LIPOSOMES: Charaterization and Apllications

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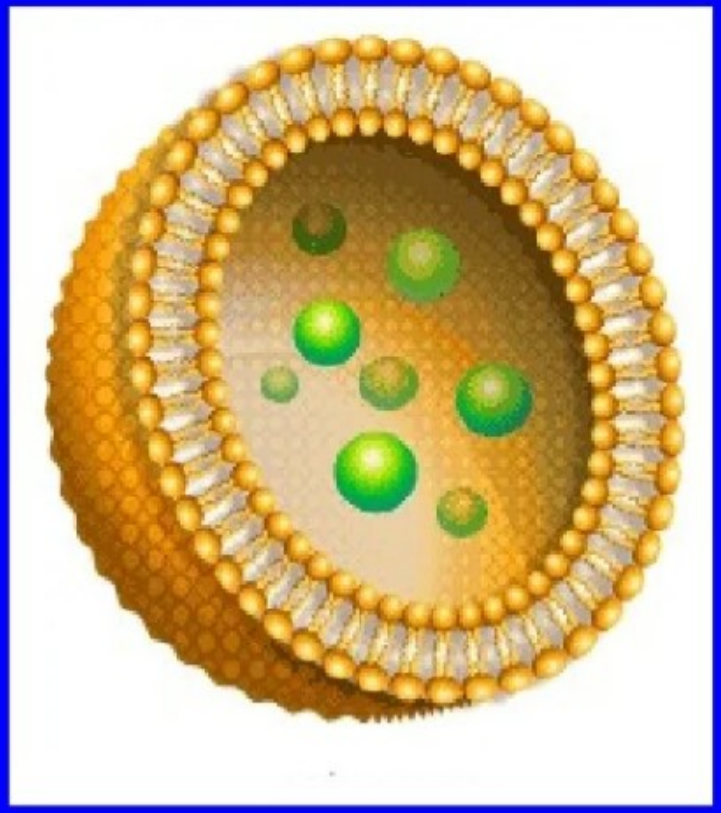
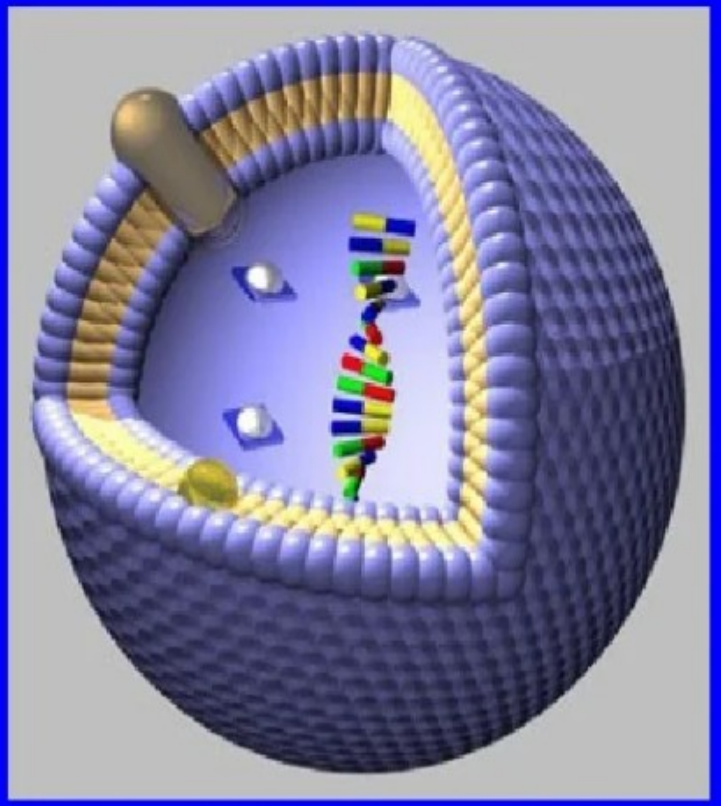
Targeted Drug Delivery System

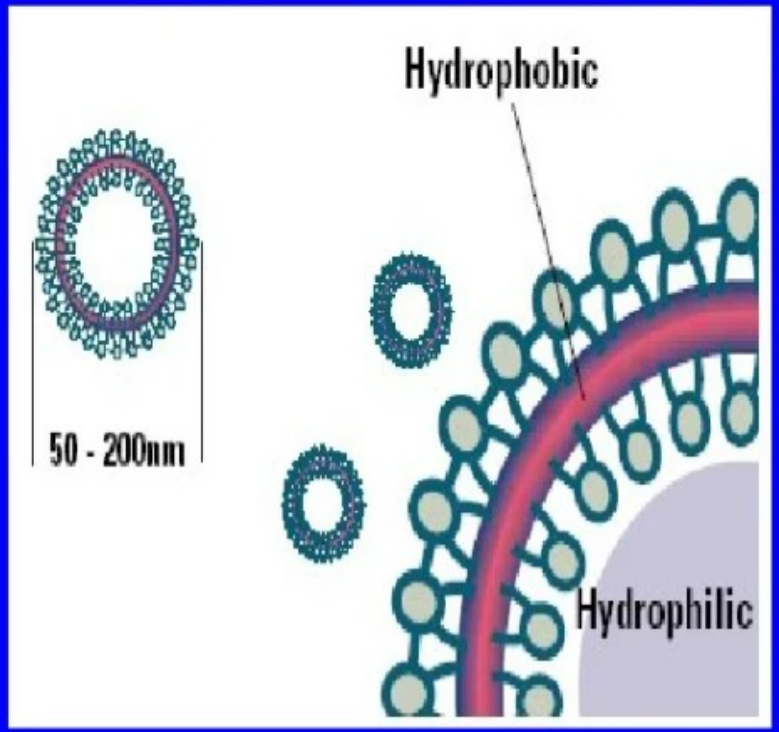
LIPOSOMES

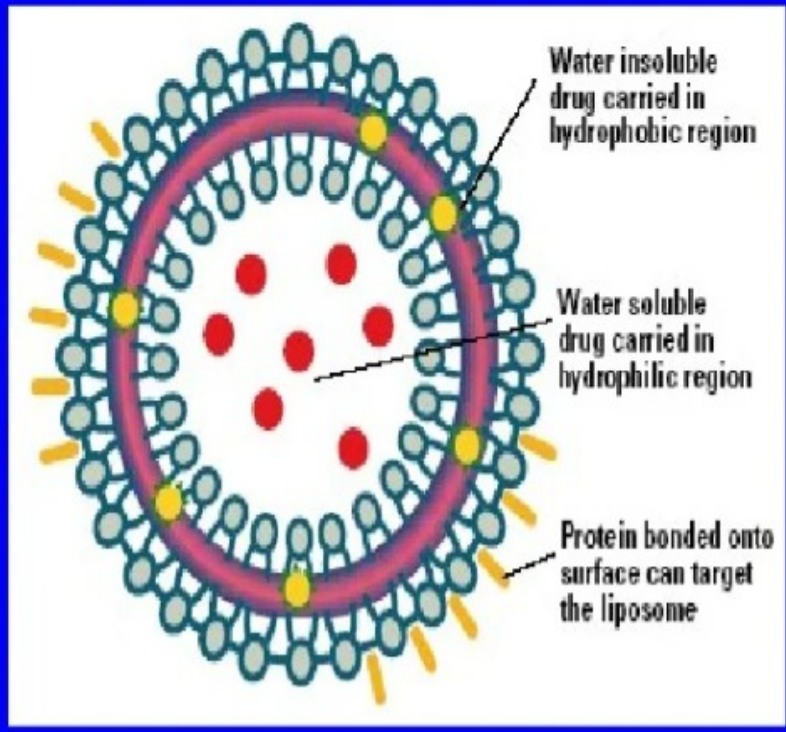




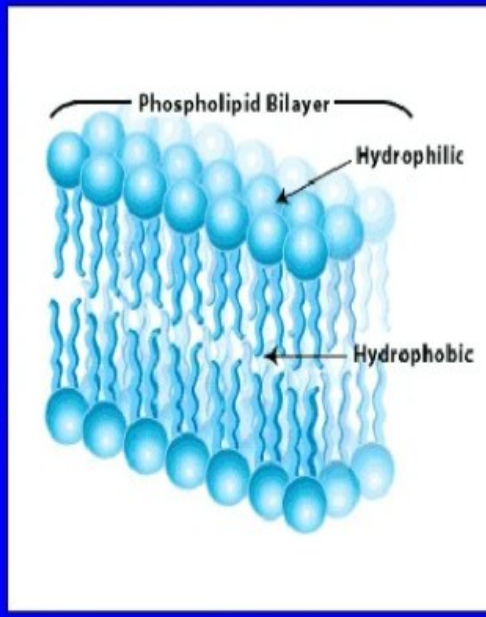
What are Liposomes?

- They are simply vesicles or 'bags' in which an aqueous volume is entirely enclosed by a membrane composed of lipid (fat) molecules, usually phospholipids.





•Structurally, liposomes are bilayered vesicles in which an aqueous volume is entirely enclosed by a membranous lipid bilayer mainly composed of natural or synthetic phospholipids.



- These vesicles can encapsulate water-soluble drugs in their aqueous spaces and lipid soluble drug within the membrane itself.

- The unique property of liposomes, namely their versatile, biodegradable, hypoallergenic nature, along with their similarity to biological membranes are the important factors in the continued efforts to develop liposomal drug delivery froms.

Advantages of liposome :

- Provides selective passive targeting to tumor tissues
- Increased efficacy and therapeutic index
- Increased stability via encapsulation
- Reduction in toxicity of the encapsulated agent.
- Improved pharmacokinetic effects
- Used as carriers for controlled and sustained drug delivery
- Can be made into variety of sizes.

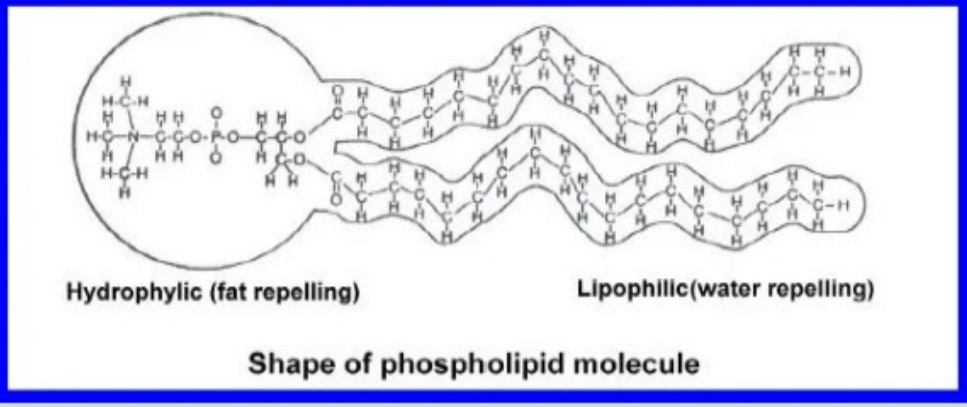
Liposomes Evolution :

- 1965 First description of closed lipid bilayer vesicles.
- 1967 introduction of the term liposomes to describe closed lipid bilayer vesicles
- 1972 liposomes first used as delivery systems of drugs
- 1974 first patients to be injected with liposomes
- 1979 liposomes first used as delivery systems of nucleic acids to cells
- 1980 first monoclonal anti body targeted liposomes termed imunoliposomes

- **1987** first synthetic cationic liposomes deliver genes to cells
- **1987** first sterically stabilized long circulating liposomes system introduced
- **1992** first lioposome based non viral vecrtor gene therapy clinical trail on cystic fibrosis patients
- **1993** frist liposome based vaccine against hepatitis A is marketed
- **1995** first long circulating immunoliposomes
- **1995** the liposomes encapsulated from of the anticancer drug doxorubicin and daunorubicin approved for human use
- **1997** first liposomes based DNA vaccine

Mechanism of liposome formation

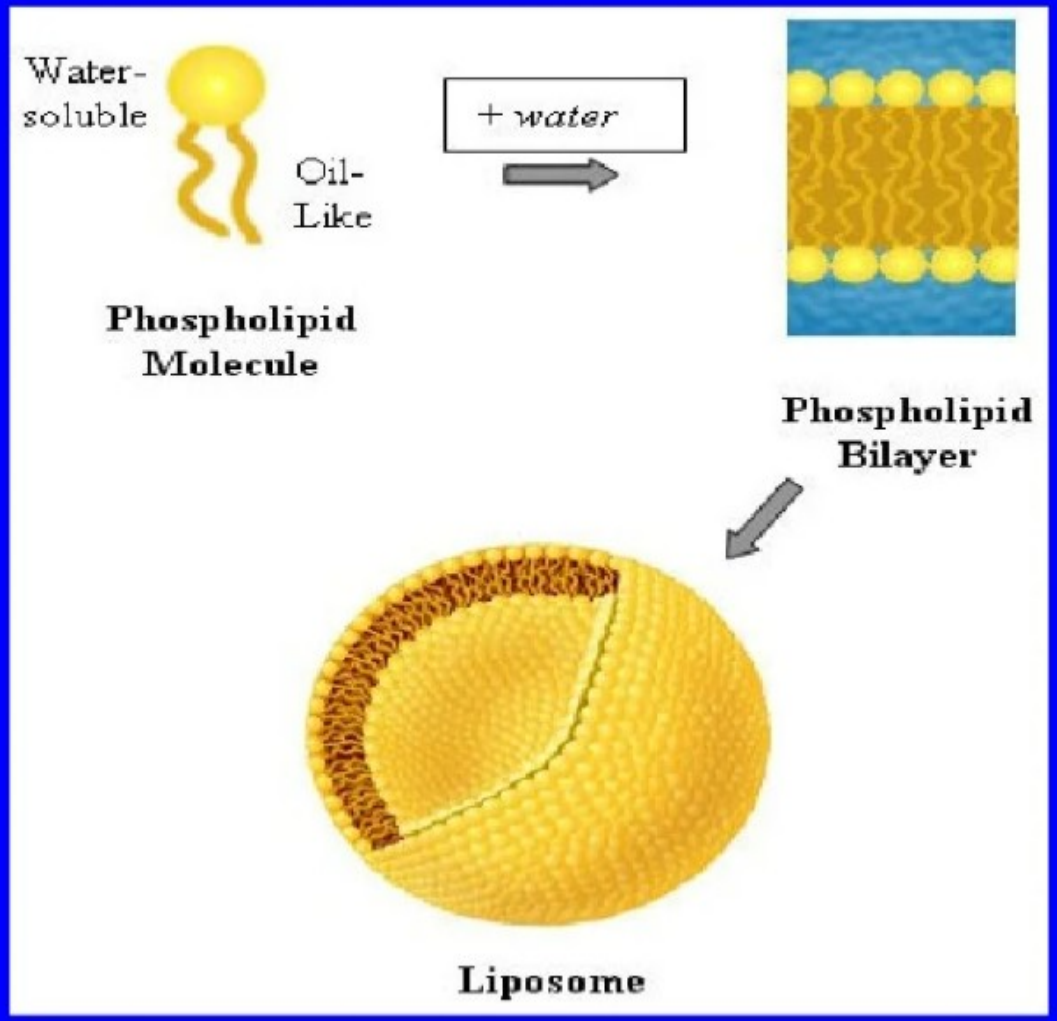
- In order to understand why liposomes are formed when phospholipids are hydrated, it requires a basic understanding of physiochemical features of phospholipids.
- Phospholipids are amphipathic molecules (having affinity for both aqueous and polar moieties) as they have a hydrophobic tail is composed of two fatty acids containing 10-24 carbon atoms and 0-6 double bonds in each chain.



Shape of phospholipid molecule

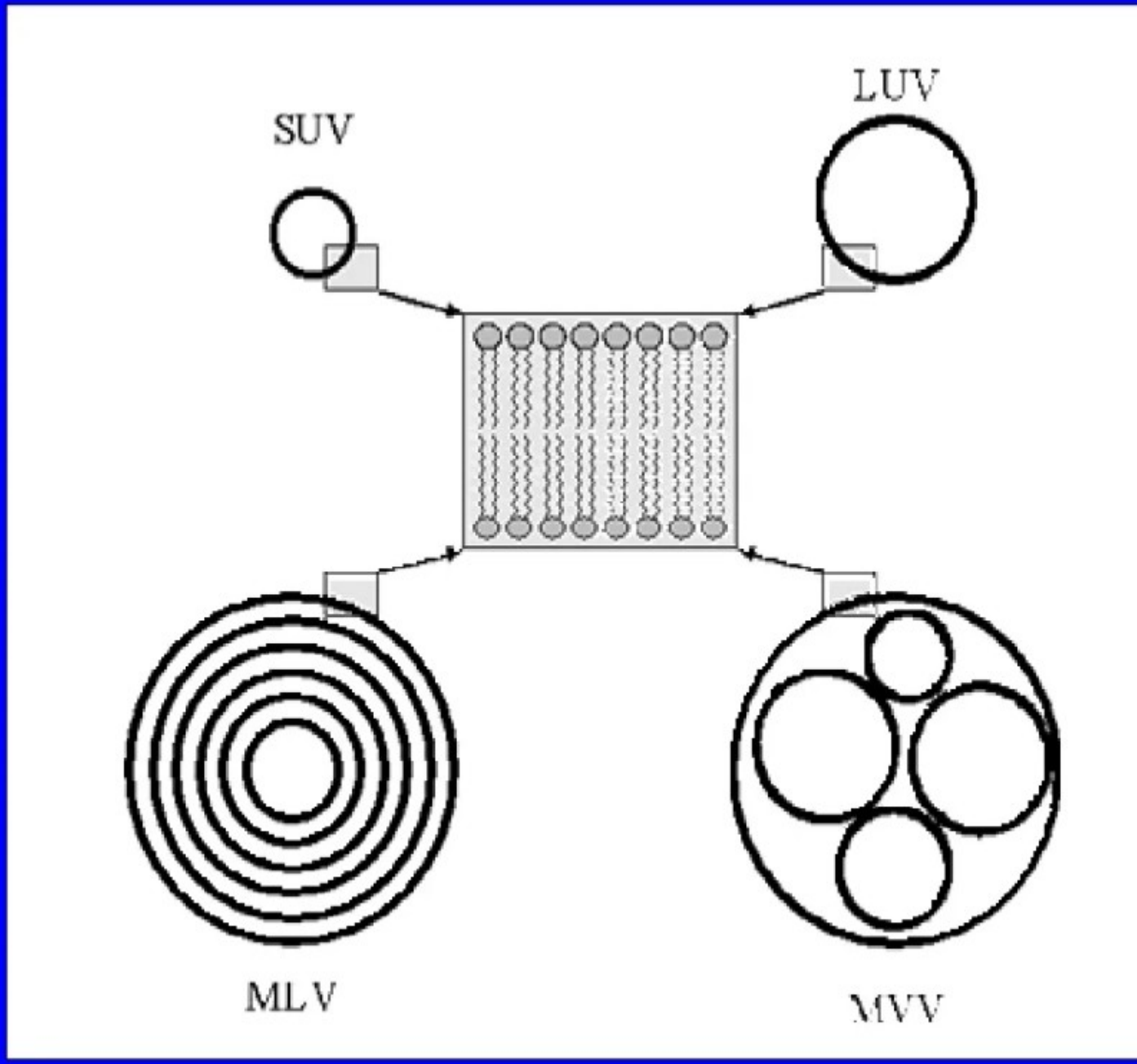
- In aqueous medium the phospholipid molecules are oriented in such a way that the polar portion of the molecule remains in contact with the polar environment and at the same shields the non-polar part.
- They align themselves closely in planer bilayer sheets to minimize the interaction between the bulky aqueous phase and long hydrocarbon fatty acyl chains.
- This alignment requires input of sufficient amount of energy (in the form of shaking, sonication, homogenization, heating, etc).

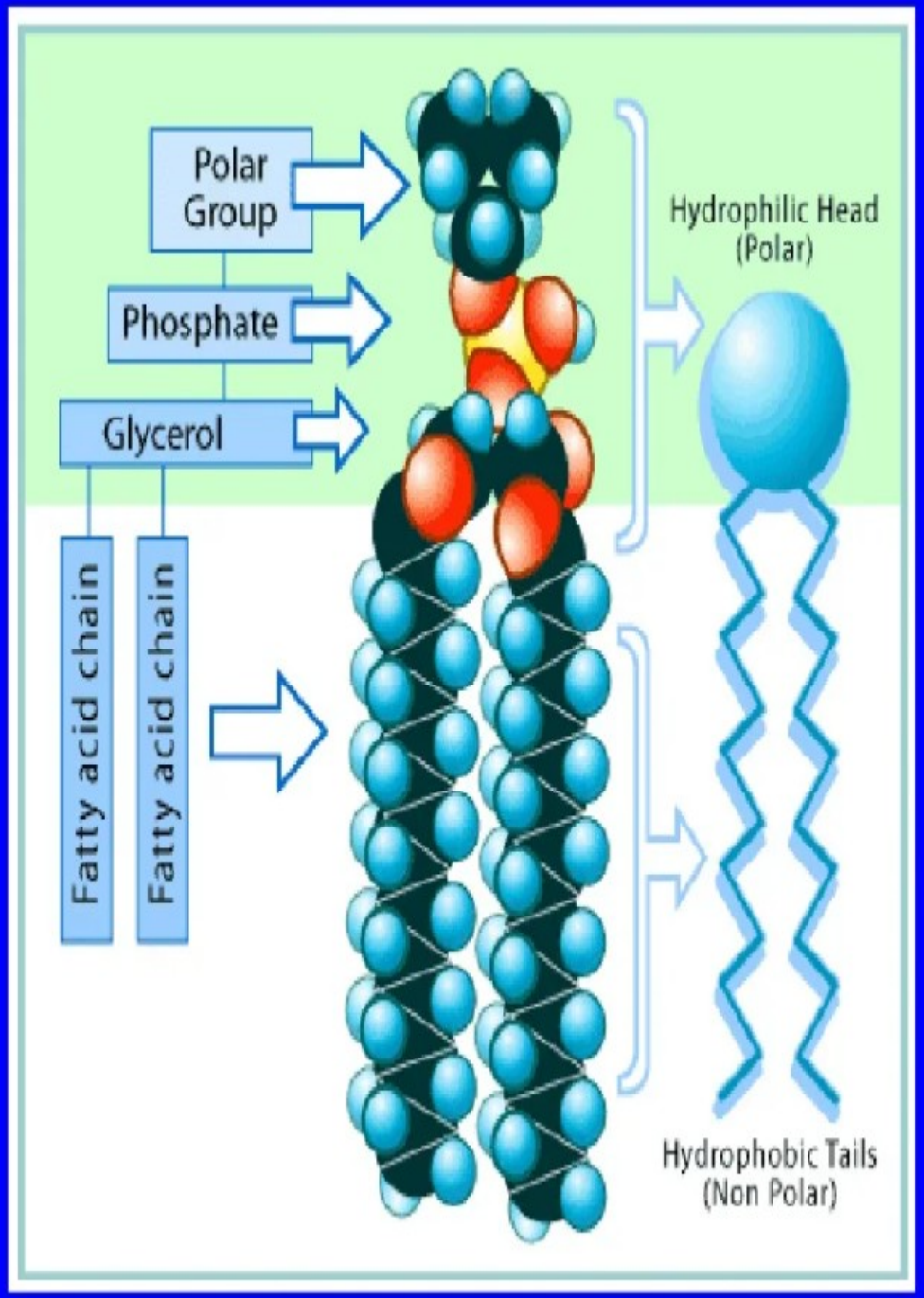
- Interactions are completely eliminated when these sheets fold over themselves to form closed, sealed and continuous bilayer vesicles

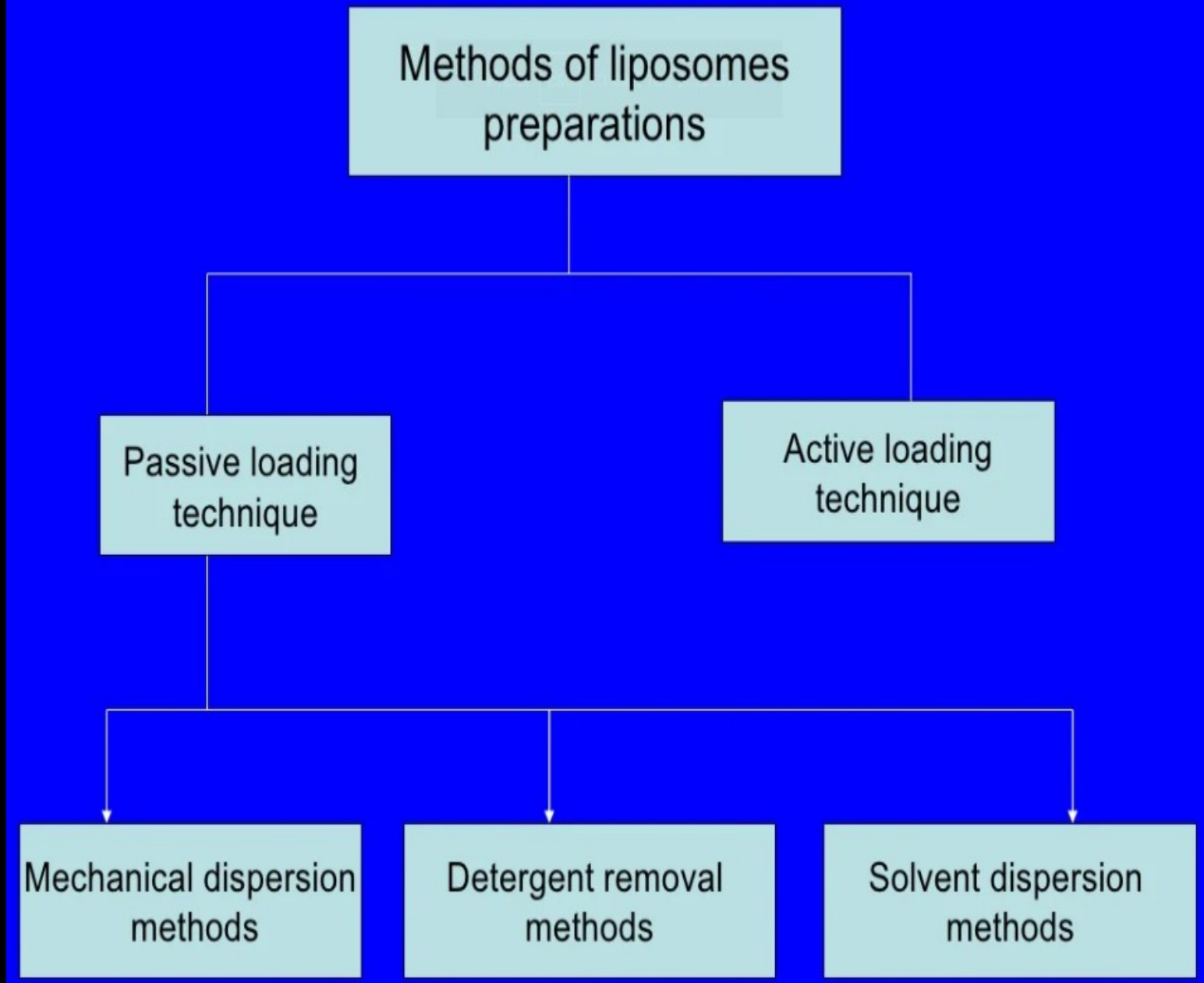


Classification of liposomes

1. Multilamellar vesicles (MLVs) : consist of several bilayers and having size ranging from 100nm-20m
2. Small unilamellar vesicles (SUVs) : composed of single lipid bilayer with dimeter ranging from 20-100nm
3. Large unilamellar vesicles (LUVs) : consist of single bilayer with diameter ranging from 0.1-1m
4. Multivesicular vesicles (MVVs) : consist of vesicles with size ranging from 100nm-20m


SUV
LUV
MLV
MVV


Polar Group
Phosphate
Glycerol
Fatty acid chain
Fatty acid chain
Hydrophilic Head (Polar)
Hydrophobic Tails (Non Polar)


Methods of liposomes preparations
Passive loading technique
Active loading technique
Mechanical dispersion methods
Detergent removal methods
Solvent dispersion methods

Physical Characterization

Parameter	Characterization method
Vesicle shape and surface morphology	Transmission electron microscopy, Freeze-fracture electron microscopy
Mean vesicle size and size distribution	Dynamic light scattering, zetasizer, Photon correlation spectroscopy, laser light scattering, gel permeation and gel exclusion
Surface charge	Free-flow electrophoresis
Electrical surface potential and surface pH	Zetapotential measurements & pH sensitive probes
Percent of free drug/ percent capture	Minicolumn centrifugation, ion-exchange chromatography, radiolabelling
Drug release	Diffusion cell/ dialysis

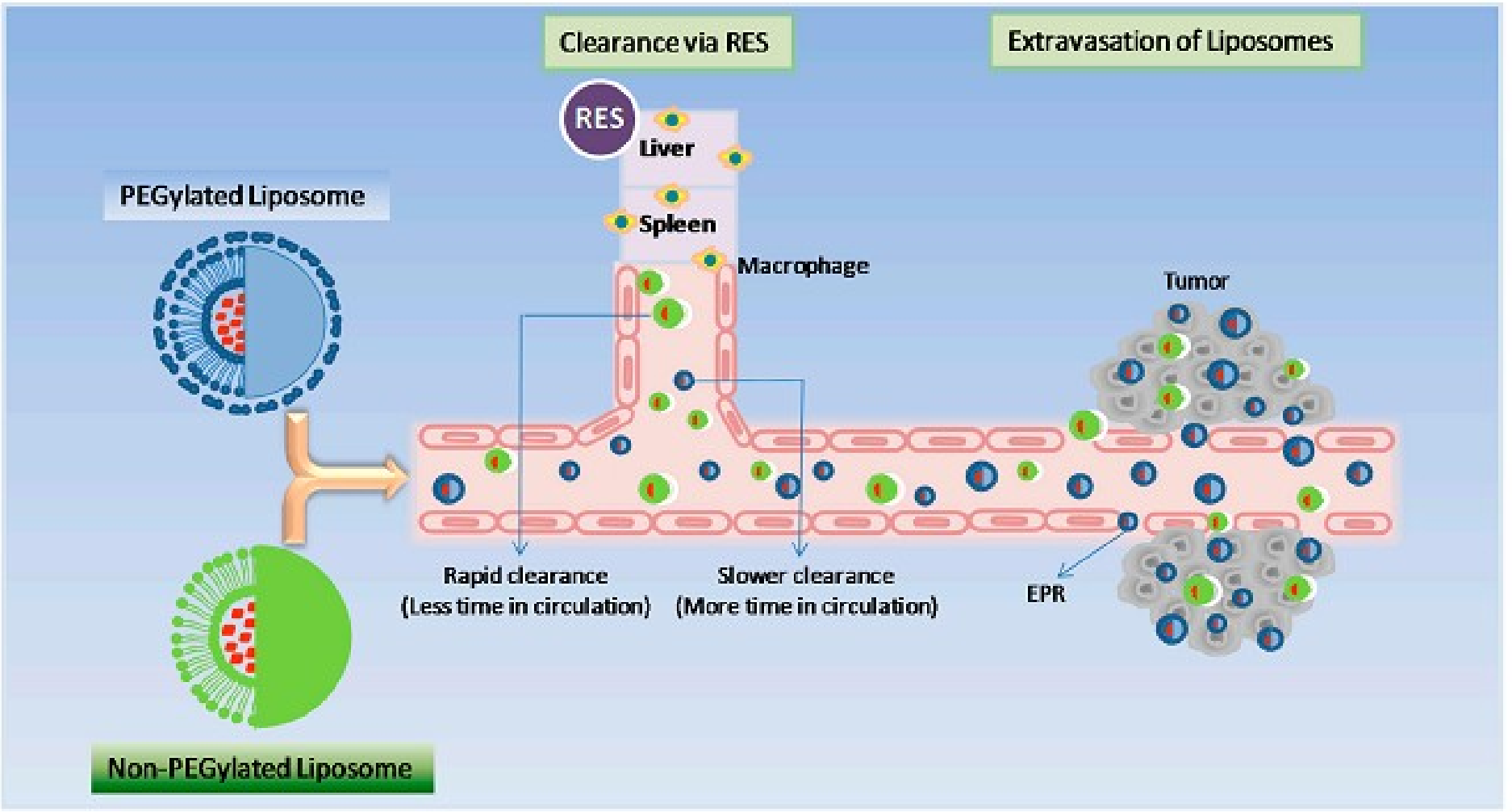
Chemical Characterization

Parameter	Characterization method
Phospholipid concentration	Barlett assay, stewart assay, HPLC
Cholesterol concentration	Cholesterol oxidase assay and HPLC
Phopholipid peroxidation	UV absorbance, Iodometric and GLC
Phospholipid hydrolysis, Cholesterol auto-oxidation	HPLC and TLC
Osmolarity	Osmometer

Biological Characterization

Parameter	Characterization method
Sterility	Aerobic or anaerobic cultures
Pyrogenicity	Limulus Amebocyte Lysate (LAL) test
Animal toxicity	Monitoring survival rates, histology and pathology

1. Liposomes in gene delivery
- Genes and antisense therapy
- Genetic (DNA) vaccination
4. Liposomes in immunology
5. Liposomes as radiopharmaceutical and radio diagnostic carrier
6. Liposomes in cosmetic and dermatology
7. Liposomes in enzyme immobilization and bioractor technology


Clearance via RES
Extravasation of Liposomes
RES
Liver
Spleen
Macrophage
PEGylated Liposome
Tumor
Rapid clearance
(Less time in circulation)
Slower clearance
(More time in circulation)
EPR
Non-PEGylated Liposome


Doxil®
DaunoXome®
Depocyt®
Mepact®
Marqibo®
Myocet®
Onivyde™
Abelcet®
Ambisome®
Amphotec®
DepoDur™
Exparel®
Visudyne®
Inflexal® V
Epaxal®
Cancer therapy
Fungal diseases
Viral vaccines
Analgesics
Photodynamic therapy
Marketed liposomes

Drug	Route of administration	Targeted Diseases
Amphotericin-B	Oral delivery	Mycotic infection
Insulin	Oral, Ocular, Pulmonary and Transdermal delivery	Diabetic mellitus
Ketoprofen	Ocular delivery	Pain muscle condition
Pentoxyfylline	Pulmonary delivery	Asthma
Tobramycin	Pulmonary delivery	Pseudomonas infection, aeruginosa

Drug	Route of administration	Targeted Diseases
Salbutamol	Pulmonary delivery	Asthma
Benzocain	Transdermal	ulcer on mucous surface with pain
Ibuprofen	Oral delivery	Rheumatoid arthritis
Adrenaline	Ocular delivery	Glucoma, Conjectivitis
Penicillin G	Pulmonary delivery	Meningococal, staphylococcal
Methotrexate	Transdermal	Cancer

Marketed product	Drug used	Target diseases	Company
DoxilTM or CaelyxTM	Doxorubicin	Kaposi's sarcoma	SEQUUS, USA
DaunoXomeTM	Daunorubicin	Kaposi's sarcoma, breast & lung cancer	NeXstar, USA
AmphotecTM	Amphotericin-B	fungal infections, Leishmaniasis	SEQUUS, USA
VENTUSTM	Prostaglandin-E1	Systemic inflammatory diseases	The liposome company, USA
ALECTM	Dry protein free powder of DPPC-PG	Expanding lung diseases in babies	Britannia Pharm, UK

Marketed product	Drug used	Target diseases	Company
Topex-Br	Terbutaline sulphate	Asthma	Ozone, USA
Depocyt	Cytarabine	Cancer therapy	Skye Pharm, USA
Novasome®	Smallpox vaccine	Smallpox	Novavax, USA
Avian retrovirus vaccine	Killed avian retrovirus	Chicken pox	Vineland lab, USA
Epaxal –Berna Vaccine	Inactivated hepatitis-A Virions	Hepatitis A	Swiss serum & vaccine institute, Switzerland

Conclusion

- Liposomes are one of the unique drug delivery system, which can be of potential use in controlling and targeting drug delivery.
- Liposomes are administrated orally, parenterally and topically as well as used in cosmetic and hair technologies, sustained release formulations, diagnostic purpose and as good carriers in gene delivery.
- Nowadays liposomes are used as versatile carriers for targeted delivery of drug.