Unit 4
Targeted drug Delivery: 2

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Introduction

- Liposomes
- Niosomes
- Monoclonal antibodies and
- their applications

Targeting is the ability to direct the drug-loaded system to the site of interest

Principle and Rationale of Drug Targeting

- exclusively delivered to preselected target cell or pharmacological receptor with maximum intrinsic activity of drug
- reduce the access of drug to irrelevant or nontarget cells
- carrier or complex or conjugates of drug ,deliver drug at controlled rate
- drug specifically bind with target cells
- carrier /complex/conjugates of drug provide bioenvironmental protection during administration of drug till it reach to the target tissues i.e. site of action.
- Restrict the distribution of drug to nontarget site
- So main aim is to get adequate concentration to its desired destination.

Carriers :

- Carrier is one of the most important entities essentially required for successful transportation of the loaded drug.
- They are drug vectors, which sequester , transport and retain drug en route , which elute or deliver it within or in the vicinity of the target.
- Carriers can do so either through an inherent characteristics or acquired (through structural modification), to interact selectively with biological targets , or otherwise they are engineered to release the drug in the proximity of the target cell lines demanding optimal pharmacological action (therapeutic index).

An ideal drug carrier should have the following features :

- should be- biochemically inert (non-toxic),
- non-immunogenic
- Both physically and chemically stable in vivo and in vitro.
- Restrict drug distribution to target cells or tissues or organs and should have uniform capillary distribution.
- Controllable and predictable rate of drug release.
- It must be able to cross anatomical barriers and in case of tumour chemotherapy tumour vasculature.
- It must be recognized specifically and selectively by the target cells and must maintain the avidity and specificity of the surface ligands.
- The linkage of the drug and the directing unit (ligand) should be stable in plasma , interstitial and other biofluids.

An ideal drug carrier should have the following features

- Carrier should biodegradable particulate or macromolecule.
- The biomodules used as carrier should not be ubiquitous (existing or being everywhere at the same time).
- After recognition and internalization, the carrier system should release the drug moiety inside the target organs, tissues or cells.

Carriers :

Based on the nature of their origin carriers are categorized as:

- **Endogenous** (low density lipoprotein, high density lipoprotein, chylomicrons , serum albumin, erythrocytes).
- **Exogenous** (microparticulates, soluble polymeric and biodegradable polymeric drug carriers).

Carriers :

1. Colloidal carriers
2. Cellular carriers
3. Supramolecular delivery systems
4. Polymer based systems
5. Macromolecular carriers

Carrier Systems Used for Targeted Drug Delivery :

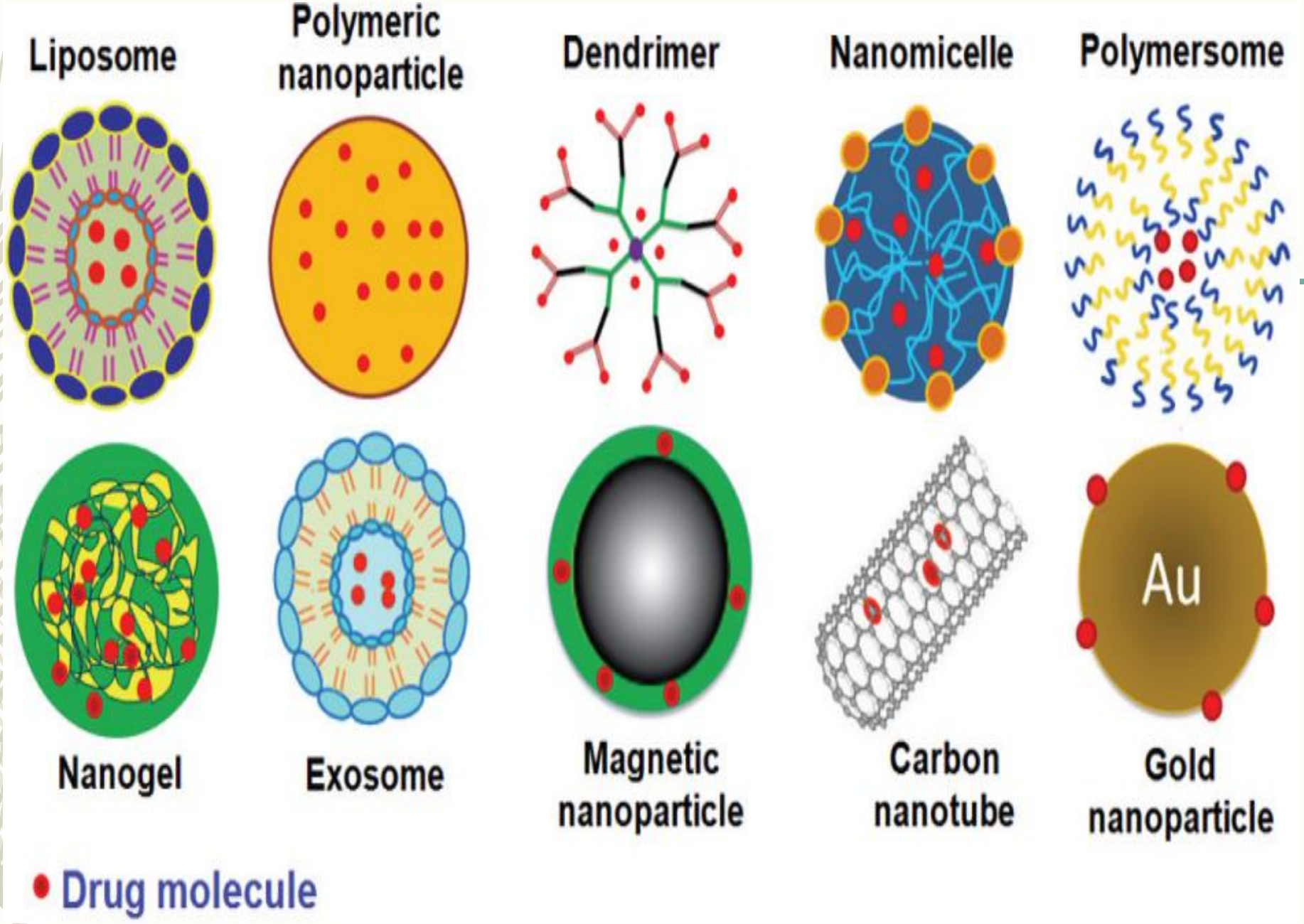
1. **Colloidal carriers**

a) Vesicular systems- Liposomes; Niosomes; Pharmacosomes; Virosomes; Immunoliposomes

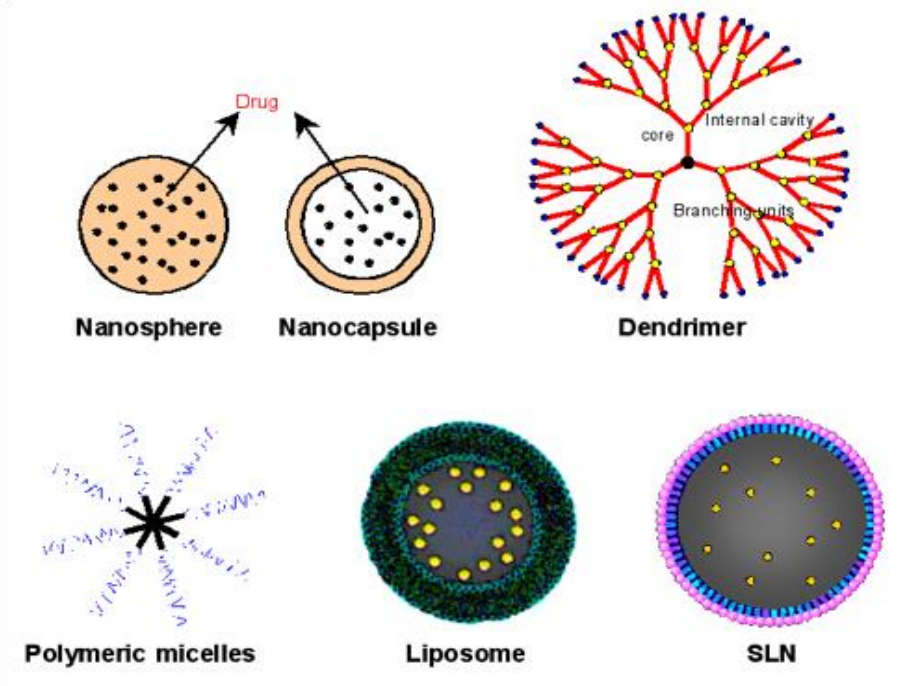
b) Particulate systems- Microparticles; Nanoparticles; Magnetic microspheres; Albumin microspheres; nanocapsules

2. **Cellular carriers**

Resealed erythrocytes; serum albumin; antibodies; platelets; leukocytes


Liposome
Polymeric nanoparticle
Dendrimer
Nanomicelle
Polymersome
Nanogel
Exosome
Magnetic nanoparticle
Carbon nanotube
Au
Gold nanoparticle
Drug molecule

Parenteral Controlled Release Systems

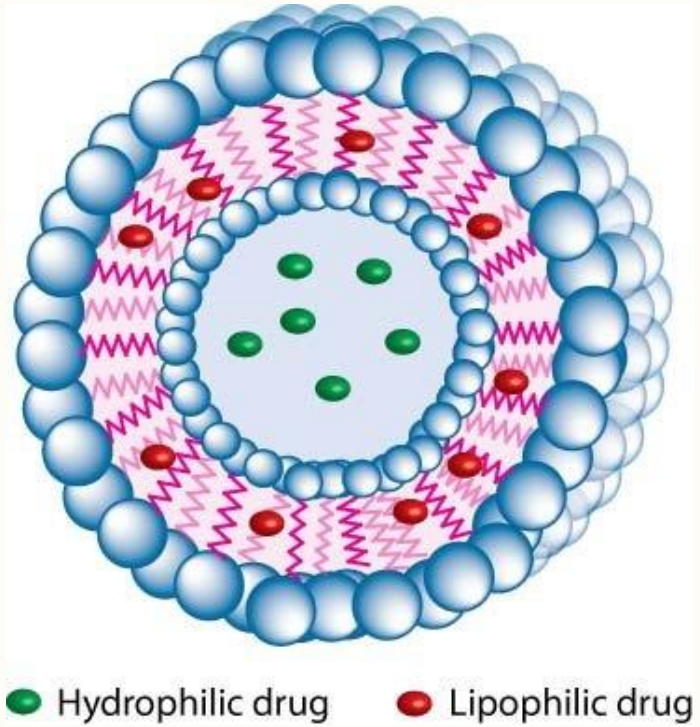


COLLOID BASED DELIVERY FOR THERAPEUTICS

Delivery system type	Typical mean particle diameter (in micrometers)	Representative systems of each type	Characteristic applications
Microspheres, Hydrogels	0.5-20	Alginate, gelatin, chitosan, polymeric hydrogels	Sustained release of therapeutics
Microparticles	0.2-5	Polystyrene, polylactide microspheres.	Targeted delivery of therapeutics
Emulsions, Microemulsions	0.15-2	o/w, w/o, lipid emulsions, o/w microemulsions.	Control and targeted delivery of therapeutics
Liposomes	30-1000	Phospholipid and polymer based bilayer vesicles.	Targeted delivery of therapeutics
Micelles	3-80	Natural and synthetic surfactant micelles.	Targeted delivery of therapeutics
Nanoparticles	2-100	Lipid, Polymer, Inorganic nanoparticles.	Targeted delivery of therapeutics, in vivo navigational devices
Nanocrystals	2-100	Quantum dots	Imaging agents

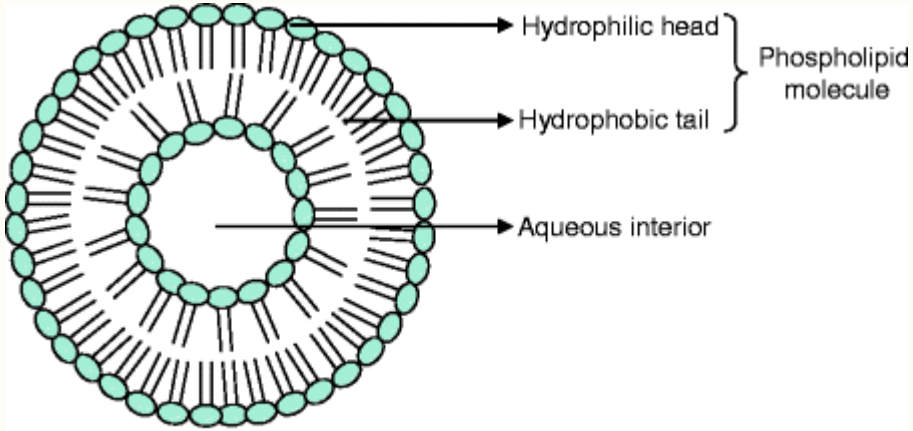
LIPOSOMES

- They are spherical vesicles with a phospholipid bilayer.
- Liposomes help improve : Therapeutic index Rapid metabolism, unfavorable pharmacokinetics low solubility, lack of stability, irritation etc.



Liposomes

- A liposome is a spherical-shaped vesicle that is composed of one or more phospholipid bilayers, which closely resembles the structure of cell membranes. The ability of liposomes to encapsulate hydrophilic or lipophilic drugs have allowed these vesicles to become useful drug delivery systems



Structure of an unilamellar liposome

Why the Liposomes?

Liposomes have the advantage of primarily consisting of **lecithin and cholesterol**, which are materials that are occur naturally in the human body.

Lecithin and cholesterol are also present in the body in large amounts and thus demand good bio acceptability.

Liposomes

- **Definition: Liposomes are targeted drug delivery systems consisting of one or more concentric spheres of lipid bilayers separated by water or aqueous buffer compartments composed of natural or synthetic phospholipids**
- **British haematologist Alec D Bangham described them first in 1961**
- **Word Liposome is derived from two Greek words: 'Lipos' meaning fat and 'Soma' meaning body.**
- **liposome can be formed in a variety of sizes with unilamellar or multilamellar construction**
- **Examples of phospholipids**

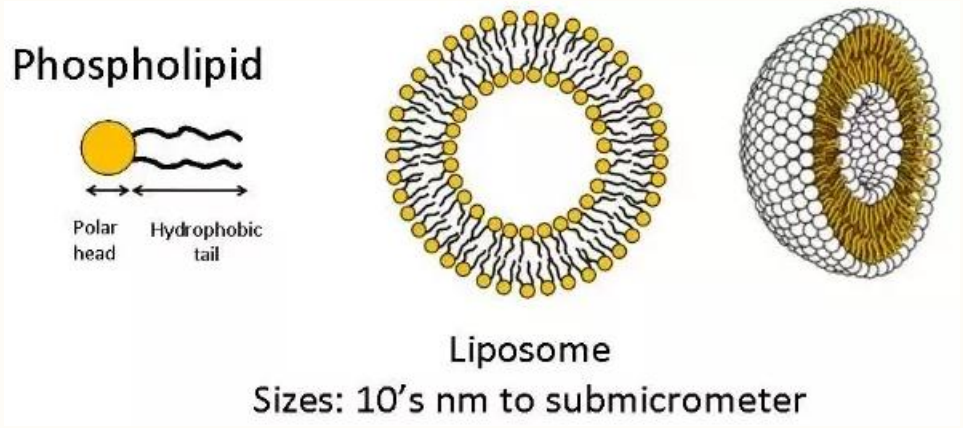
Phosphatidyl choline (Lecithin) – PC

Phosphatidyl ethanolamine (cephalin) – PE

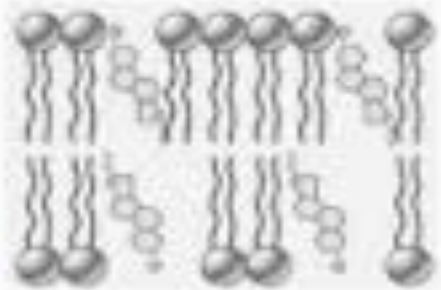
Phosphatidyl serine (PS)

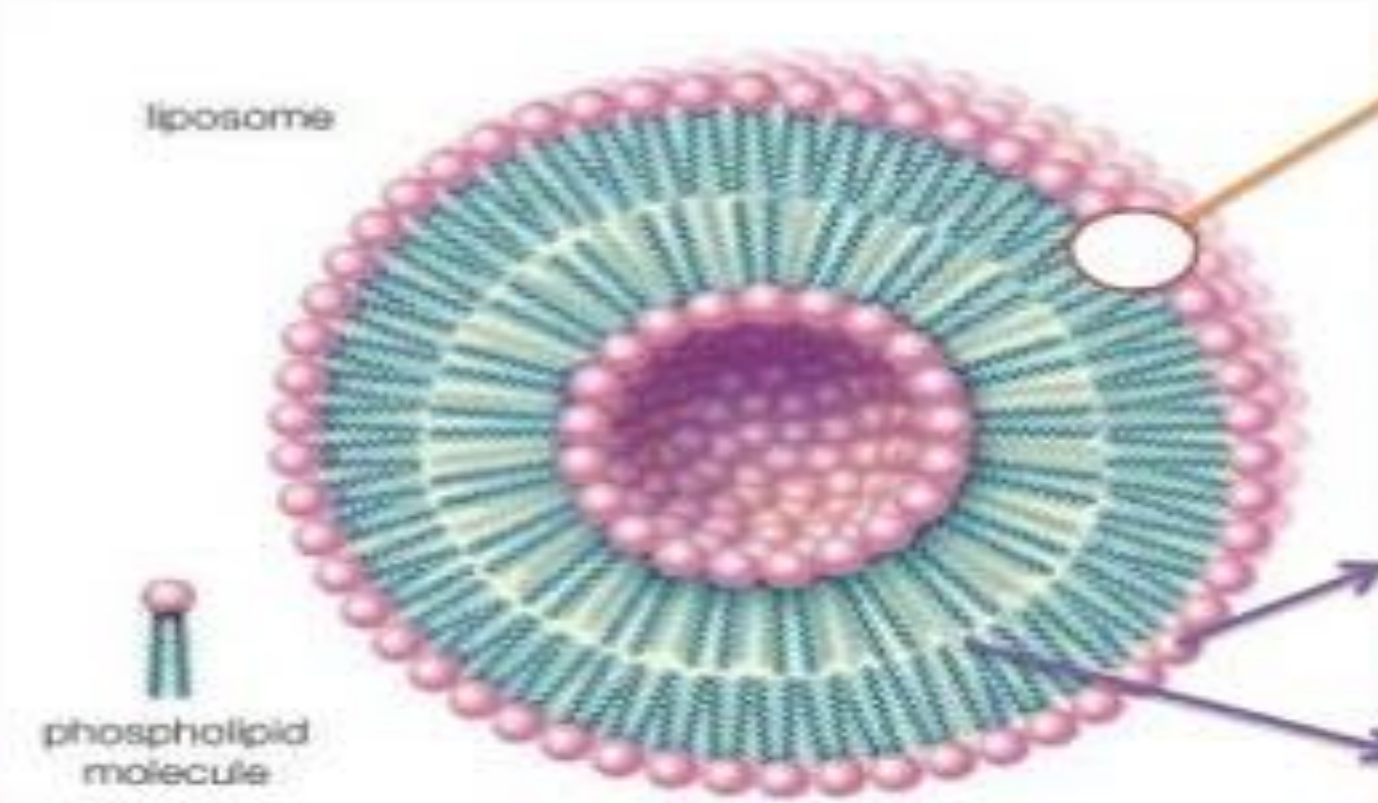
Phosphatidyl inositol (PI)

Phosphatidyl Glycerol (PG)



Structure Of Liposome





Liposome =Phospholipid+ cholesterol

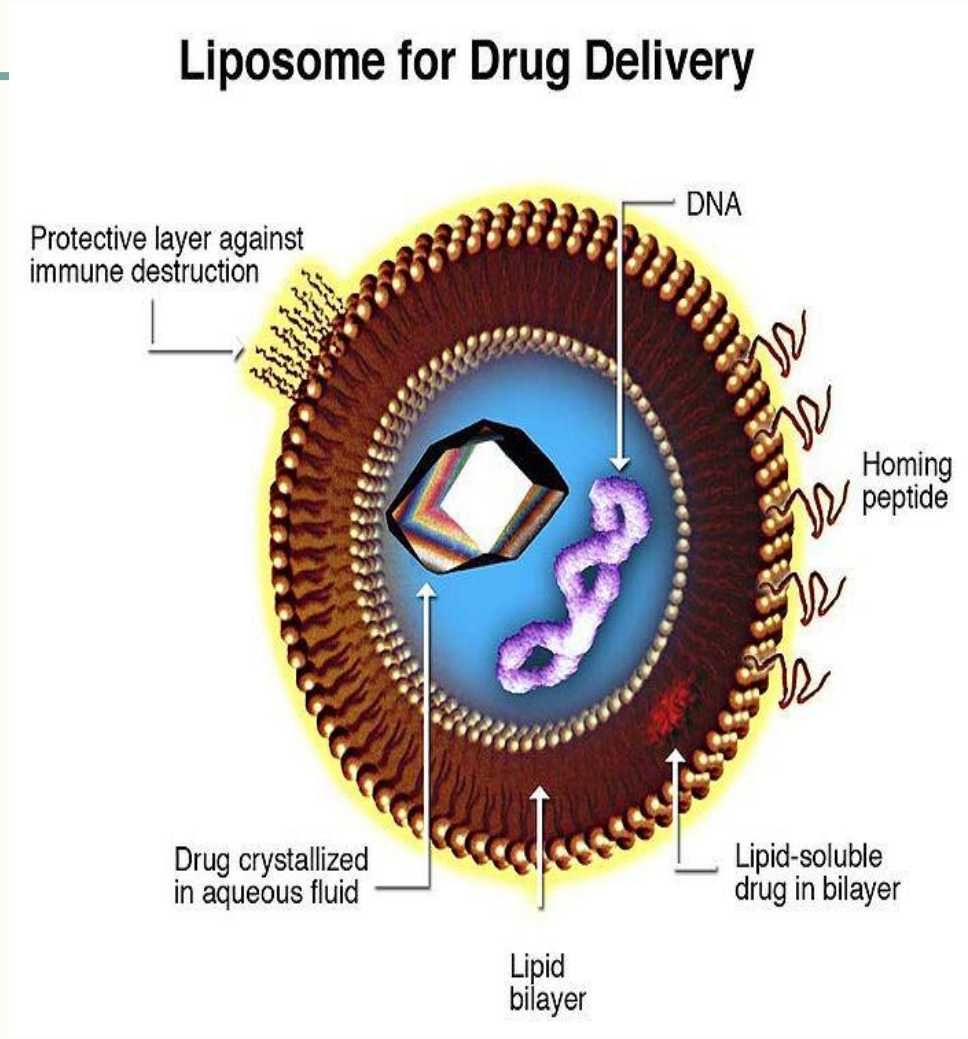
Hydrophillic head

Hydrophobic tail

The lipid moecules are usually phospholipids-amphipathic moieties with a hydrophilic head group and two hydrophobic tails.

Liposomes

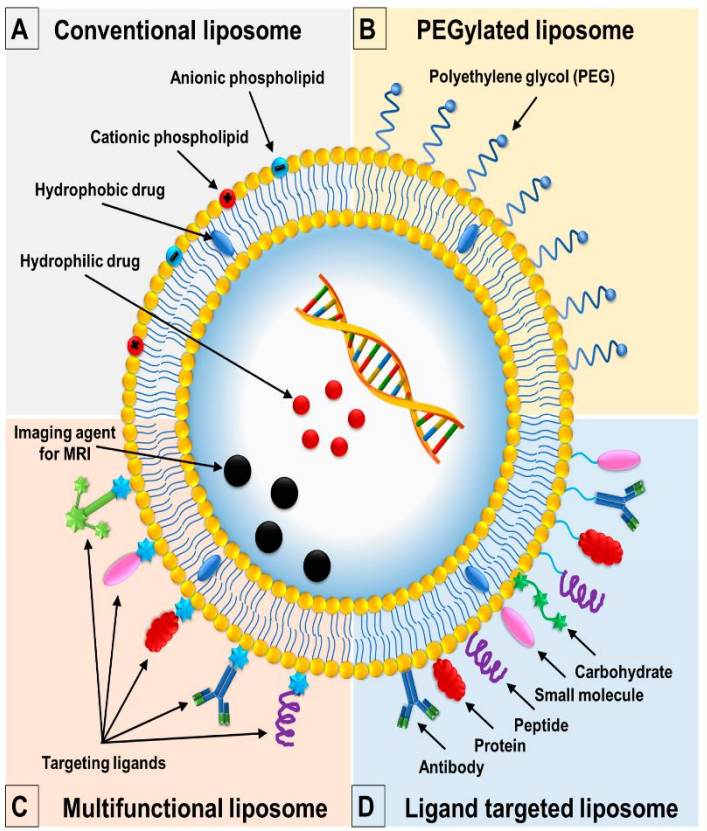
- Liposomes is assembled vesicles which can carry
  - hydrophilic molecules inside the core and
  - hydrophobic molecules in between the bilayer.



Liposomes: Classification

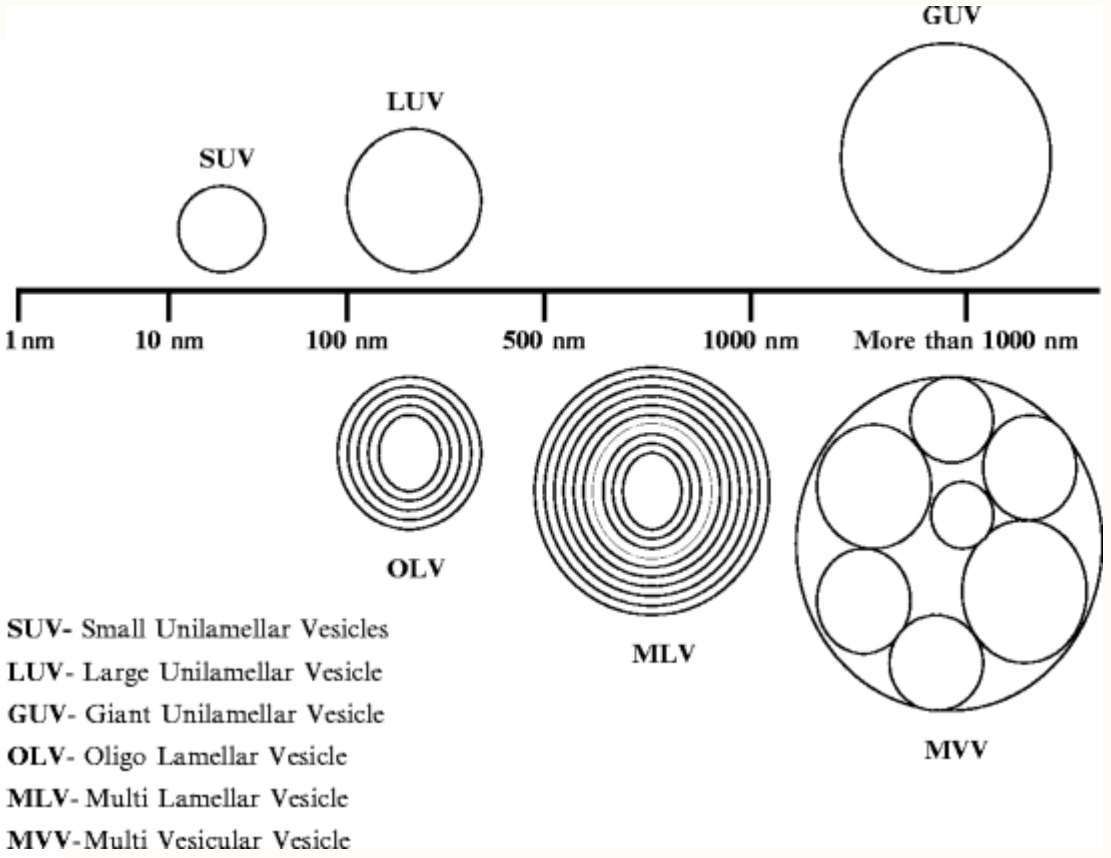
Based on work and mechanism of intracellu

1. **Conventional liposomes**
2. **pH sensitive liposomes**
3. **Cationic liposomes**
4. **Immune liposomes**
5. **Long circulating liposomes**



Liposomes: Classification based on Structure

Vesicle Type	Abbreviation	Hydrodynamic Diameter	No. of Lipid Bilayers
Small unilamellar vesicles	SUV	20–50 nm	1
Large unilamellar vesicles	LUV	100–1000 nm	1
Giant unilamellar vesicles	GUV	>1000 nm	1
Multilamellar vesicles	MLV	>500 nm	5–25
Oligolamellar vesicles	OLV	100–1000 nm	~5
Multivesicular vesicles	MVL	>1000 nm	Multicompartmental structure



Advantages of Liposomes

1. **Liposomes can complex both with negatively and positively charged molecules.**
2. **Liposomes offer a degree of protection to the DNA from degradative processes.**
3. **Liposomes can carry large pieces of DNA, possibly as big as a chromosome.**
4. **Liposomes can be targeted to specific cells or tissues.**
5. **Non toxic, biocompatible, and completely biodegradable;**
6. **Enhanced bioactivity and efficacy;**
7. **Increased stability via encapsulation processes;**
8. **Reduced toxicity of encapsulated drugs;**
9. **Flexibility for active targeting.**

Disadvantages of Liposomes

1. **Production cost is high.**
2. **Leakage and fusion of encapsulated drug / molecules.**
3. **Sometimes phospholipid undergoes oxidation and hydrolysis-like reactions.**
4. **Short half-life.**
5. **Low solubility.**
6. **Fewer stables.**

Liposomes :Applications

1. **Protection**: active drug are protected by a membrane barrier from metabolism or degradation.
2. **Sustained release**: release is dependent on the ability to vary the permeability characteristics of the membrane by control of bilayer composition and lamellarity.
3. **Controlled release**: Drug release is enabled by utilizing lipid phase transitions in response to external triggers (activators) such as changes in temperature or pH.
4. **Targeted delivery**: targeted delivery to specific cells or organs can be achieved by: Modifying on natural characteristics such as liposome size and surface charge to effect passive delivery to body organs. Incorporating antibodies or other ligands to aid delivery to specific cell types.
5. **Internalization**: This occurs by encouraging cellular uptake via endocytosis or fusion mechanisms, to deliver genetic materials into cells.

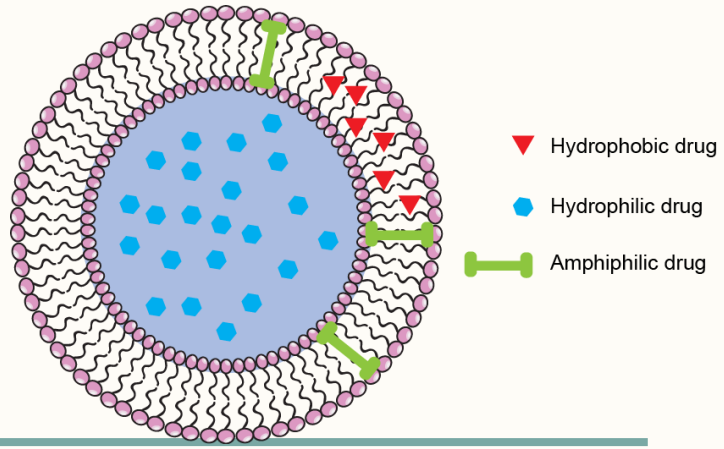
Applications

1.Cancer chemotherapy	• Liposomes are successfully used to entrap anticancer drugs. This increases circulation life time, protects from metabolic degradation.
2.Liposomes as carrier of drug in oral treatment	• Steroids used for arthritis can be incorporated into large MLVs. • Alteration in blood glucose levels in diabetic animals was obtained by oral administration of liposome encapsulated insulin.
3.Liposomes for topical applications	• Drugs like triamcilone, methotrexate, benzocaine, corticosteroids etc can be successfully incorporated as topical liposome
4. Liposomes for pulmonary delivery	Inhalational devices like nebulizers are use to produce an aerosol of droplets containing liposomes.

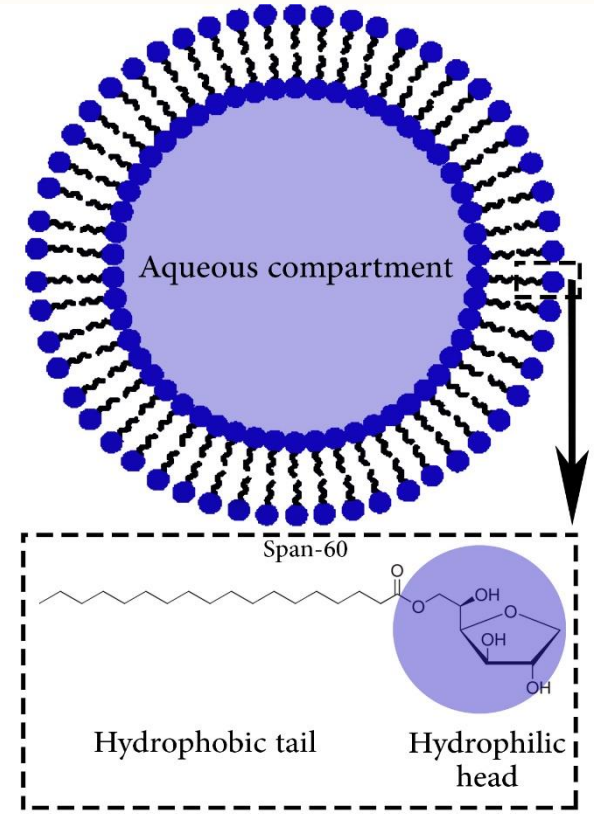
Niosomes

Niosomes

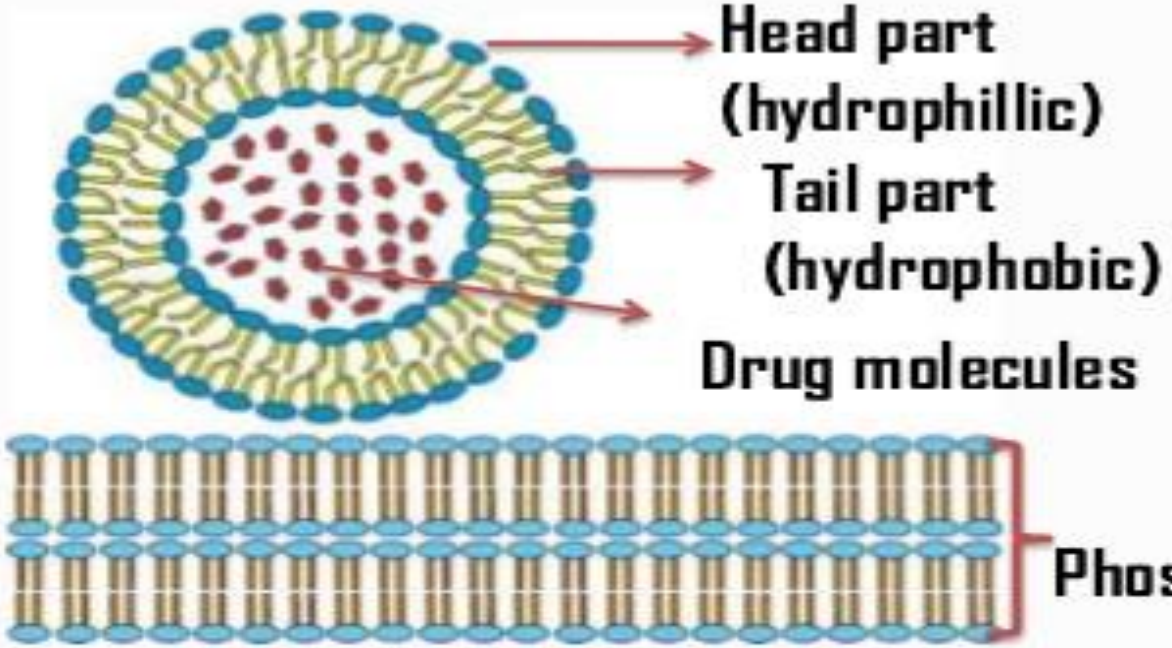
Niosomes

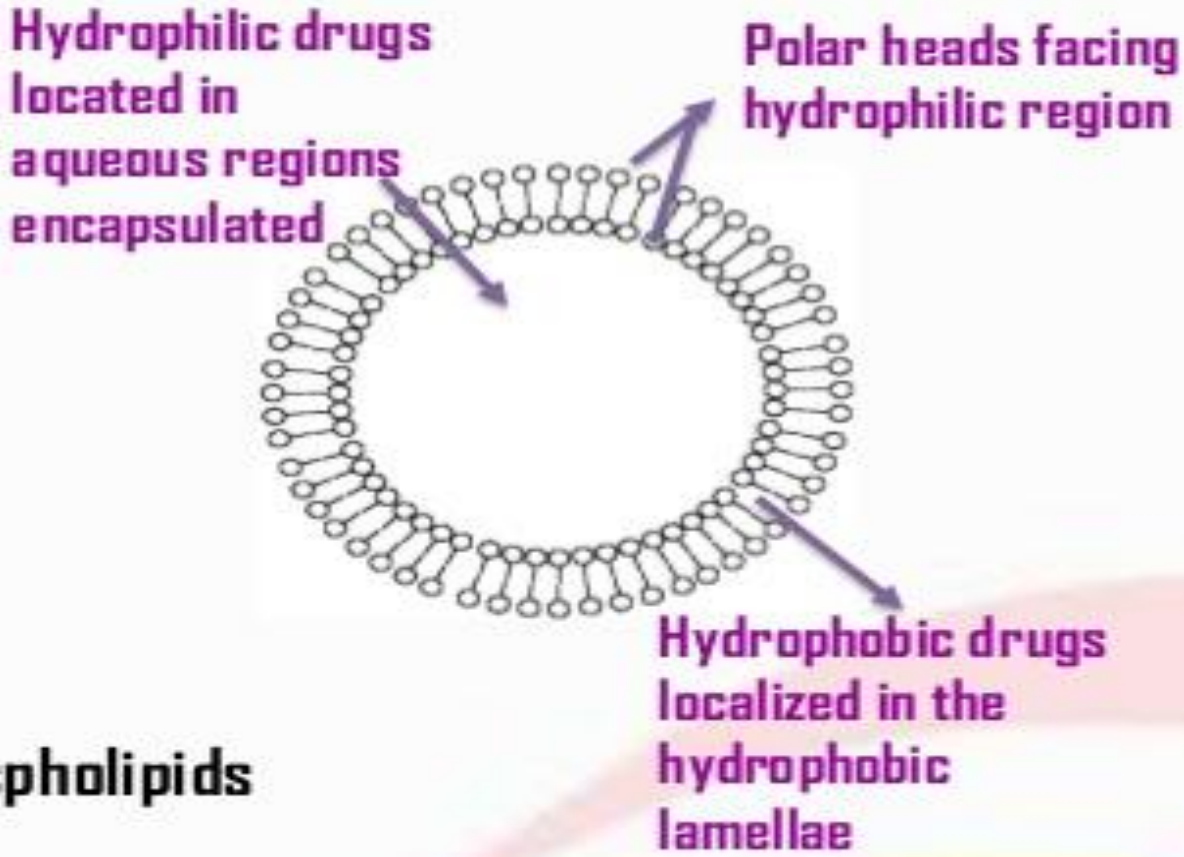


- Niosomes are a novel drug delivery system, in which the medication is encapsulated in a vesicle, consisting of one or more bilayer of non-ionic surface active agents along with cholesterol
- Vesicles are prepared from self assembly of hydrated non ionic surfactants molecules
- Basic structural components are
  - Non ionic surfactant
  - Cholesterol
  - Charge inducing molecule



Structure of niosomes:





- These vesicular systems are similar to liposomes that can be used as carriers of amphiphilic and lipophilic drugs.

- It is less toxic and improves the therapeutic index of drug by restricting its action to target cells.

Niosomes: Advantages

- Biocompatible, biodegradable, non-toxic, non immunogenic and non-carcinogenic
- Targeted drug delivery can be achieved
- Reduced dose is required to achieve the desired effect
- Subsequent decrease in the side effects
- The therapeutic efficacy of the drugs is improved by reducing the clearance rate, targeting to the specific site and by protecting the encapsulated drug
- Niosomes are amphiphilic i.e. both hydrophilic and lipophilic in nature and can accommodate a large number of drugs with a wide range of solubilities
- Improve the oral bioavailability of poorly soluble drugs
- Enhance the skin permeability of drugs when applied topically
- provide advantage of usage through various routes viz. oral, parentral, topical, ocular etc.
- The bilayers of the niosomes protect the enclosed Active pharmaceutical ingredient from the various factors present both inside and outside the body

Niosomes: Disadvantages

- Aqueous suspension of niosome may exhibit fusion, aggregation leaching or hydrolysis of entrapped drug, thus limiting the shelf life of niosomes dispersion.
- Time consuming
- Requires specialized equipment
- Inefficient drug loading

Niosomes :Applications

- The applications of niosomes can be mainly classified into ***three*** categories

1) **For Controlled Release of Drugs**
2) **To Improve the Stability and Physical Properties of the Drugs**
3) **For Targeting and Retention of Drug in Blood Circulation**

Applications Of Niosomes

- It is used as Drug Targeting.
- It is used as Anti- Neoplastic Treatment i.e. Cancer Disease.eg.Methotrexate
- It is used as Leishmaniasis i.e. Dermal and Mucocutaneous infections e.g. Sodium stibogluconate.
- It is used act as Delivery of Peptide Drugs.
- It is used in Studying Immune Response.
- Niosomes as Carriers for Hemoglobin.
- Transdermal Drug Delivery Systems Utilizing Niosomes. eg.Erythromycine
- It is used in Ophthalmic drug delivery. eg.Cyclopentolate

Applications

1) **Targeting of bioactive agents**

a) **To reticulo-endothelial system**

b) **To organs other than RES**

2) **Neoplasia**

Doxorubicin Niosomal delivery of Doxorubicin to mice bearing S-180 tumor increased their life span and decreased the rate of proliferation of sarcoma

3) **Leishmaniasis**

4) **Delivery of peptide drugs**

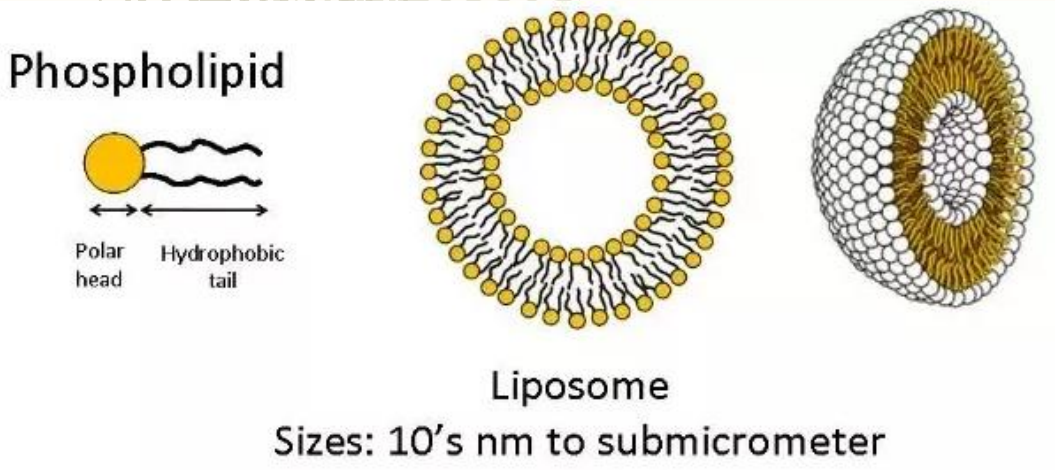
Oral delivery of 9-desglycinamide, 8-arginine vasopressin entrapped in niosomes increase stability of peptide significantly.

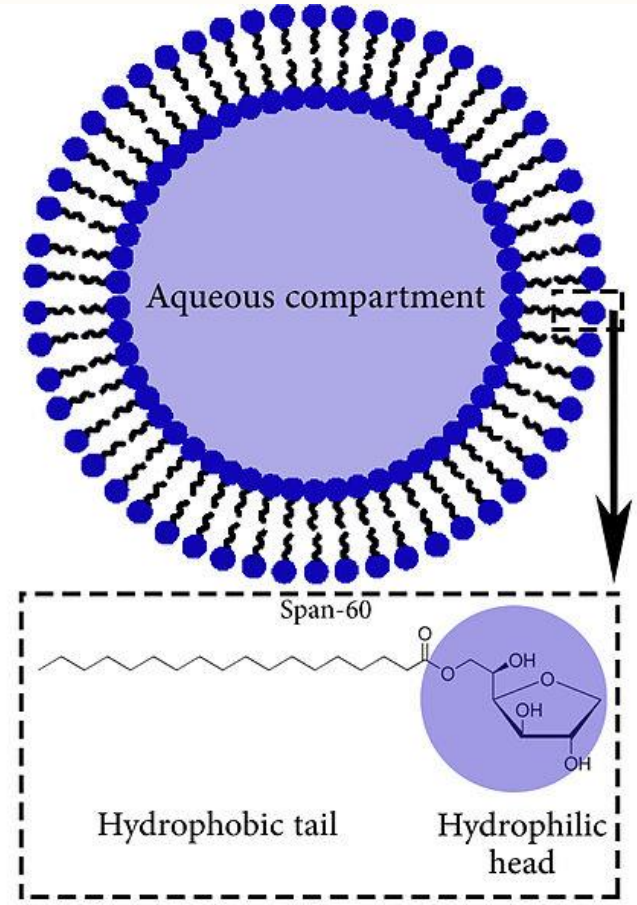
Comparisition between liposomes & niosomes:

Sl. No.	Liposomes	Niosomes
1.	Vesicles made up of concentric bilayer of phospholipids	Vesicles made up of surfactants with or without incorporation of cholesterol.
2.	Size ranges from 10-3000nm	Size ranges from 10-100nm
3.	Comparatively expensive	Inexpensive
4.	Special storage condition are required	No such special requirement
5.	Phospholipids used are unstable	Non-ionic surfactants are stable
6.	Comparatively more toxic	Less toxic

Comparison Between Liposomes & Niosomes

- liposomes are made up of phospholipids, which contain two hydrophobic tails whereas niosomes are made up of non-ionic surfactants, which usually contain a single hydrophobic tai





Monoclonal Antibodies

What are Antibodies?

- **Antibody, also called immunoglobulin(proteins), a protective protein produced by the immune system in response to the presence of a foreign substance, called an antigen.**
- **Antibodies recognize and latch onto antigens in order to remove them from the body.**

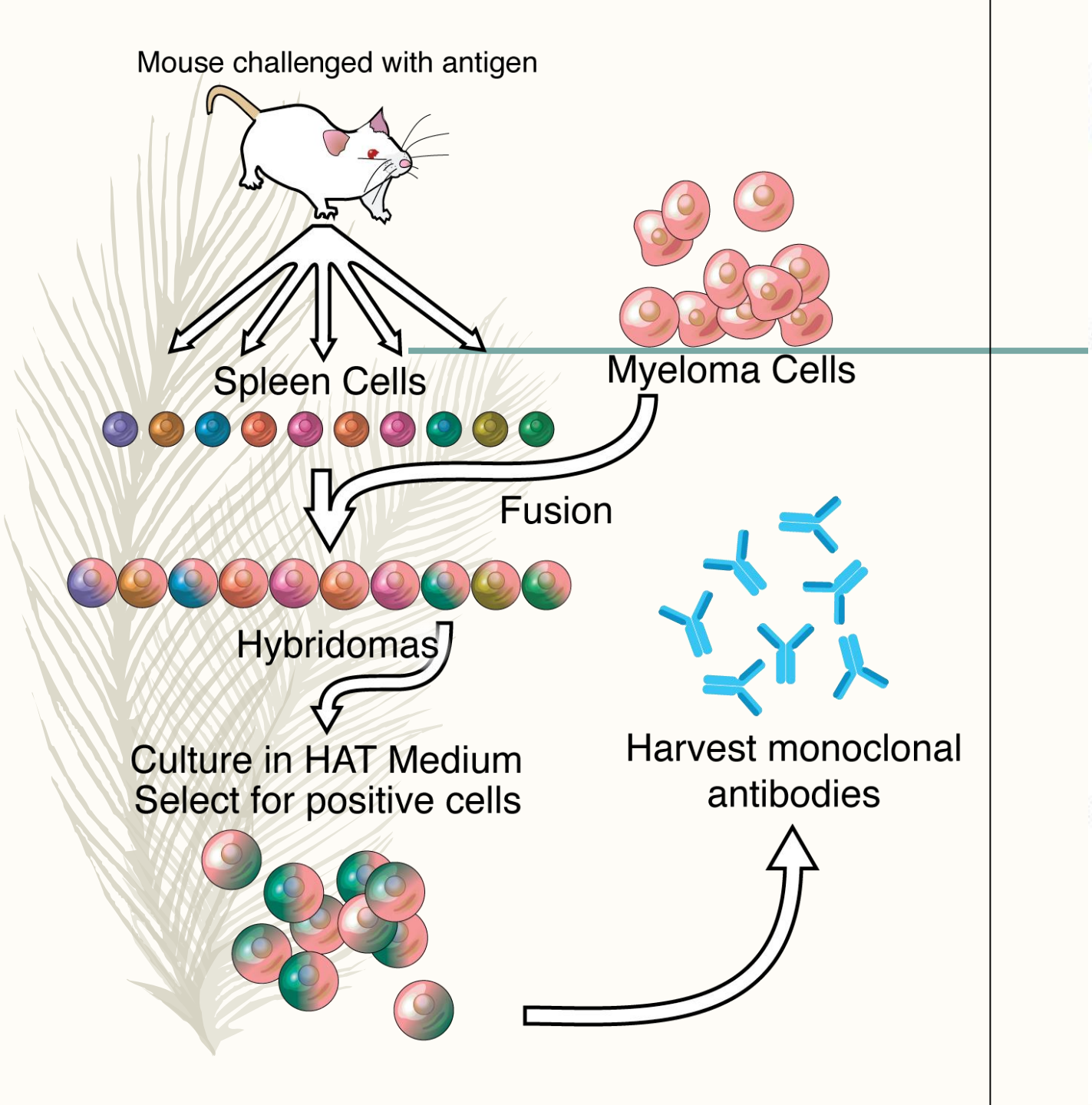
What are Monoclonal antibodies (mAb or moAb)?

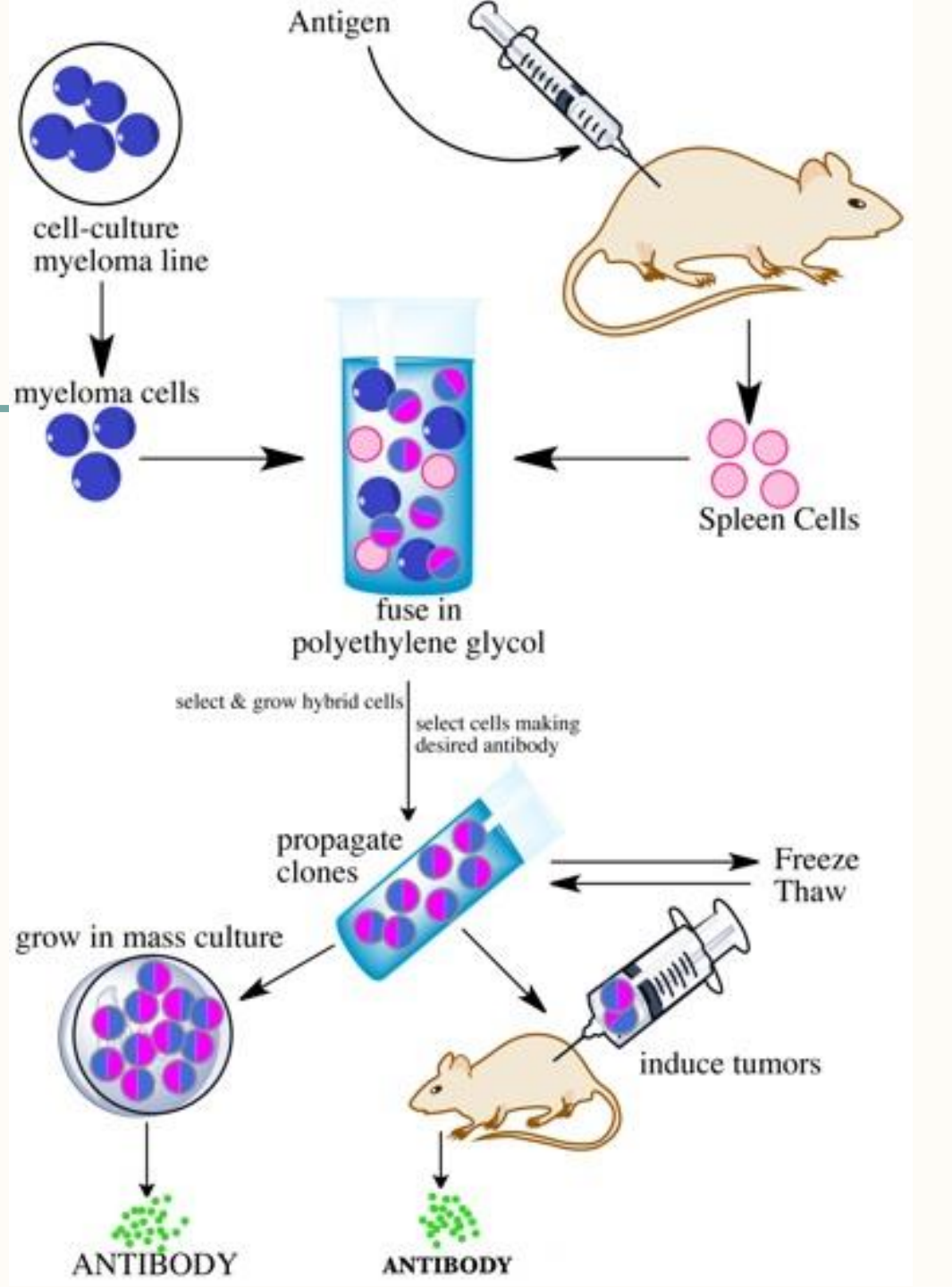
- Monoclonal antibodies (mAb or moAb) are identical immunoglobulin (proteins), generated from a single B-cell clone.
- These antibodies recognize unique epitopes, or binding sites, on a single antigen.
- Derivation from a single B-cell clones and subsequent targeting of a single epitope is what differentiates monoclonal antibodies from polyclonal antibodies.
- Polyclonal antibodies are antibodies that are derived from different cell lines. They differ in amino acid sequences

Monoclonal antibodies

- **Monoclonal antibodies (mAB) are antibody that are identical and are directed against a specific epitope (antigen, antigenic determinant) and are produced by B-cell clones of a single parent or a single hybridoma cell line.**
- **A hybridoma cell line is formed by the fusion of one B-cell lymphocyte with a myeloma cell.**
- **Some myeloma cell synthesize single mAB antibodies naturally.**

How to make monoclonal antibodies?


Mouse challenged with antigen
Spleen Cells
Myeloma Cells
Fusion
Hybridomas
Culture in HAT Medium
Select for positive cells
Harvest monoclonal
antibodies


Antigen
cell-culture
myeloma line
myeloma cells
Spleen Cells
fuse in
polyethylene glycol
select & grow hybrid cells
select cells making
desired antibody
propagate
clones
Freeze
Thaw
grow in mass culture
induce tumors
ANTIBODY
ANTIBODY

How to make monoclonal antibodies?

1. **Lymphocyte Harvest**

Monoclonal antibodies production requires the collection of the antibody producing cells found in the spleen or lymph nodes.

2. **Fusion to Create Hybridoma Cells**

As spleen cells have limited survival times in culture, they require fusion with myelomas, cancerous B-Cells, to create an immortalized hybrid that can undergo many passages *in vitro*. This is achieved through polyethylene glycol (PEG) or electric pulses both of which disrupt cell membrane and allow merging of two adjacent cells.

3. **Selecting for Fused Hybrids**

B-Cell and myeloma fusion is not 100% efficient. Therefore, selecting for myeloma-lymphocyte hybrids is required. Hypoxanthine-aminopterin-thymidine medium (HAT) inhibits DNA synthesis via aminopterin. B-Cells and fused hybrids can overcome culturing in HAT medium as they posses thymidine kinase which allows them to synthesize requisite DNA polymerase precursors from the HAT medium supplied thymidine. Myelomas do not produce thymidine kinase, and consequently do not survive in HAT medium. Although mortal B-Cells contain thymidine kinase, they eventually die off due to limited in vitro replication ability.

How to make monoclonal antibodies?

- The population of cells which survive selection is still heterogeneous, containing both multiple clones specific to the target antigen and clones producing antibodies with irrelevant specificity. Single cells are required to assure clonality

WHAT ARE THE USES FOR MONOCLONAL ANTIBODIES?

- The use of monoclonal antibodies to treat diseases is called immunotherapy because each type of monoclonal antibody will target a **specific** targeted antigen in the body.

- Uses for monoclonal antibodies includes: cancer, MS ,Crohn's disease, ulcerative colitis, rheumatoid arthritis, systemic lupus erythematosus …

Applications of Monoclonal antibodies

- Diagnostic Applications

- Detects protein of interest either by blotting or immunoflouroscence
- Cardiovascular diseases
- Deep vein thrombosis
- Location of 1^0 and 2^0 metastatic tumours
- Immunosuppressive therapy
- Pregnancy testing kits

- Therapeutic Applications

- Radioisotope immunoconjugates
- Toxin and drug immunoconjugates
- Immunoliposome based kits
- In cancer

Monoclonal antibodies for Cancer

- Monoclonal antibodies are laboratory-produced molecules engineered to serve as substitute antibodies that can restore, enhance or mimic the immune system's attack on cancer cells.
- They are designed to bind to antigens that are generally more numerous on the surface of cancer cells than healthy cells.