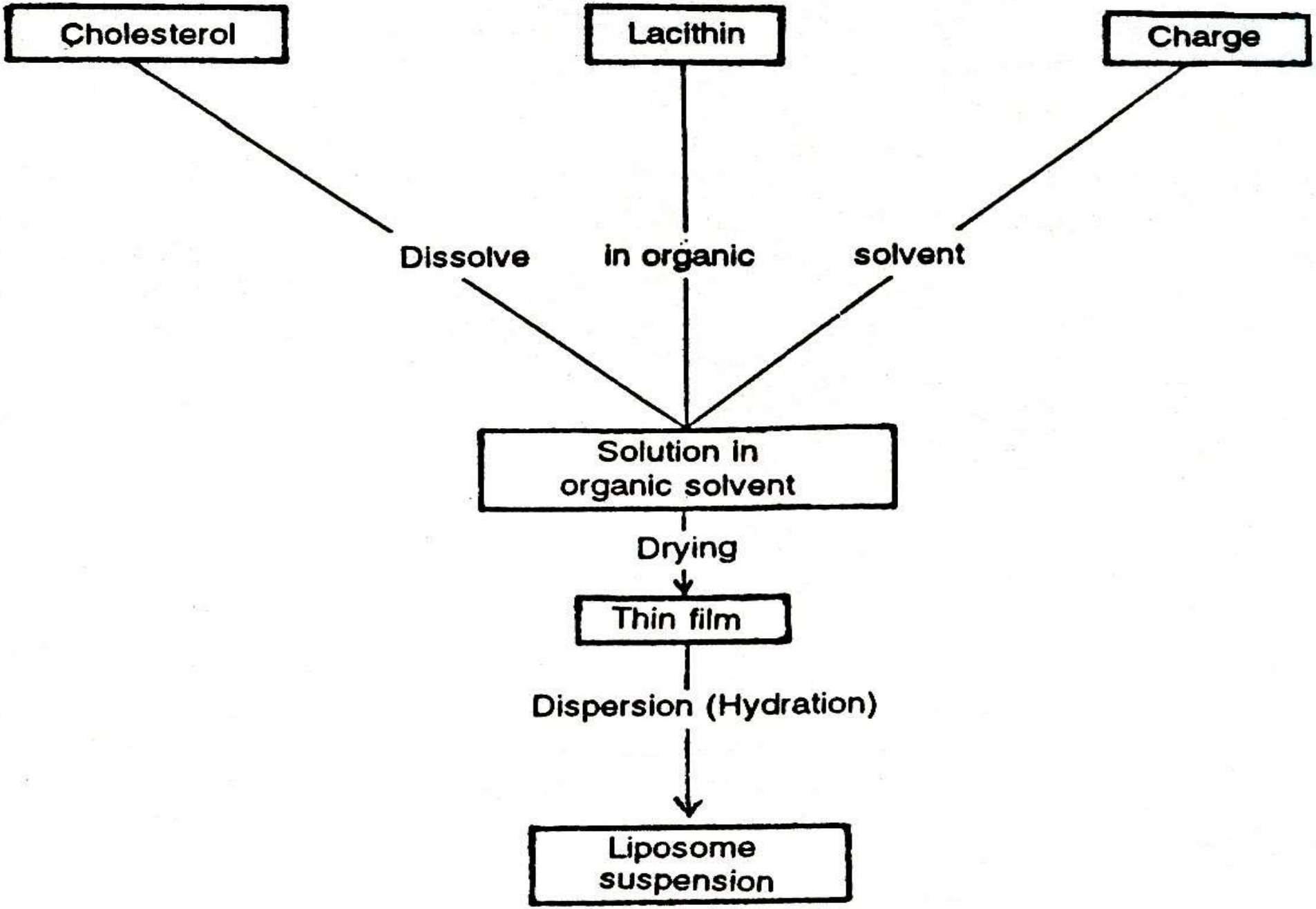
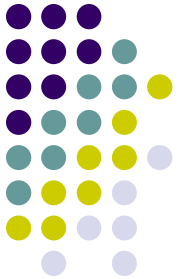


Fig. 15.4. Common stages of all methods of preparation of liposomes.

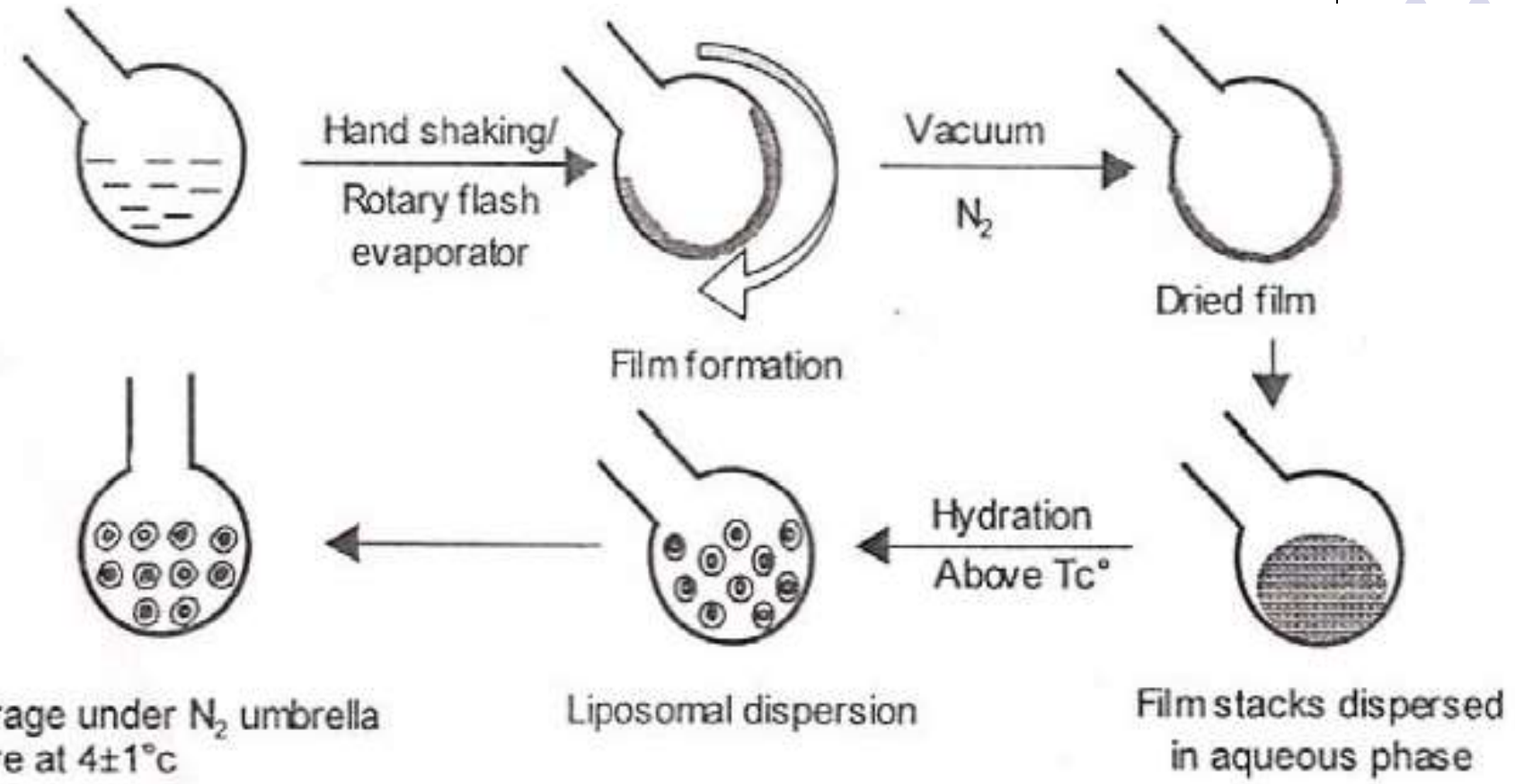
Method of Preparation of Liposomes



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

HAND SHAKING METHOD:



Multilamellar Vesicles (MLVs) Formed by either Hand Shaking Technique or Using Rotary Flash Evaporator

Micro Emulsification Liposomes (Mel):

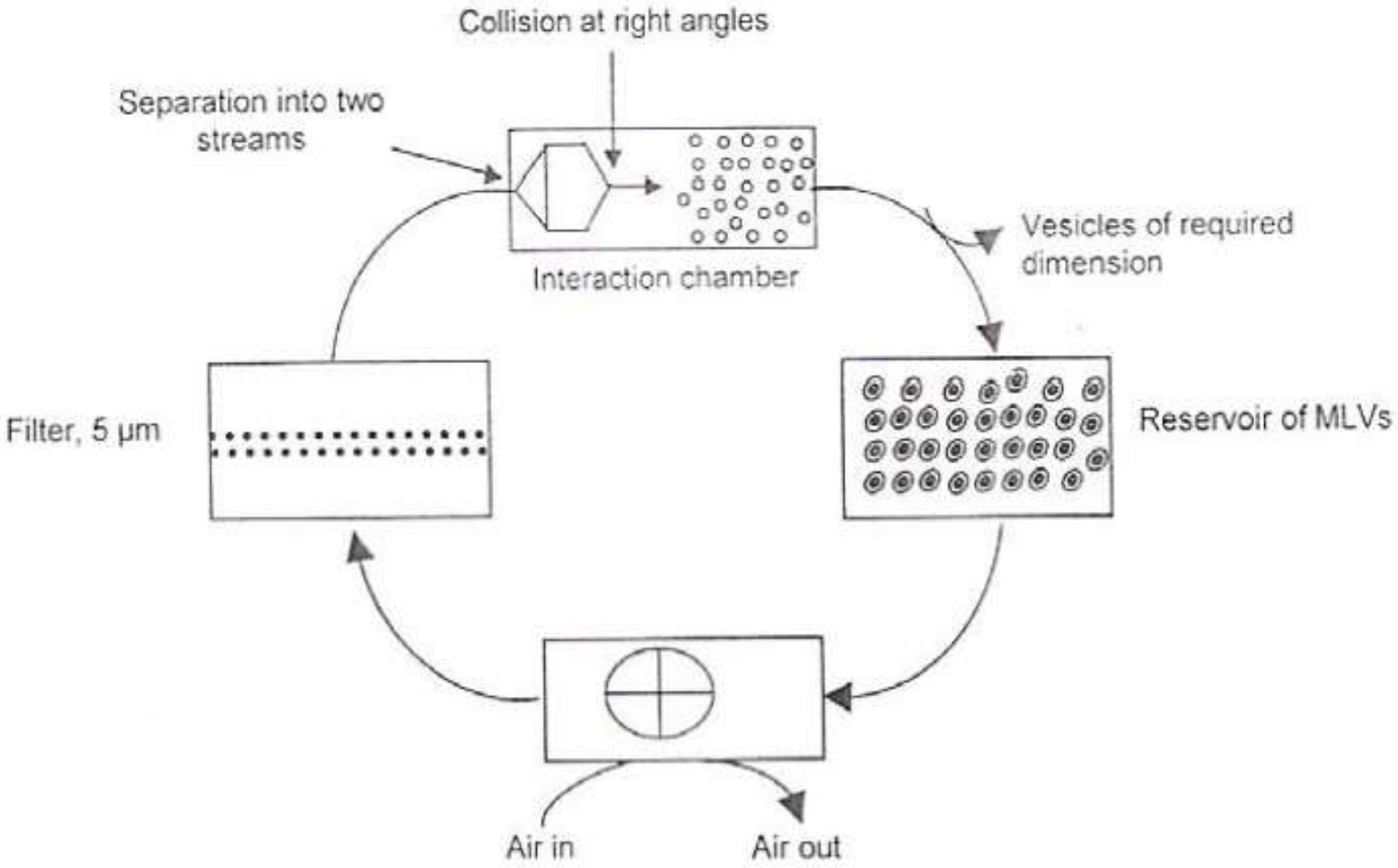


Fig. 5-10. Representation of Use of Micro-Fluidizer to Prepare Small Unilamellar Vesicles (SUVs) From MLVs

Sonicated unilamellar vesicles (SUVs)

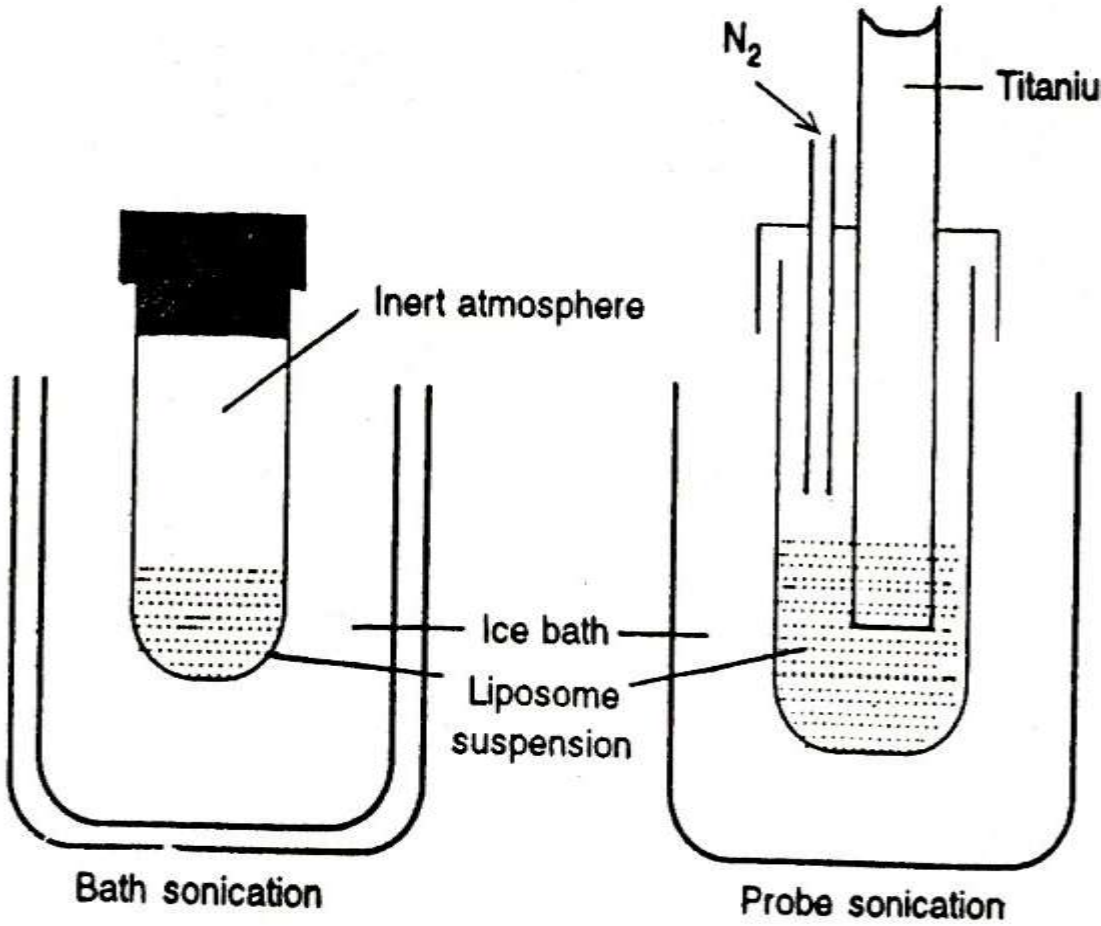


Fig 15.6. Preparation of liposomes by sonication.

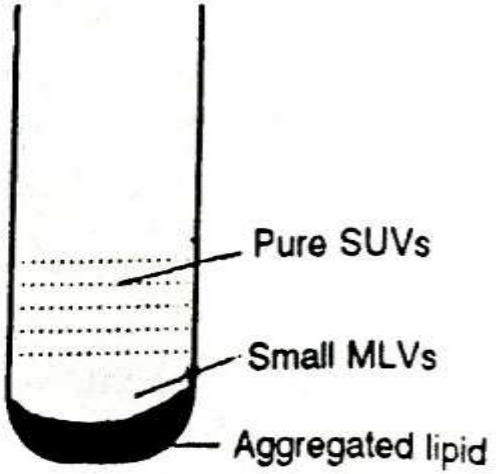


Fig. 15.7. Purification of small unilamellar vesicles.

FRENCH PRESSURE CELL

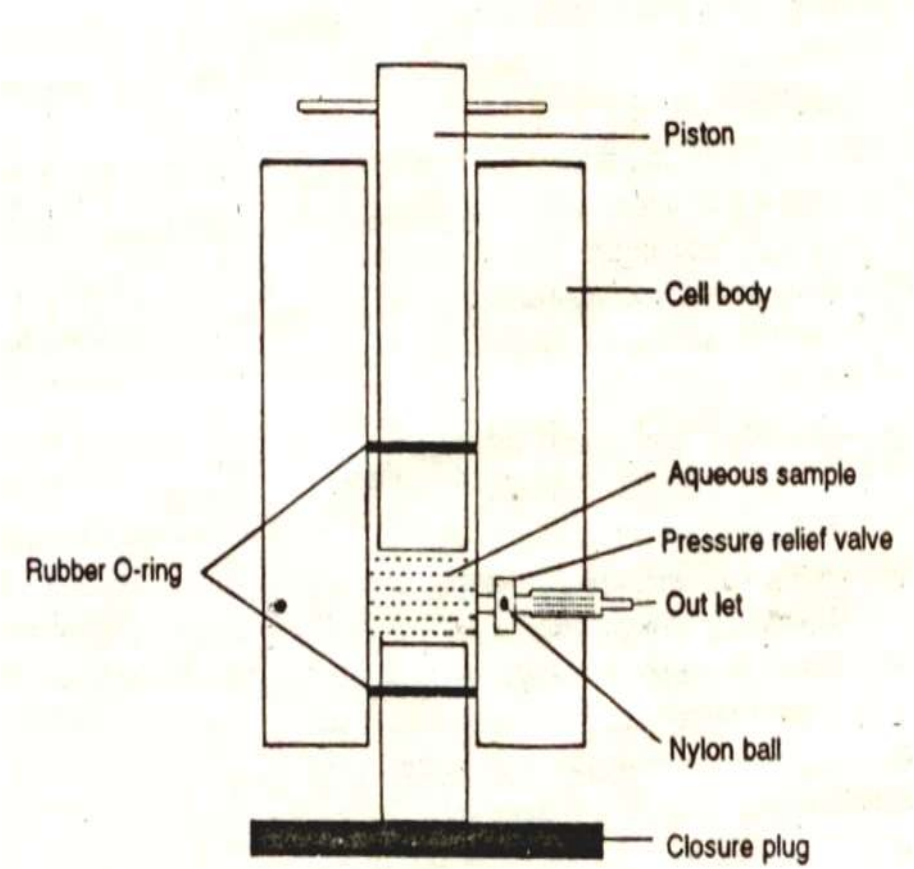


Fig. 15.8. French pressure cell.

Solvent Dispersion methods

- Ethanol injection method
- Ether injection method

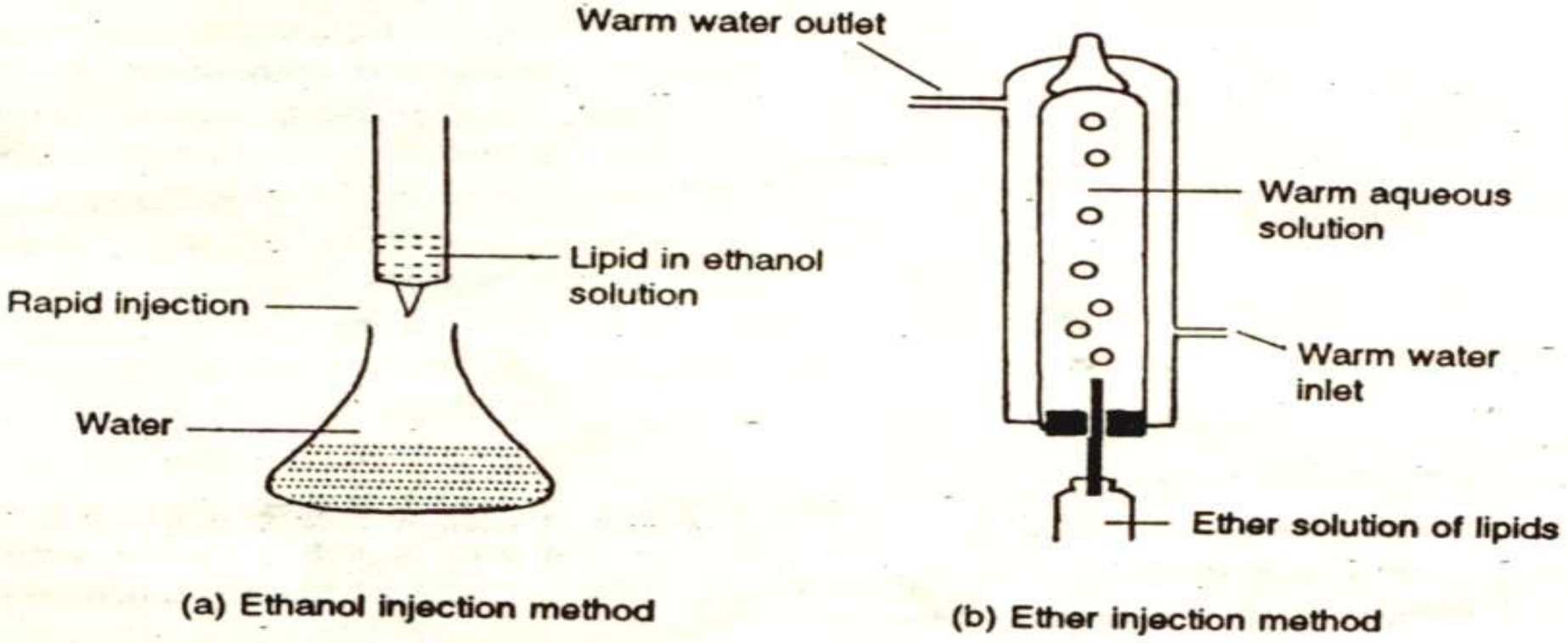


Fig. 15.10. Solvent dispersion techniques for liposome preparation.

CHARACTERISATION OF LIPOSOMES:

	Characterization parameters	Analytical method/Instrument
1.	Vesicle shape and surface morphology	Transmission electron microscopy, Freeze-fracture electron microscopy
2.	Mean vesicle size and size distribution (submicron and micron range)	Dynamic light scattering, zetasizer, Photon correlation spectroscopy, laser light scattering, gel permeation and gel exclusion
3.	Surface charge	Free-flow electrophoresis
4.	Electrical surface potential and surface pH	Zetapotential measurements & pH sensitive probes
5.	Phase behavior	Freeze-fracture electron microscopy, Differential scanning colorimetery
6.	Percent of free drug/ percent capture	Minicolumn centrifugation, ion-exchange chromatography, radiolabelling
7.	Drug release	Diffusion cell/ dialysis

2. CHEMICAL CHARACTERISATION

	Characterization parameters	*Analytical method/Instrument*
1.	Phospholipid concentration	Barlett assay, stewart assay, HPLC
2.	Cholesterol concentration	Cholesterol oxidase assay and HPLC
3.	Phopholipid peroxidation	UV absorbance, Iodometric and GLC
4.	Phospholipid hydrolysis, Cholesterol auto-oxidation.	HPLC and TLC
5.	Osmolarity	Osmometer

3. BIOLOGICAL CHARACTERISATION

	Characterization parameters	*Analytical method/Instrument*
1.	Sterility	Aerobic or anaerobic cultures
2.	Pyrogenicity	Limulus Amebocyte Lysate (LAL) test
3.	Animal toxicity	Monitoring survival rates, histology and pathology

Marketed product	*Drug used*	*Target diseases*	*Company*
Doxil™ or Caelyx™	Doxorubicin	Kaposi's sarcoma	SEQUUS, USA
DaunoXome™	Daunorubicin	Kaposi's sarcoma, breast & lung cancer	NeXstar, USA
Amphotec™	Amphotericin-B	fungal infections, Leishmaniasis	SEQUUS, USA
Fungizone®	Amphotericin-B	fungal infections, Leishmaniasis	Bristol-squibb, Netherland
VENTUS™	Prostaglandin-E_1	Systemic inflammatory diseases	The liposome company, USA
ALEC™	Dry protein free powder of DPPC-PG	Expanding lung diseases in babies	Britannia Pharm, UK
Topex-Br	Terbutaline sulphate	Asthma	Ozone, USA
Depocyt	Cytarabine	Cancer therapy	Skye Pharm, USA
Novasome®	Smallpox vaccine	Smallpox	Novavax, USA
VincaXome	Vincristine	Solid Tumours	NeXstar, USA

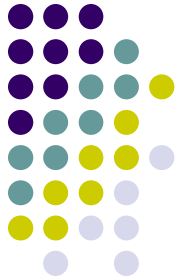
Liposomes as carrier of antigens

Antigen	Route of administration & animal species	Major observations
Diptheria toxoid	i.v , s.c mice	-ve liposome's with PA elicited greater absorption. DCP is superior to PA .s.c & i.m administration showed greater absorption.
Herpes simplex virus type 1 antigen	i.p mice	Enhanced immunogenicity with liposomal antigen.Max. absorption with liposome bound antigen containing lipid A. No CMP with liposome preparation.

Liposome's for topical application

DRUG	RESULTS
Benzocain gel	Shows prolonged anesthesia as compared to plain benzocaine cream
Hydrocortisone	High concentration of drug in individual layers of human skin than control ointment
Diclofenac gel	Increase concentration of drug in subcutaneous tissue and increased permeation through the skin

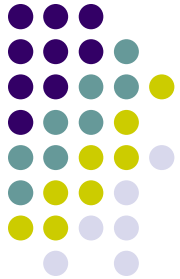
Liposome's for pulmonary delivery



DRUG	RESULTS
Enriroxine	Liposome encapsulated drug was observed to be 10 – 50 times less toxic to tissue culture cells than free drug
Atropine glutathione	Maintained much higher level of entrapped drug in lung than solution form
Tobramycin	Liposome entrapped drug shows pulmonary level 3 times higher than those of free drug
Salbutamol	Dehydrated liposome entrapped drug ensured an effective sustained release system following inhalation

Liposome's for ophthalmic delivery

DRUG	RESULTS
Idoxuridine	Improved efficacy of liposome's encapsulated drug in the treatment of Herpes simplex keratitis
Benzyl penicillin indoxol	Ocular bioavailability enhanced by delivery in liposome's
Penicillin G	Flux was enhanced by positive charged unilamellar liposome's



THANK YOU