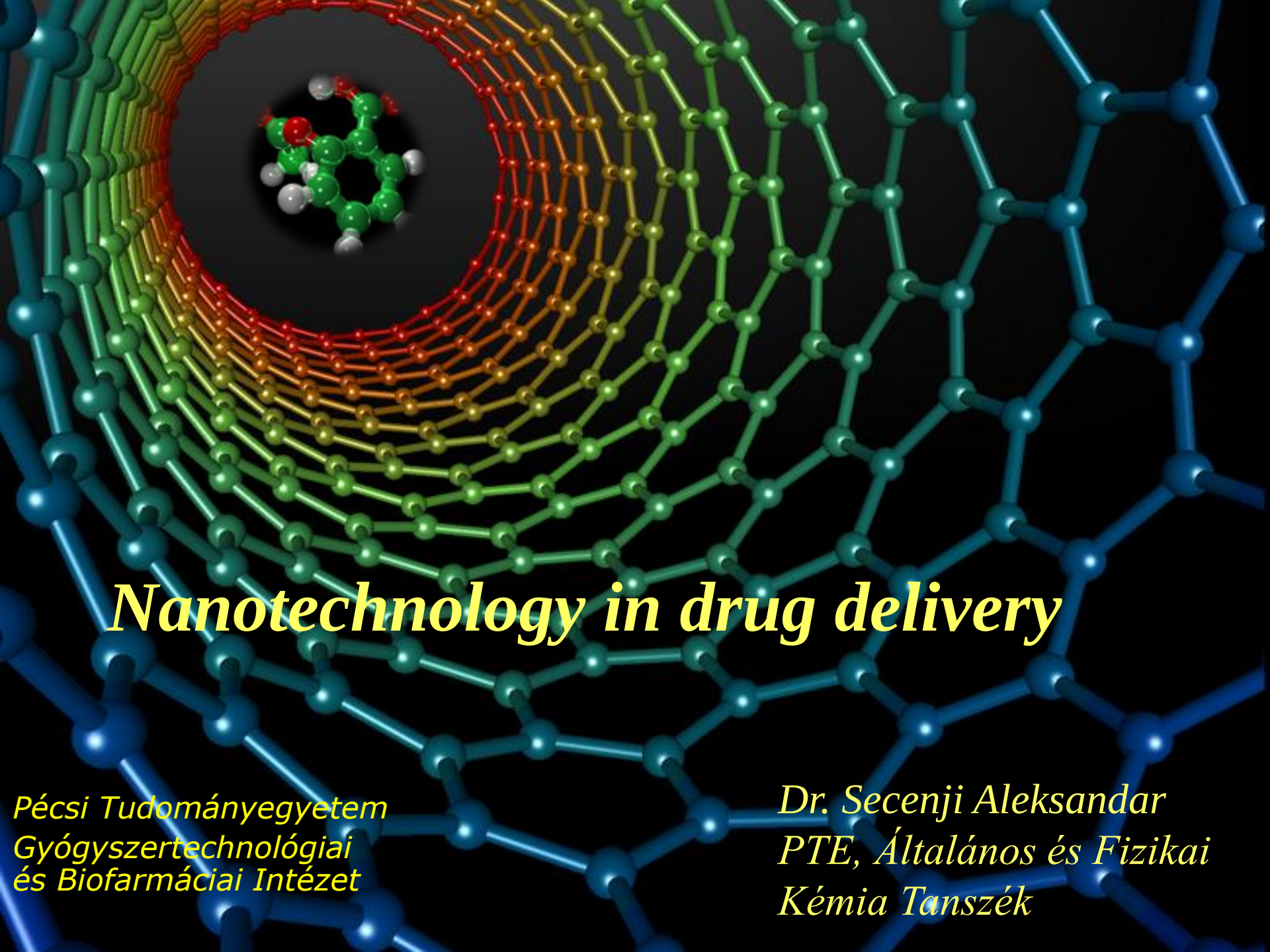
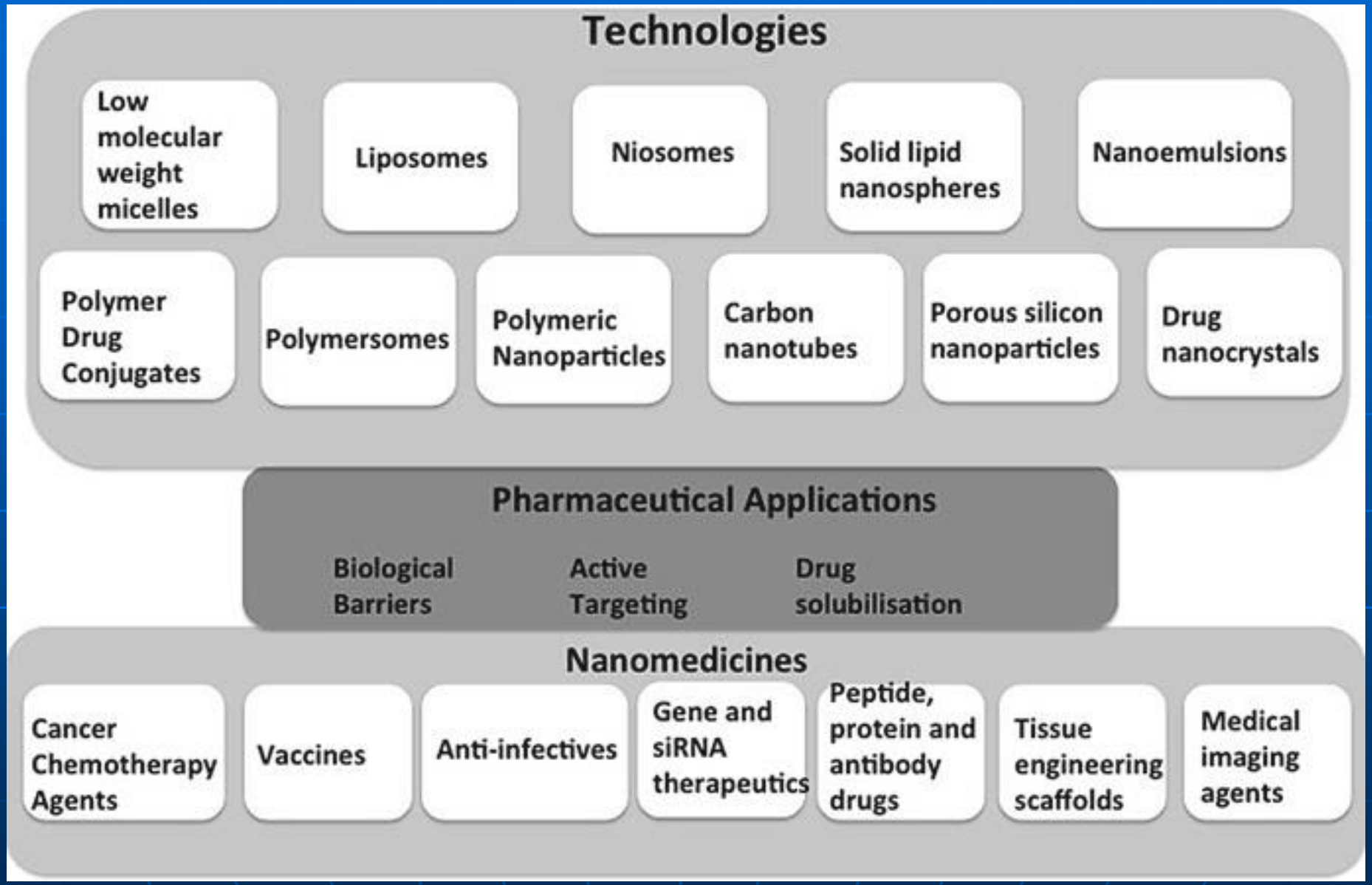


Nanotechnology in drug delivery

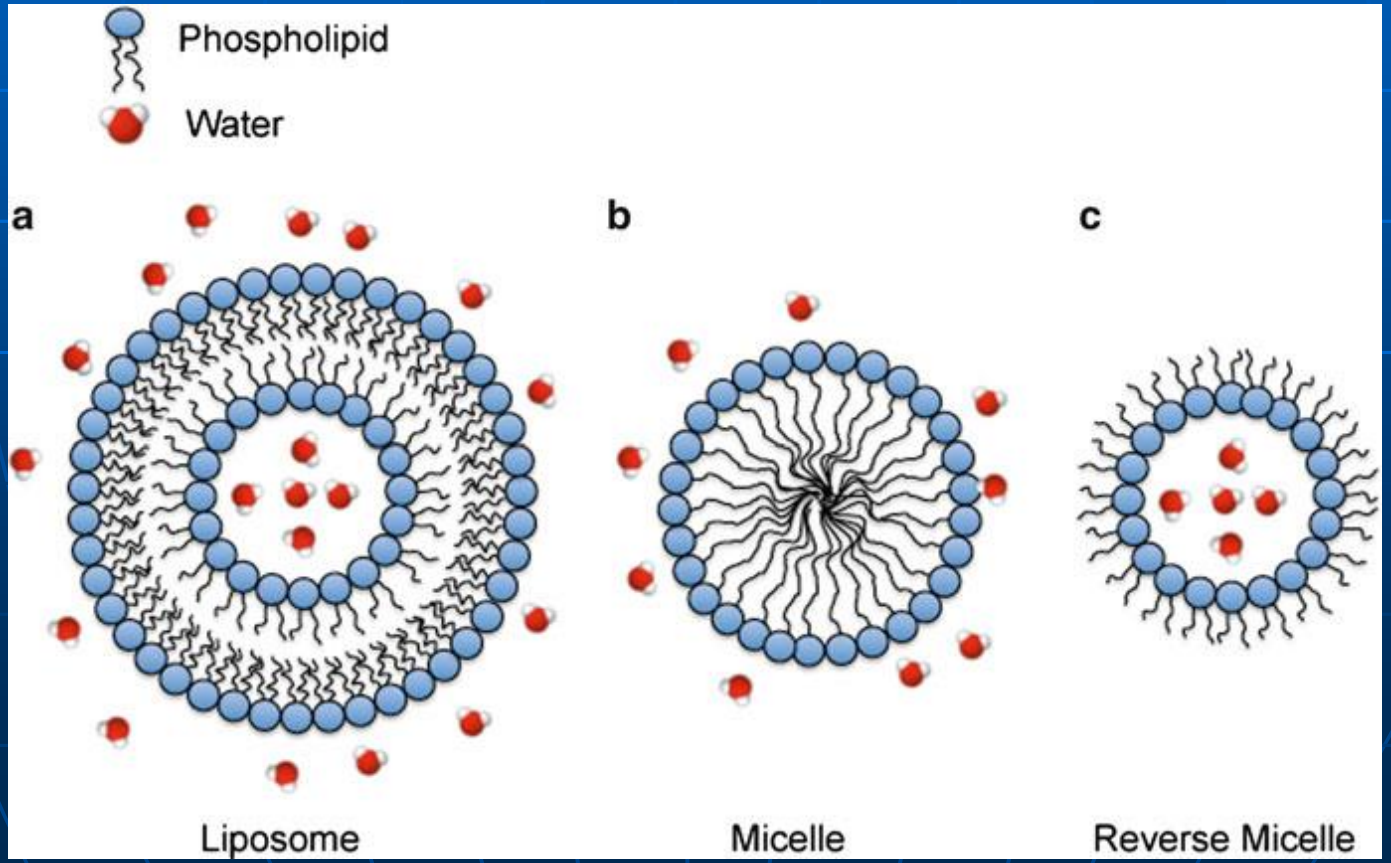
Pécsi Tudományegyetem
Gyógyszertechnológiai
és Biofarmáciai Intézet

Dr. Secenji Aleksandar
PTE, Általános és Fizikai
Kémia Tanszék


Technologies
Low molecular weight micelles
Liposomes
Niosomes
Solid lipid nanospheres
Nanoemulsions
Polymer Drug Conjugates
Polymersomes
Polymeric Nanoparticles
Carbon nanotubes
Porous silicon nanoparticles
Drug nanocrystals
Pharmaceutical Applications
Biological Barriers
Active Targeting
Drug solubilisation
Nanomedicines
Cancer Chemotherapy Agents
Vaccines
Anti-infectives
Gene and siRNA therapeutics
Peptide, protein and antibody drugs
Tissue engineering scaffolds
Medical imaging agents

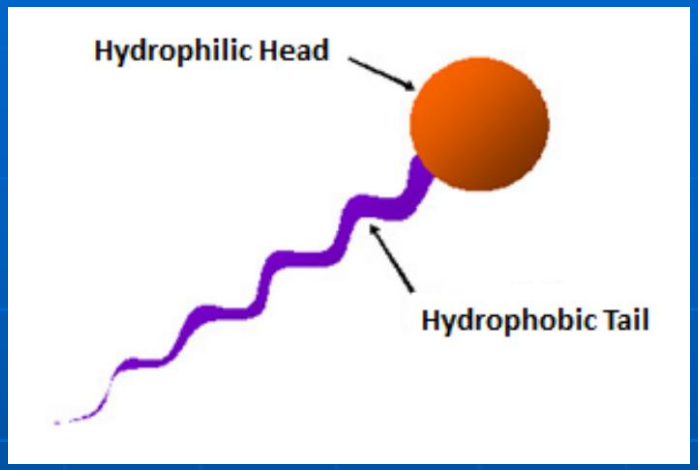
Vesicular drug delivery systems

- The vesicular systems are highly ordered assemblies of one or several concentric lipid bilayers formed, when certain amphiphillic building blocks are confronted with water.



Critical Packing Parameter (CPP) (Israelachvili 2011) is defiened by the relative sizes of the hydrophobic and hydrophilic regions of the molecule

$$CPP = \frac{v}{a_0 l_c}$$

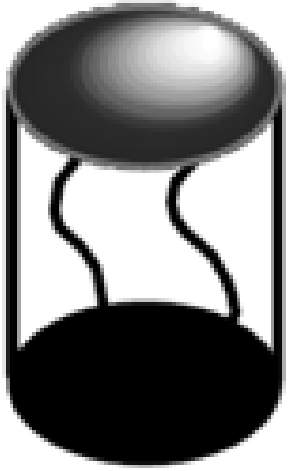
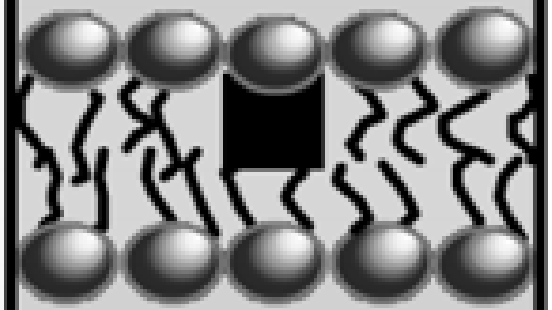
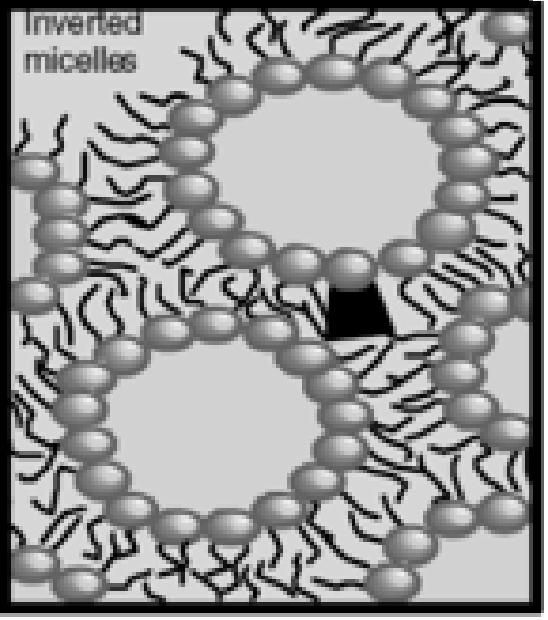


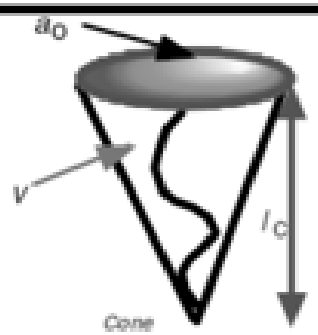
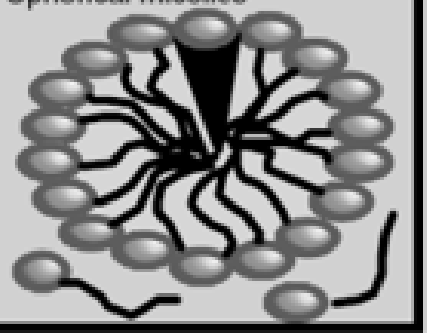
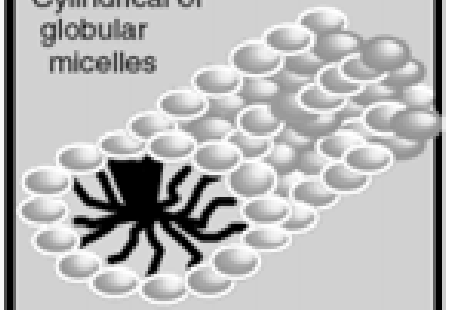
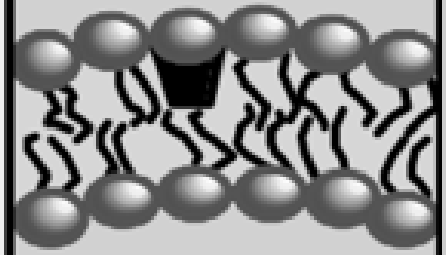
v = the volume of the hydrocarbon,
a_0 = the hydrophilic head group area
l_c = chain length

CPP < 0.33 (symptomatic of relatively hydrophilic molecules) leads to the formation of spherical micelles within aqueous media

0,5 < CPP < 1 (applicable to relatively hydrophobic molecules) leads to the formation of closed bilayers — ultimately vesicles, such as liposomes and niosomes in aqueous media.

1 < CPP of above 1 (indicative of extremely hydrophobic molecules) results in the formation of reverse micelles in non-aqueous media.

Lipid	Critical packing parameter $\frac{v}{a_o l_c}$	Critical packing shape	Structures formed
Double-chained lipids with small head-group areas: anionic lipids high salt, saturated frozen chains. (e.g. phosphatidyl ethanolamine, phosphatidyl serine + Ca^{2+})	~1	 *Cylinder*	Planar bilayers 
Double-chained lipids having small head groups (e.g. non-ionic lipids, poly(cis) unsaturated chains, phosphatidic acid + Ca^{2+})	> 1	 *Inverted truncated cone*	Inverted micelles 

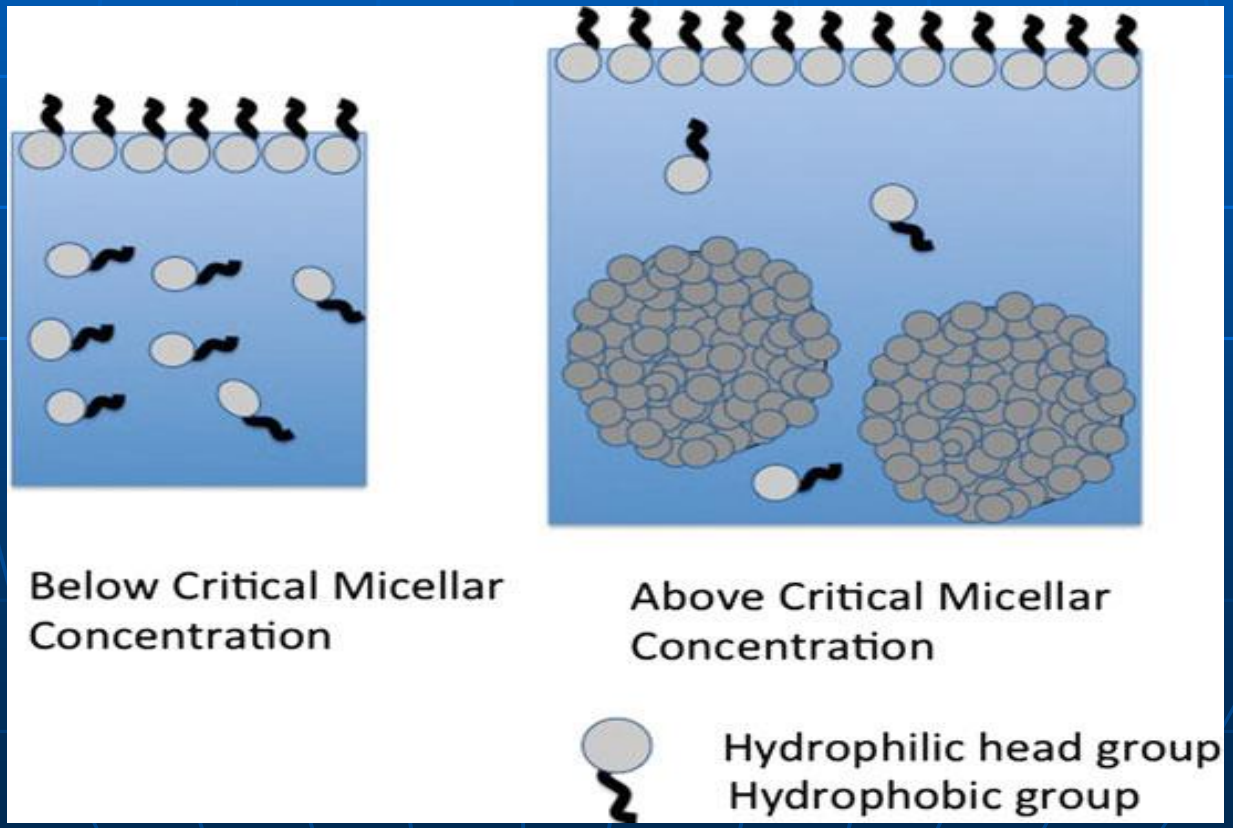
Lipid	Critical packing parameter $\frac{v}{a_0 l_c}$	Critical packing shape	Structures formed
Single-chained lipids with large head-group areas. (e.g. NaDS in low salt and some lysophospholipids)	$< \frac{1}{3}$		Spherical micelles 
Single-chained lipids with small head-group areas. (e.g. NaDS in high salt, lysolecithin and non-ionic surfactants)	$\frac{1}{3} - \frac{1}{2}$		Cylindrical or globular micelles 
Double-chained lipids with large head-group and fluid chains. (e.g. lecithin, dialkyl dimethyl ammonium salts, sphingomyelin, DGDG, phosphatidylserine, phosphatidyl inositol)	$\frac{1}{2} - 1$		Flexible bilayers, vesicles 

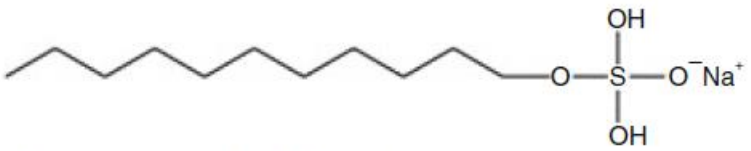
Vesicular drug delivery systems

- 1. Prolong the existence of the drug in systemic circulation and perhaps, reduces the toxicity if selective uptake can be achieved due to the delivery of drug directly to the site of infection.
- 2. Improves the bioavailability especially in the case of poorly soluble drugs.
- 3. Both hydrophilic and lipophilic drugs can be incorporated.
- 4. Delays elimination of rapidly metabolizable drugs and thus function as sustained release systems

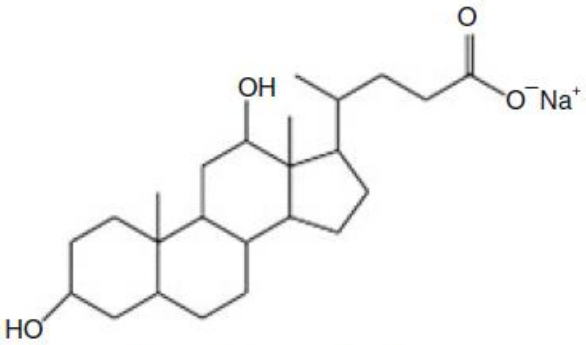
Low Molecular Weight Micelles

- Low molecular weight amphiphile micelles are formed from the selfassembly of comparatively hydrophilic amphiphiles (molecular weight <1,500 Da).

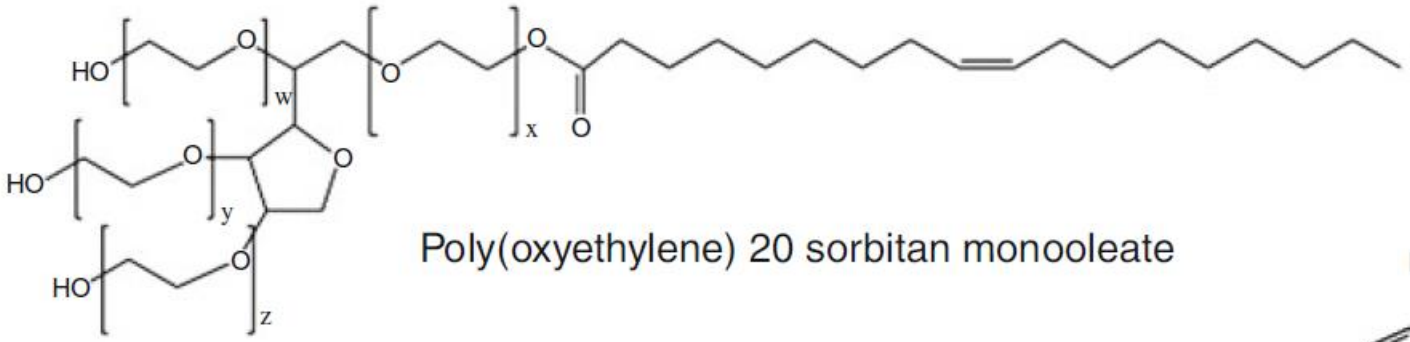




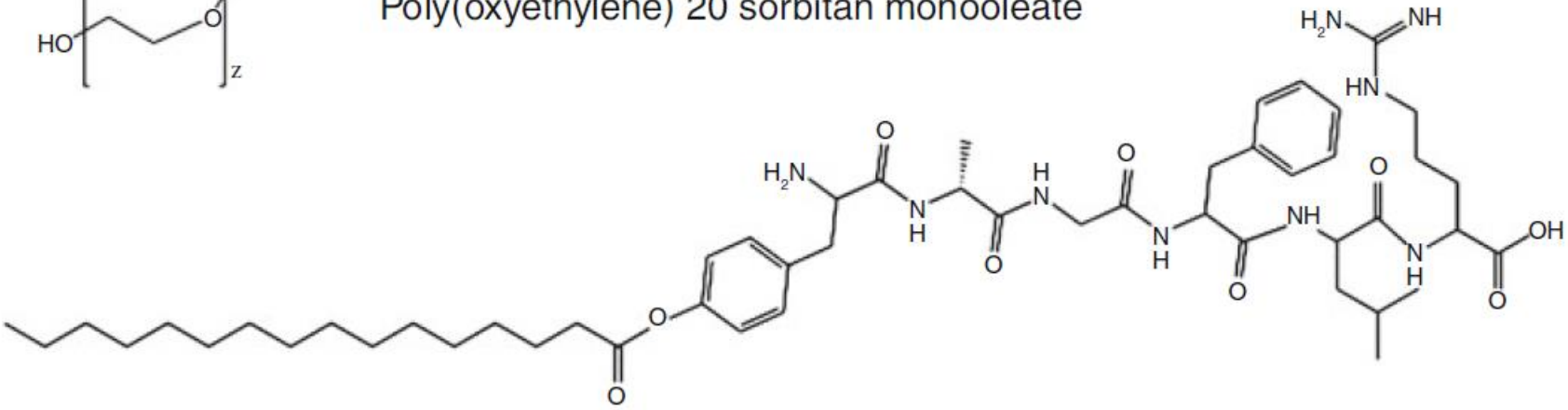
Sodium dodecyle sulphate



Sodium deoxycholate



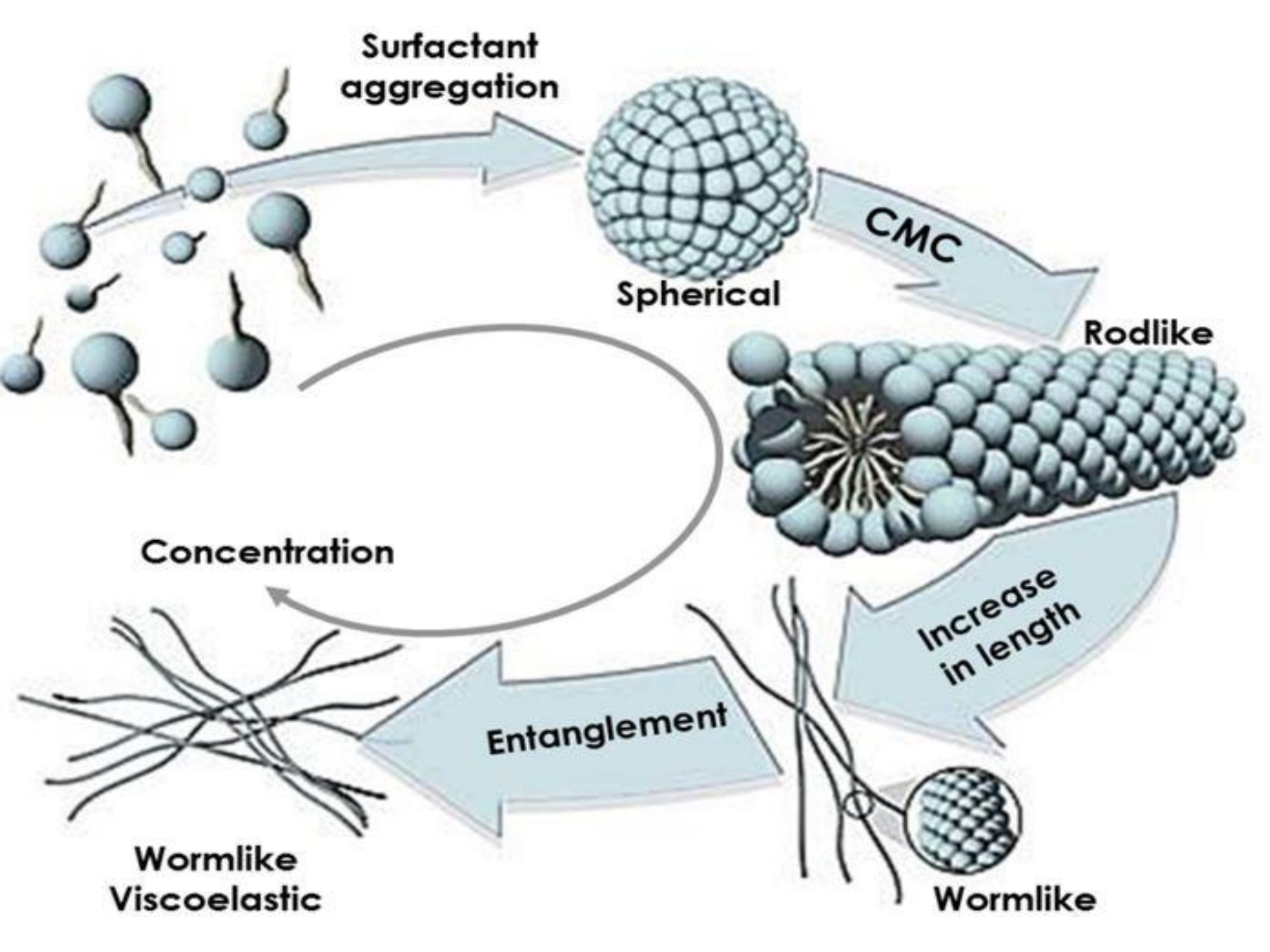
Poly(oxyethylene) 20 sorbitan monooleate



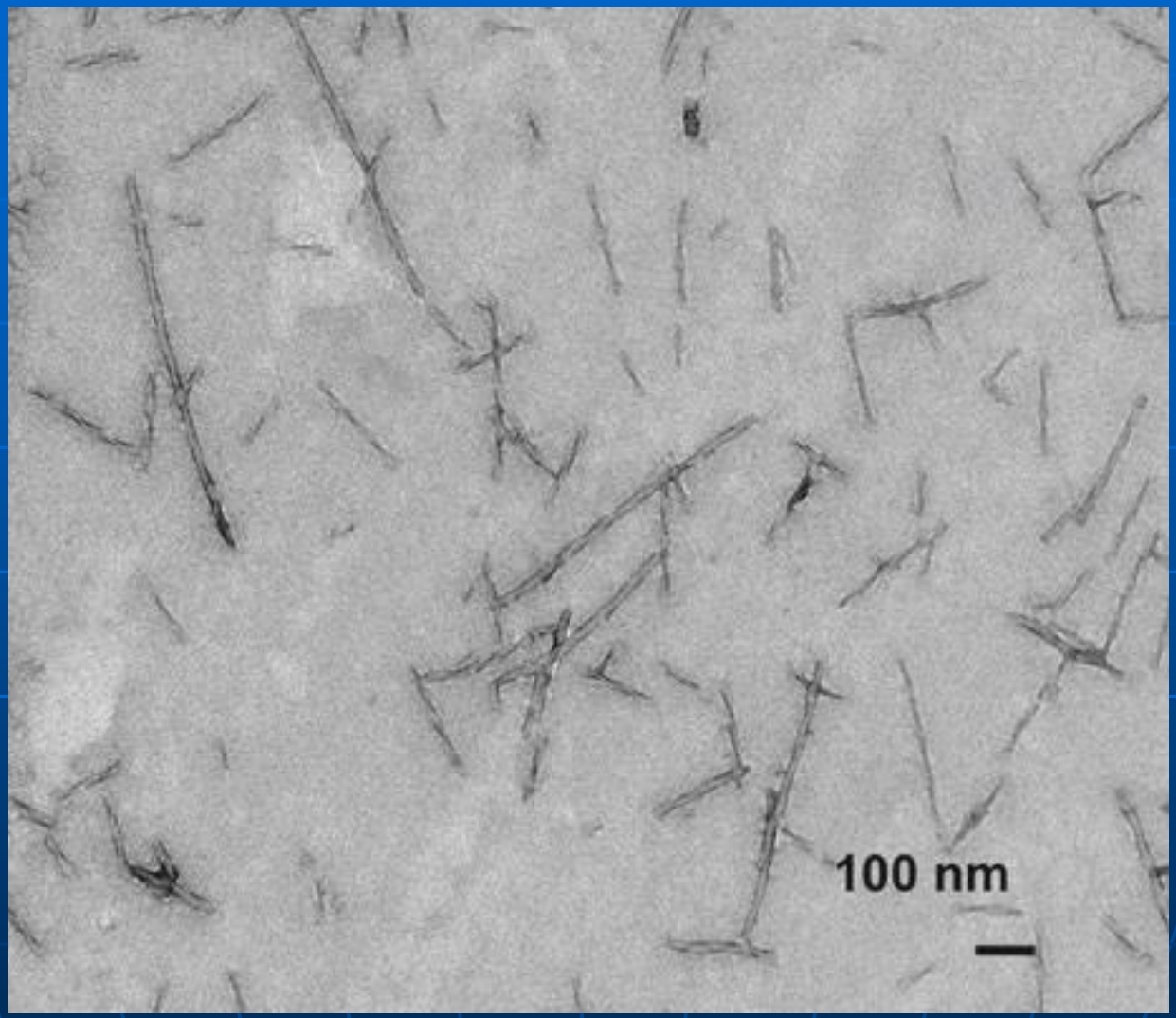
Palmitoyl dalargin

Table 2.1 Methods for the determination of the critical micelle concentration

Method	Principle	Reference	Advantages	Comments
Surface tension	Measures the change in surface tension caused by progressively more molecules associating at the air—aqueous interface; a limiting value signals the onset of micellisation	Nagadome et al. (1992)	Simple methodology	Large quantities of sample required (>100 mg)
Conductivity	Measures the change in conductivity as ionic surfactants aggregate into micelles	Mehrotra and Jain (1992); Okano et al. (2000)	Simple methodology	Only suitable for ionic amphiphiles
Capillary electrophoresis	Measures the conductivity change when ionic amphiphiles aggregate into micelles	Cifuentes et al. (1997)	Low sample requirements <10 mg	Only suitable for ionic amphiphiles
Cyclic voltammetry	Measures the change in current associated with an electrochemical probe, at varying potential and in the presence of a micelle forming amphiphile	Mandal et al. (1988)	Relatively simple experimentation	Only suitable for ionic amphiphiles
Isothermal calorimetry	Measures the enthalpy of demicellisation as the micelles are diluted	Hildebrand et al. (2004)	Accurate method provides additional information on other thermodynamic parameters (e.g. the entropy and free energy change of micellisation)	Expensive instrumentation
Nuclear magnetic resonance spectroscopy	Measures the chemical shift changes of a relevant proton as the amphiphile self assembles into micelles	Zhao and Fung (1993)	Small sample requirements — mg	Expensive instrumentation
Colorimetry—methyl orange	Measures the change in the absorption spectrum, the hypsochromic shift, experienced by methyl orange as it associates with the hydrophobic micelle core	Karukstis et al. (1998); Buwalda and Engberts (2001); Wang et al. (2004)	Rapid analysis	Tends to overestimate the CMC value (Siew et al. 2012)
Flourescence spectroscopy—pyrene	Measures the change in the emission spectra of pyrene as it associates with the hydrophobic core of the micelle	Kalyanasundaram and Thomas (1977); Chooi et al. (2010)	Rapid analysis	Errors may arise if pyrene associates with the monomers in solution, leading to an underestimation of the CMC (Chooi et al. 2010)


Surfactant aggregation
Spherical
CMC
Rodlike
Concentration
Increase in length
Entanglement
Wormlike Viscoelastic
Wormlike

Drug	Trade name	Indication(s)	Manufacturer	Formulation and administration	Reference
Calcitriol	Calcijex	Hypocalcemia associated with chronic renal dialysis	Abbott	A micellar dispersion containing: calcitriol (1 μg mL^{-1}), poly(oxyethylene) 20 sorbitan monolaurate (4 mg mL^{-1}), sodium ascorbate (10 mg mL^{-1}), sodium chloride (1 mg mL^{-1}), ethylene diamine tetraacetic acid (1.1 mg mL^{-1}), sodium phosphate (9.2 mg mL^{-1}), pH=6.5–8.0 Administered as an intravenous bolus	Strickley (2004)
Doxercalciferol	Hectorol	Secondary hyperparathyroidism associated with chronic renal dialysis	Bone care	A micellar dispersion containing: doxercalciferol (2 μg mL^{-1}), poly(oxyethylene) 20 sorbitan monooleate (4 mg mL^{-1}), sodium ascorbate (10 mg mL^{-1}), sodium chloride (1.5 mg mL^{-1}), disodium ethylene diamine tetraacetic acid (1.1 mg mL^{-1}), sodium phosphates (9.2 mg mL^{-1}) Administered as an intravenous bolus	Strickley (2004)
Amphotericin B	Fungizone	Life-threatening fungal infections, mucocutaneous leishmaniasis	Bristol Myers Squibb	A lyophilised solid containing: amphotericin B (50 mg), sodium desoxycholate (41 mg), sodium phosphate (20.2 mg) Reconstituted in 10 mL water for injection and further diluted to an amphotericin B concentration of 0.1 mg mL^{-1} in dextrose (5 % w/v) injection. Administered as a slow infusion	Dailymed (2012)

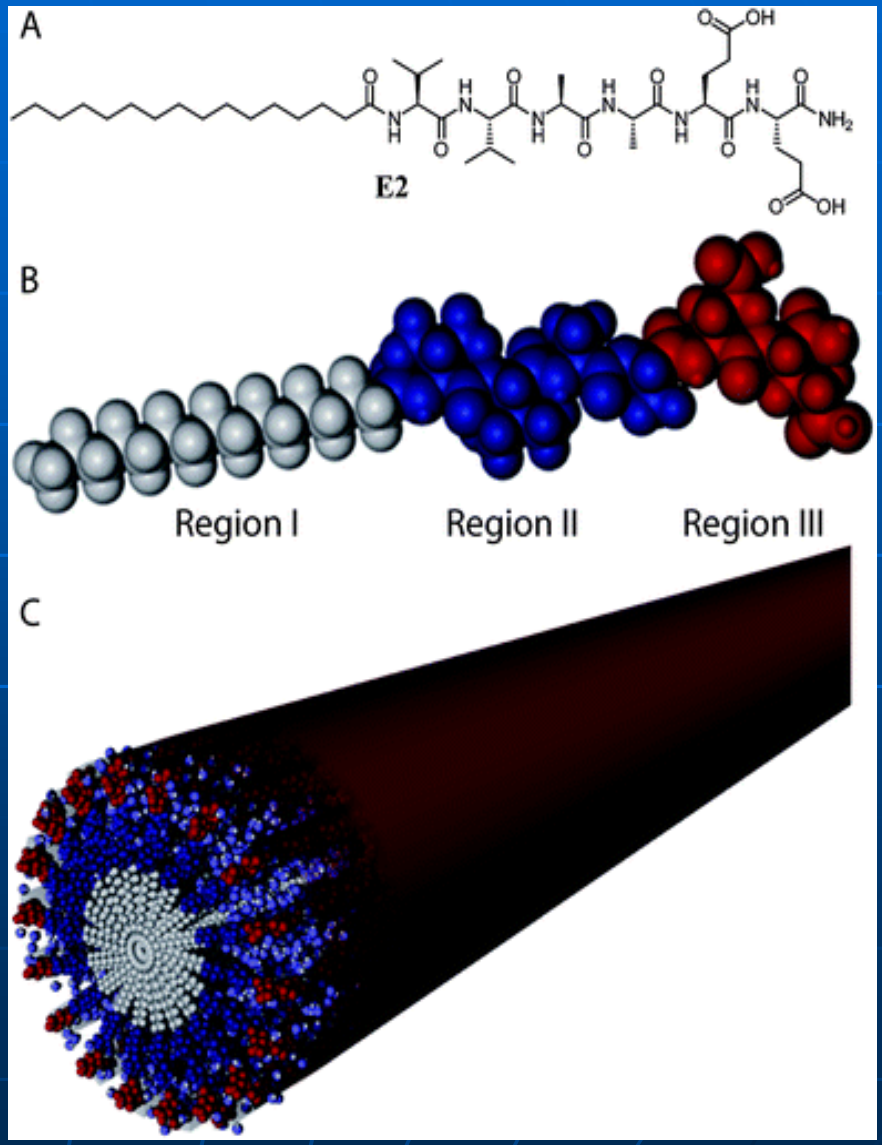


Palmitoyl dalargin peptide nanofibres Mazza et al. 2013

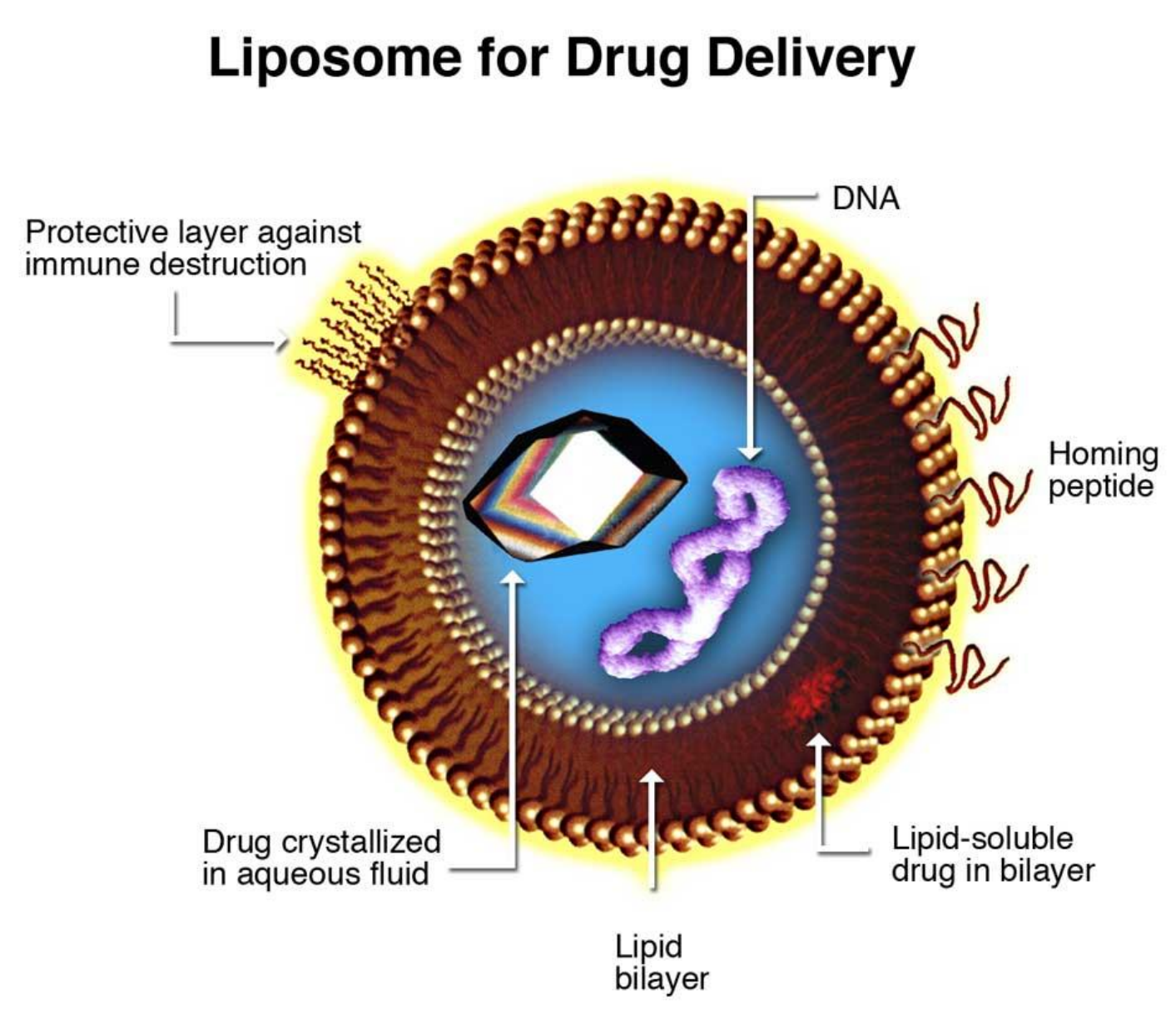
Delivery of hydrophilic peptides

EP2590682A1, US20130203647

A composition comprises nanofibres for the delivery of a peptide across the blood brain barrier in a method of therapy of the human or animal body, wherein the nanofibres comprise a peptide conjugated to a lipophilic group. Further, a compound comprises a Dalargin or a derivative having one or more substituted, deleted or inserted aminoacyl units, and, conjugated to an aminoacyl group preferably via a side chain, a lipophilic group, optionally via a linker.



Liposomes


Liposome for Drug Delivery
DNA
Protective layer against immune destruction
Homing peptide
Drug crystallized in aqueous fluid
Lipid-soluble drug in bilayer
Lipid bilayer

Classification Based on structural parameters

TYPE	SPECIFICATIONS
MLV	Multilamellar large vesicles- >0.5 μm
OLV	Oligolamellar vesicles- 0.1-1 μm
UV	Unilamellar vesicles (all in size)
SUV	Small unilamellar vesicles-20-100nm
MUV	Medium sized unilamellar vesicles
LUV	Large unilamellar vesicles->100nm
GUV	Giant unilamellar vesicles->1 μm
MV	Multivesicular vesicles->1 μm

Structure of liposmes

(SUV - small unilamellar vesicle)

(LUV - large unilamellar vesicle)

(MLV – multilamellar large vesicle)

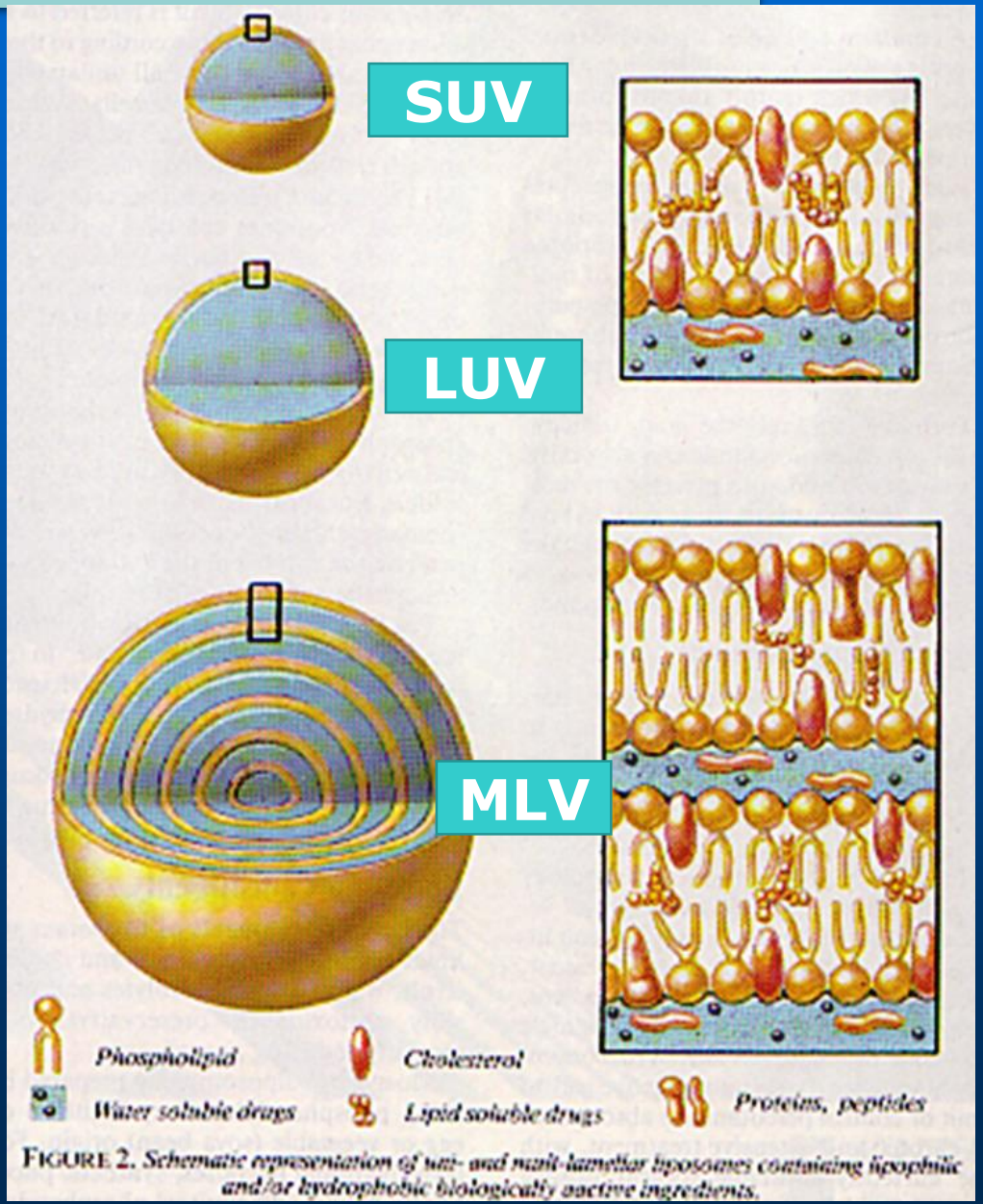


FIGURE 2. Schematic representation of uni- and multi-lamellar liposomes containing lipophilic and/or hydrophobic biologically active ingredients.

Designing of the drug in the vesicular system has brought a new life to the old pre-existing drugs and thus has improved their therapeutic efficacies by controlling and sustaining the actions.

-liposomes,
-niosomes,
-transfersomes, -
-pharmacosomes,
-ethosomes,
-sphinosomes,
-colloidosomes,
-herosomes
-cubosomes

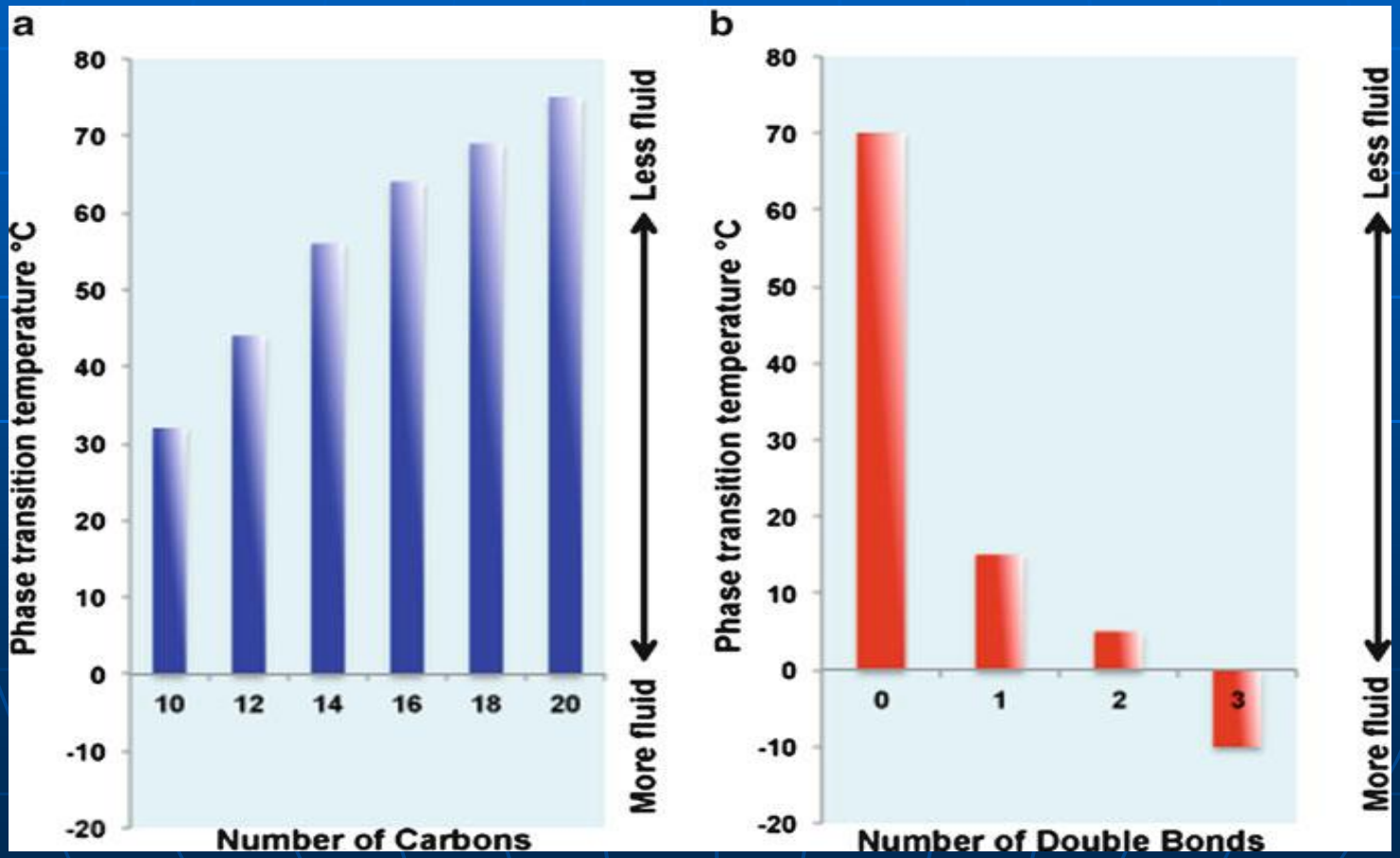
-aquasomes,
-cryptosmes,
-discomes,
-emulsomes,
-enzymosome,
-genosomes,
-photosomes,
-virosomes,
-vesosomes,
-proteosomes

provesicular drug delivery, coating of vesicles, layerosomes, ufosomes system etc

- **Liposomes** consist of one or more concentric lipid bilayers, which enclose an internal aqueous volume(s). For drug delivery applications liposomes are usually unilamellar, range in diameter from about 50 – 150nm
- **Niosomes** are formations of vesicles by hydrating mixture of cholesterol and nonionic surfactants.
- **Pharmacosomes** are defined as colloidal dispersions of drugs covalently bound to lipids and may exist as ultrafine vesicular, micellar or hexagonal aggregates, depending on the chemical structure of drug-lipid complex
- **Ethosomes** are noninvasive delivery carriers that enable drugs to reach the deep skin layers and/or the systemic circulation.
- **Transfersomes** are specially optimized, ultradeformable (ultraflexible) lipid supramolecular aggregates, which are able to penetrate the mammalian skin intact.
- **Colloidosomes** are the hollow shell microcapsules consisting of coagulated or fused particles at interface of emulsion droplets.

Phase Transition Temperature (T c)

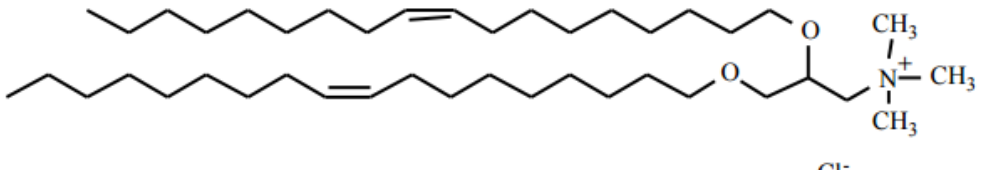
The *T* c is the temperature at which lipids move from a gel phase to a liquid crystalline phase.



Head Group and Surface Charge

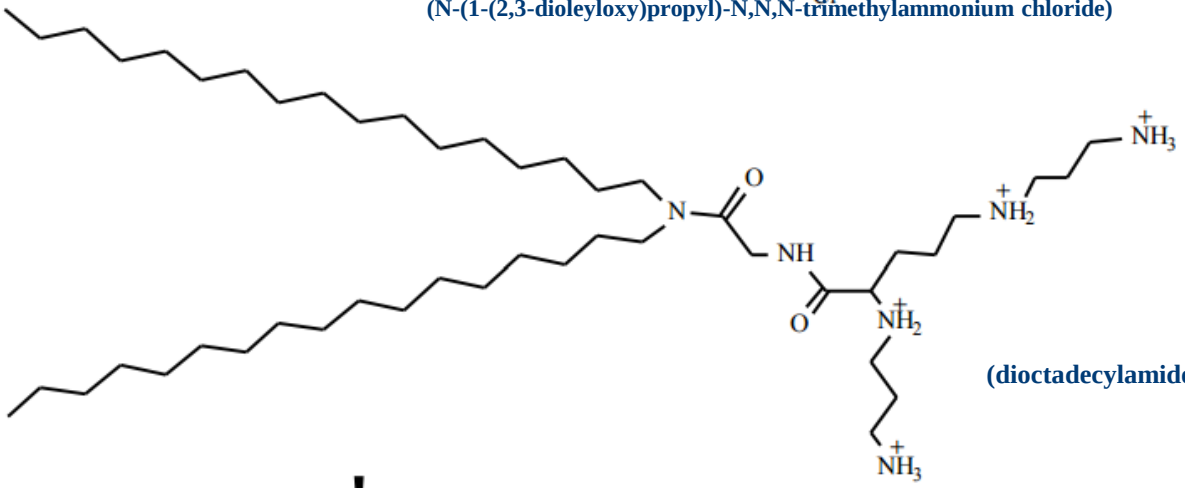
Neutral, anionic, and cationic liposomes may be formed depending on the nature of the head group on the chosen lipid

Neutral	Negative
Phosphatidylcholine	Phosphatidylglycerol
$R–PO_4–CH_2CH_2N(CH_3)_3$	$R–PO_4–H_2CHOHCH_2OH$
Phosphatidylethanolamine	Phosphatidylserine
$R–PO_4–CH_2CH_2NH_3$	$R–PO_4–CH_2CHCOONH_3$
	Phosphatidic acid
	$R–PO_2–(OH)_2$



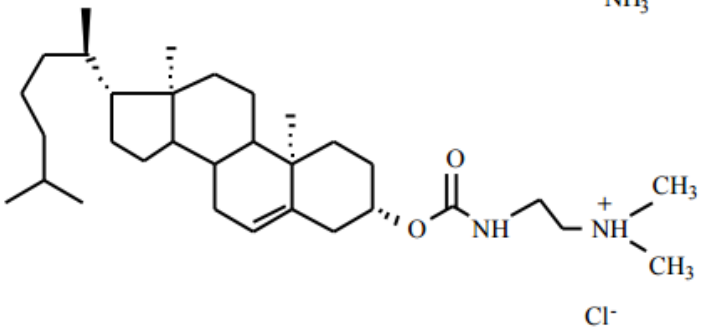
DOTMA

(N-(1-(2,3-dioleyloxy)propyl)-N,N,N-trimethylammonium chloride)



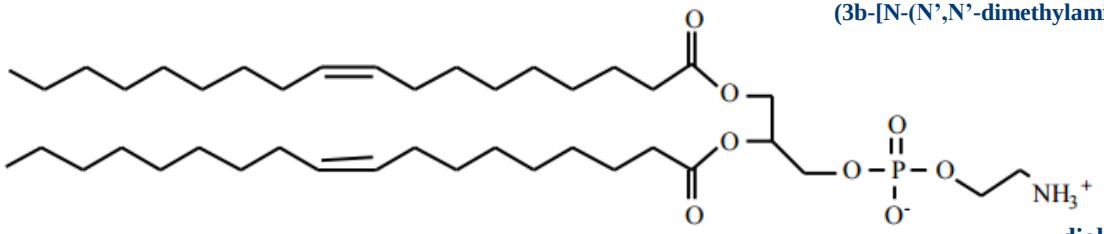
DOGS

(dioctadecylamido-glycylspermine)



DC-Chol

(3b-[N-(N',N'-dimethylaminoethyl)carbamoyl) cholesterol)



DOPE

dioleoyl phosphatidylethanolamine

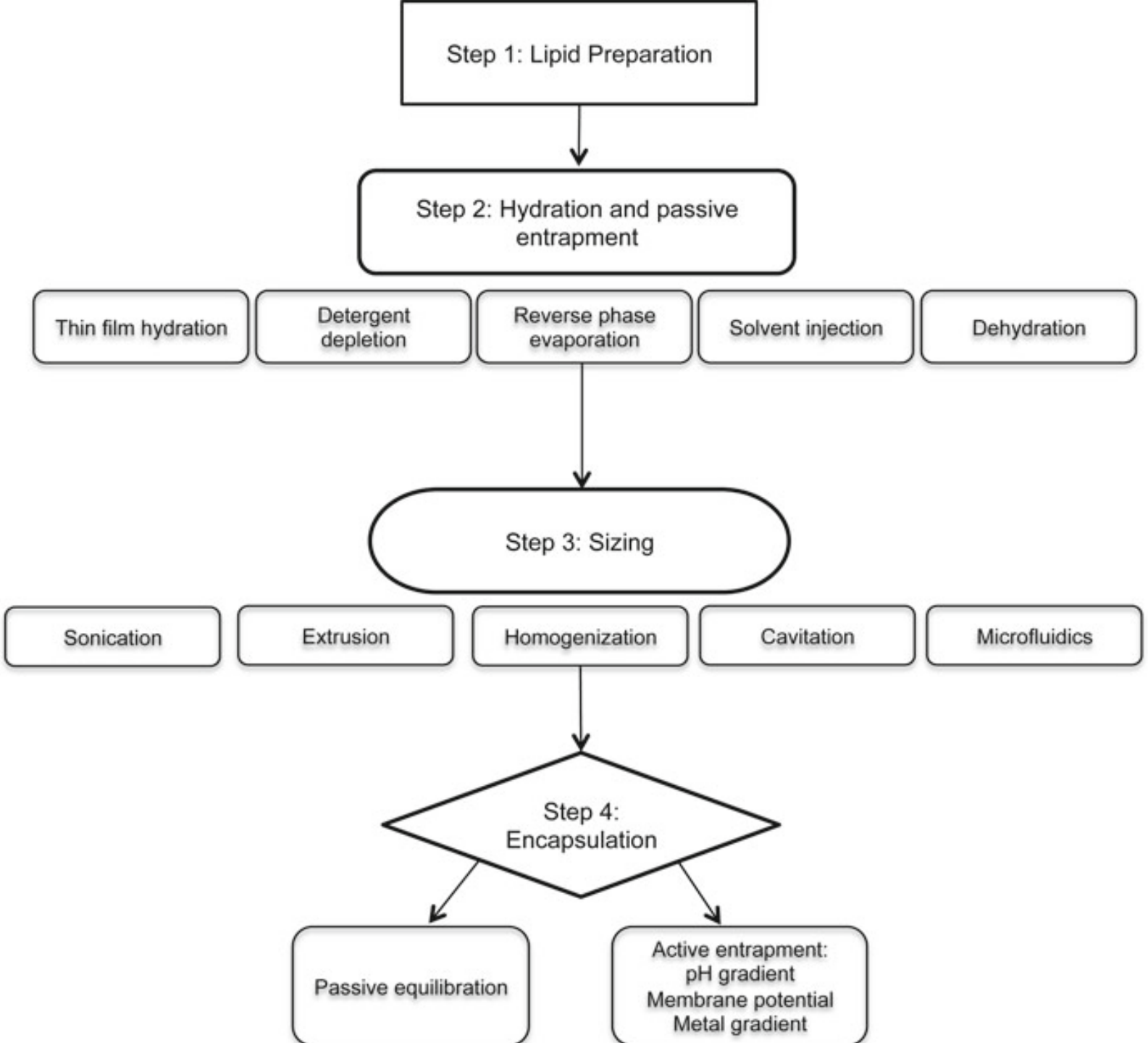
Table 3.1 Lipids used in the formation of liposomes and their properties

Name	Abbreviation	Fatty acid	Transition temperature	Net charge at pH 7.4
Egg phosphatidylcholine	EPC		–15–17	0
Dilauryloylphosphatidylcholine	DLPC	12:0	–1	0
Dimyristoylphosphatidylcholine	DMPC	14:0	23	0
Dipalmitoylphosphatidylcholine	DPPC	16:0	41	0
Distearoylphosphatidylcholine	DSPC	18:0	55	0
I-Myristoyl-2-palmitoylphosphatidylcholine	MPPC	14:0,16:0	27	0
I-Palmitoyl-2-myristoyl phosphatidylcholine	PMPC	16:0, 14:0	35	0
I-Palmitoyl-2-stearoyl phosphatidylcholine	PSPC	16:0,18:0	44	0
I-Stearoyl-2-palmitoyl phosphatidylcholine	SPPC	18:0, 16:0	47	0
Dioleoylphosphatidylcholine	DOPC	18:1	–20	0
1,2-dioleoyl-sn-glycero-3-phosphoethanolamine	DOPE	18:1	–16	0
1,2-dioleoyl-sn-glycero-3-phosphate	DOPA	18:1	–8	–1.3
Dilauryloylphosphatidylglycerol	DLPG		4	–1
Dimyristoylphosphatidylglycerol	DMPG	14:0	23	–1
Dipalmitoylphosphatidylglycerol	DPPG	16:0	41	–1
Distearoylphosphatidylglycerol	DSPG		55	–1
Dioleoylphosphatidylglycerol	DOPG	18:1	–18	–1
Dimyristoyl phosphatidic acid	DMPA	14:0	50	–1.3
Dipalmitoyl phosphatidic acid	DPPA	16:0	67	–1.3
Dimyristoyl phosphatidylethanolamine	DMPE		50	0
Dipalmitoyl phosphatidylethanolamine	DPPE		63	0
Dimyristoyl phosphatidylserine	DMPS	14:0	35	–1
Dipalmitoyl phosphatidylserine	DPPS	16:0	54	–1
	DOPS	18:1	–11	–1
Brain phosphatidylserine	PS		6–8	–
Brain sphingomyelin	BSP		32	0
Dipalmitoyl sphingomyelin	DPSP		41	0
Distearoyl sphingomyelin	DSSP		57	0

Adapted from (Szoka and Papahadjopoulos 1980) (http://avantilipids.com)

Liposome Manufacturing

- The general steps of the manufacturing procedure are;
  - (1) Preparation of the lipids for hydration;
  - (2) Hydration;
  - (3) Sizing to a homogeneous distribution of vesicles;
  - (4) Encapsulation (in cases where the agent of interest is encapsulated/associated passively the agent is added while the lipids are being hydrated).

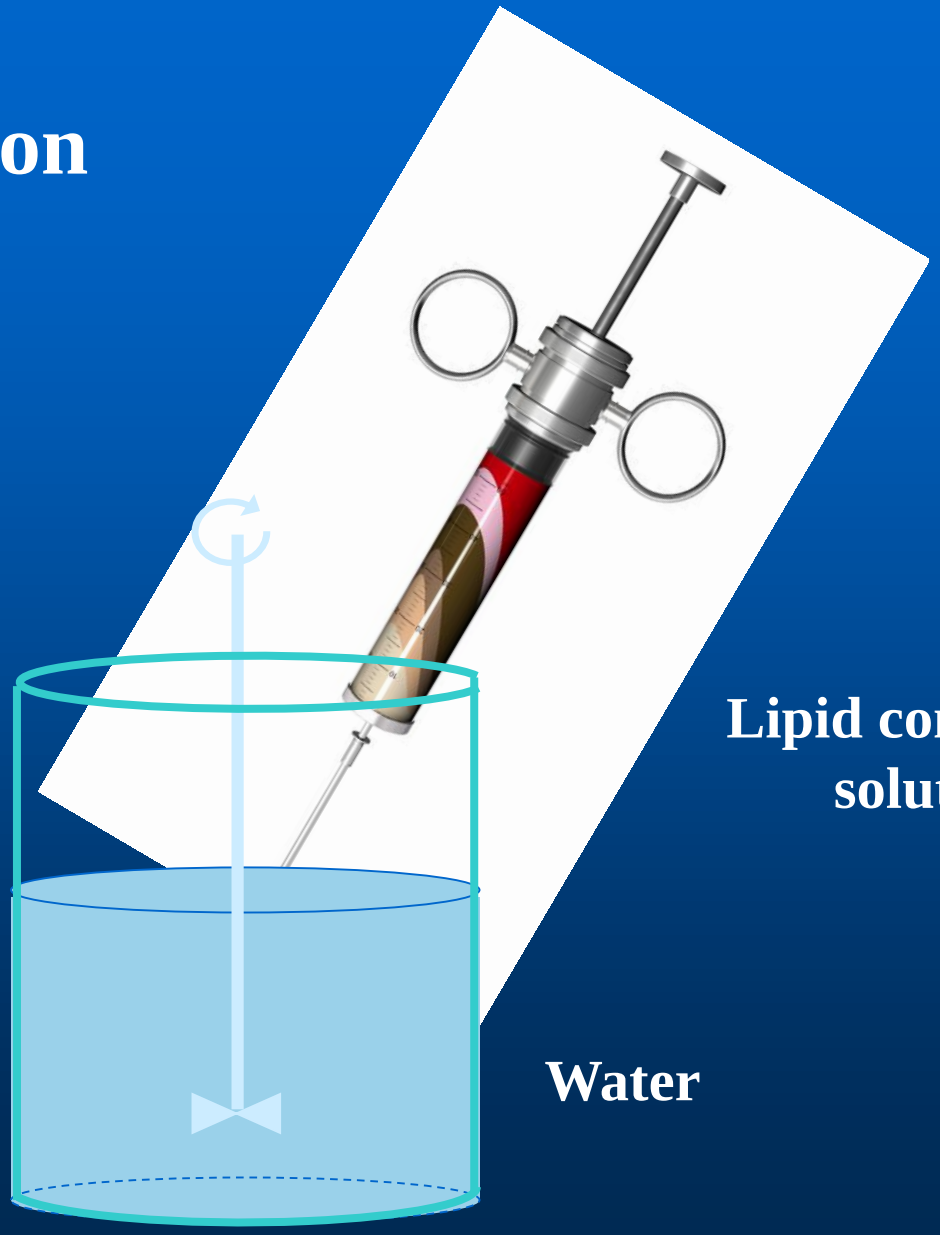

Step 1: Lipid Preparation
Step 2: Hydration and passive entrapment
Thin film hydration
Detergent depletion
Reverse phase evaporation
Solvent injection
Dehydration
Step 3: Sizing
Sonication
Extrusion
Homogenization
Cavitation
Microfluidics
Step 4: Encapsulation
Passive equilibration
Active entrapment:
pH gradient
Membrane potential
Metal gradient

Classification Based on the method of liposome preparation

TYPE	SPECIFICATIONS
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

Liposome Manufacturing

Lipid hydratation by injection



Lipid contaning solution

Water

Solvent evaporation

Dissolved lipids and lipophylic API in organic solvent.

Heating to phase transition temperature and evaporation of solvent and hidratation on the same temperature.

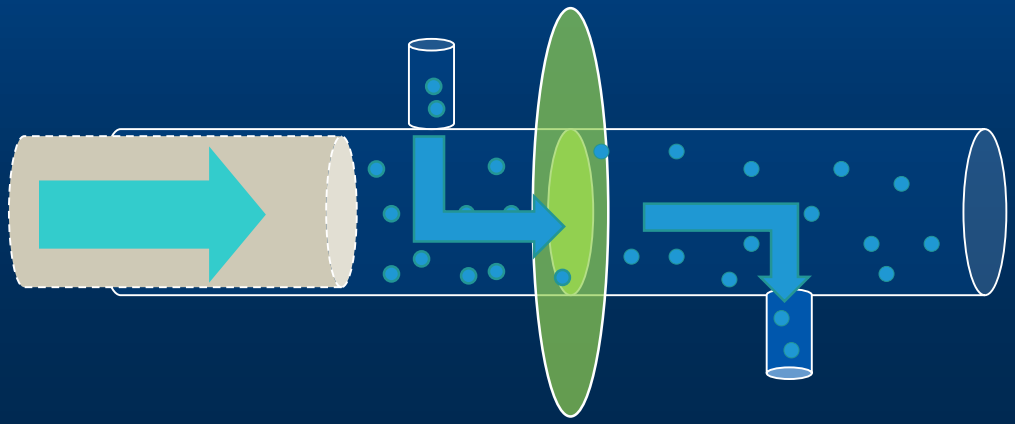
Cooling and separation

Liposome Manufacturing

Extrusion

MLV → Extrusion → SUV

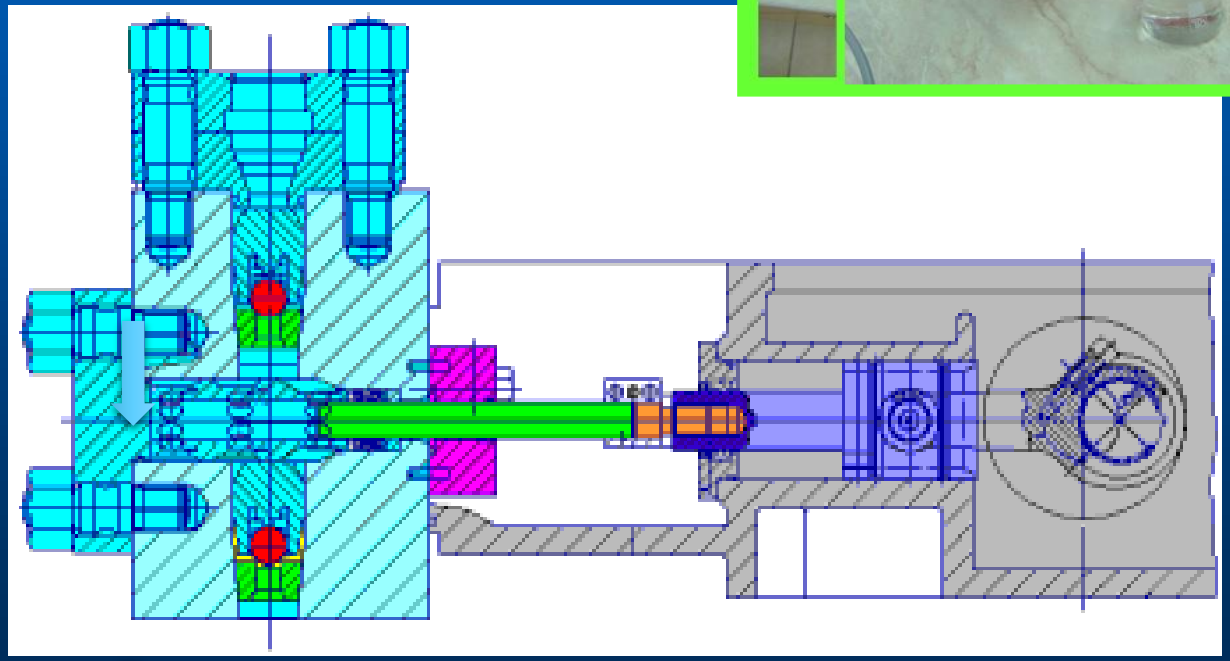
Micro sieve



Liposome Manufacturing

High pressure extrusion

MLV →SUV

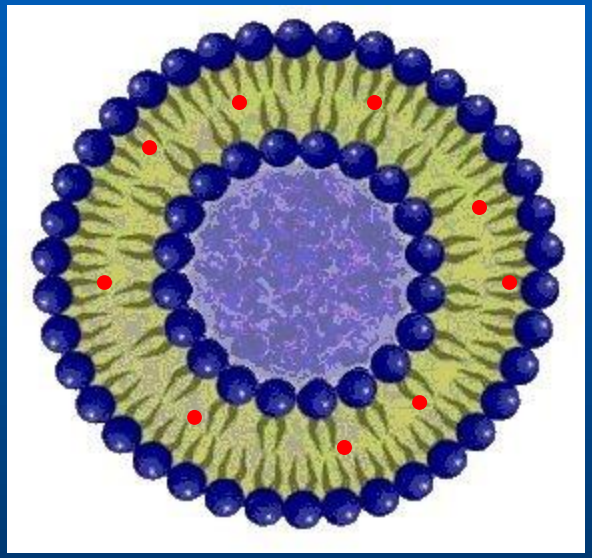


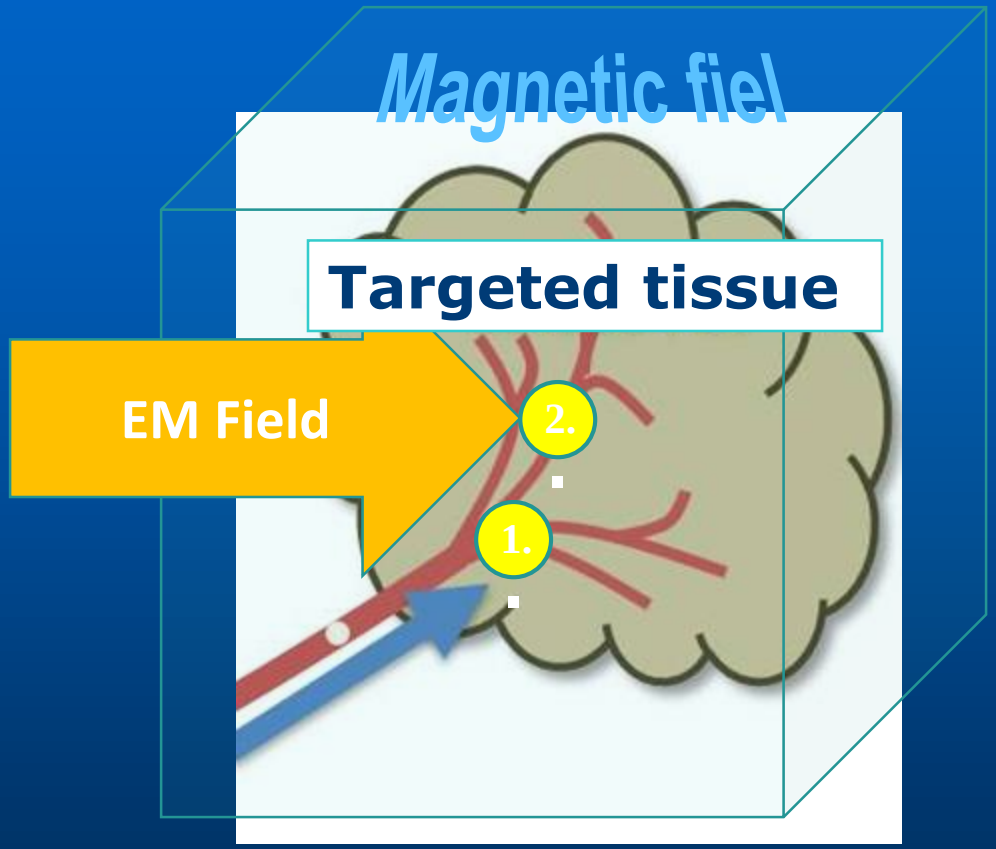
Extrusion chamber

Excentric high pressure pump

Liposome Manufacturing

Magnetic liposomes





1. Magnetic liposomes concentration by magnetic field
2. Liberation of API by alternating magnetic fileld

Liposome Manufacturing

Aseptic manufacturing

Liposome Manufacturing

Materials

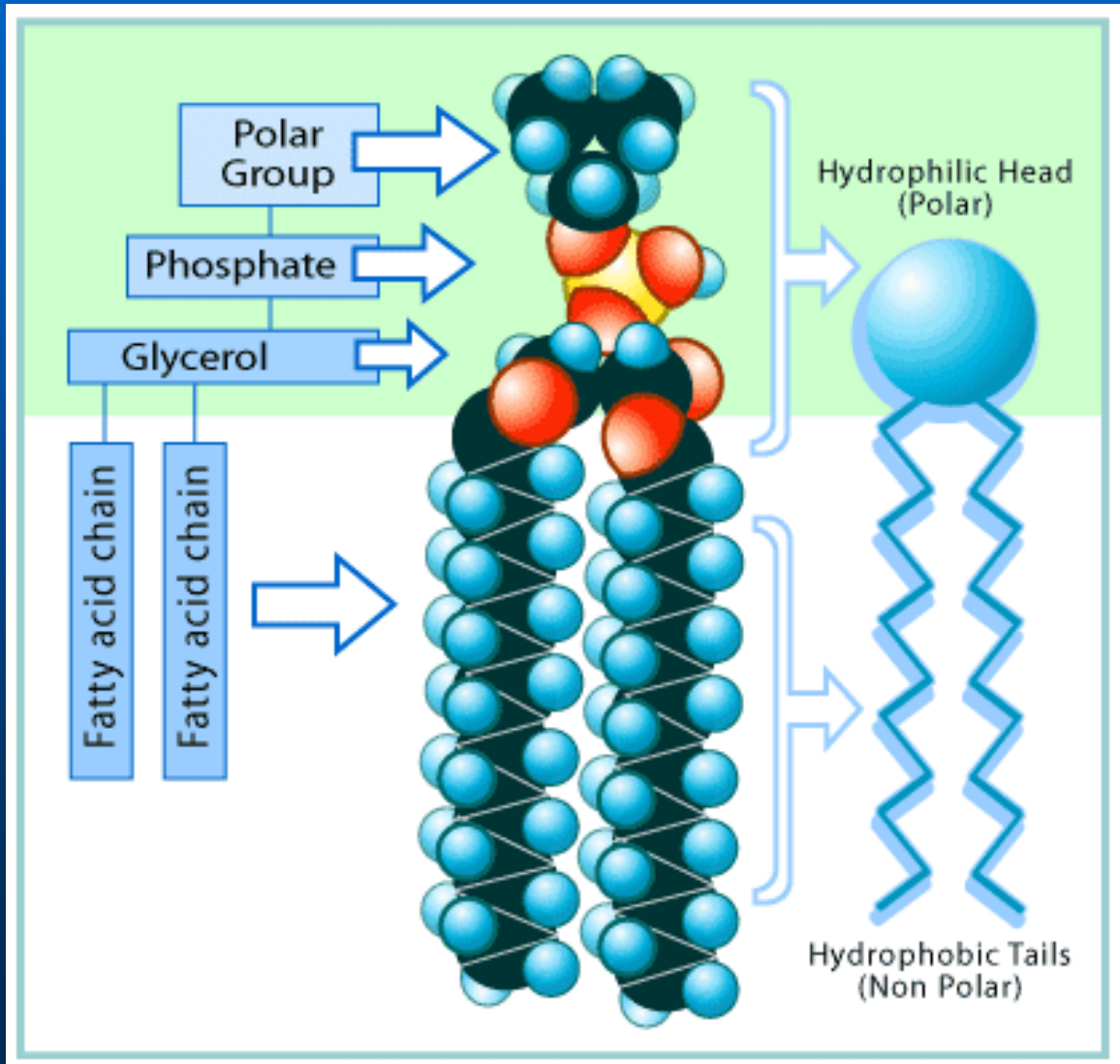
There are number of components of liposomes however **phospholipids** and **cholesterol** being main components.

Phospholipids are the major structural components of biological membranes, where two types of phospholipids exist – phosphodiglycerides and sphingolipids, together with their corresponding hydrolysis products.

The most common phospholipid is phosphatidylcholine (PC) molecule

Liposome Manufacturing

Phospholipids structure

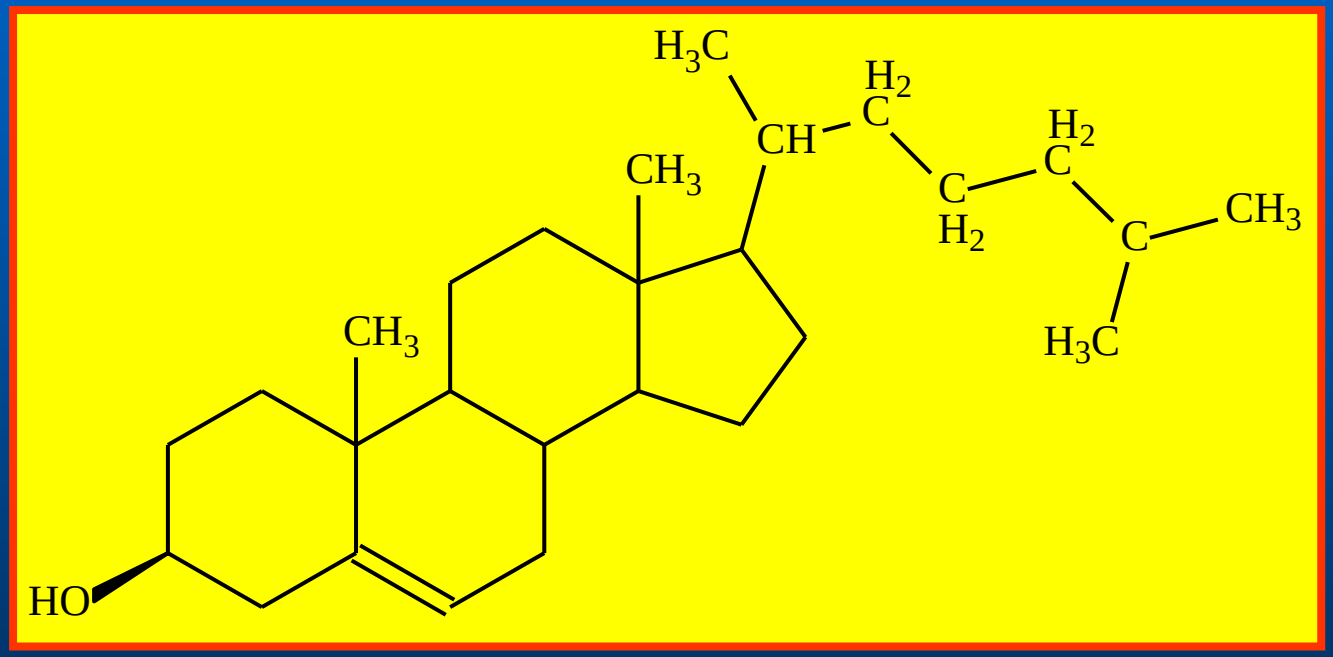


Liposome Manufacturing

- Commonly used synthetic Phospholipids:
- DOPC = Dioleoyl Phosphatidylcholine
- DOPE = Dioleoyl phosphatidylethanolamine
- DSPC = Distearoyl phosphatidylcholine
- DSPE = Distearoyl phosphatidylethanolamine
- DLPC = Dilauryl phosphatidylcholine
- DMPC = Dimyristoyl phosphatidylcholine
- DLPE = Dilauryl phosphatidylethanolamine

Liposome Manufacturing

Cholesterole
Stabilyzing agent

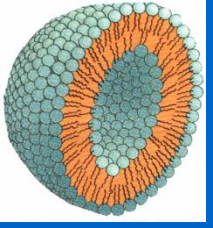


Liposomes

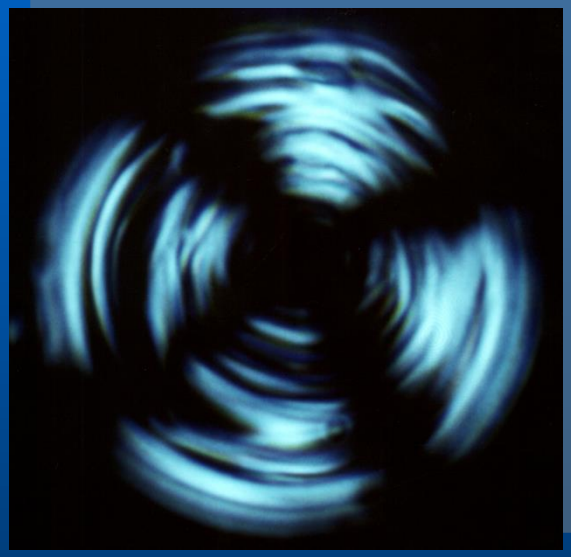
Examination

Physical Characterization

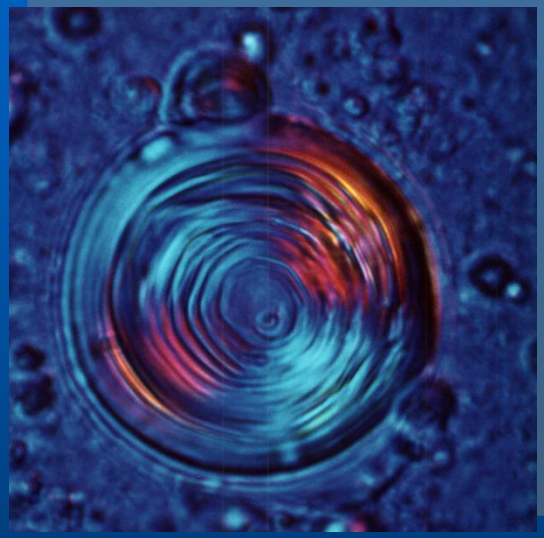
Characterization parameters	**Analytical methods**
Vesicle shape and surface morphology	Transmission electron microscopy, freeze-fracture electron microscopy.
Surface charge	Free-flow electrophoresis. EDLS
Lamellarity	Small angle X-ray scattering, freeze-fracture electron microscopy, ^{31}P-NMR.
Phase behavior	Freeze-fracture electron microscopy, differential scanning calorimetry
Percent capture/percent of free drug	Minicolumn centrifugation, gel exclusion, ion-exchange chromatography, radiolabelling



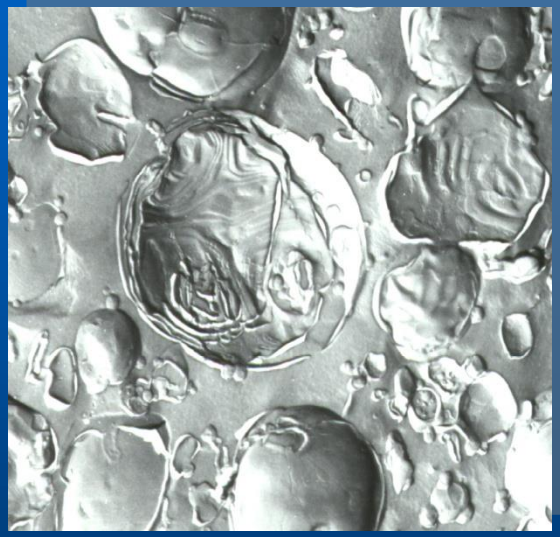
Liposomes examination



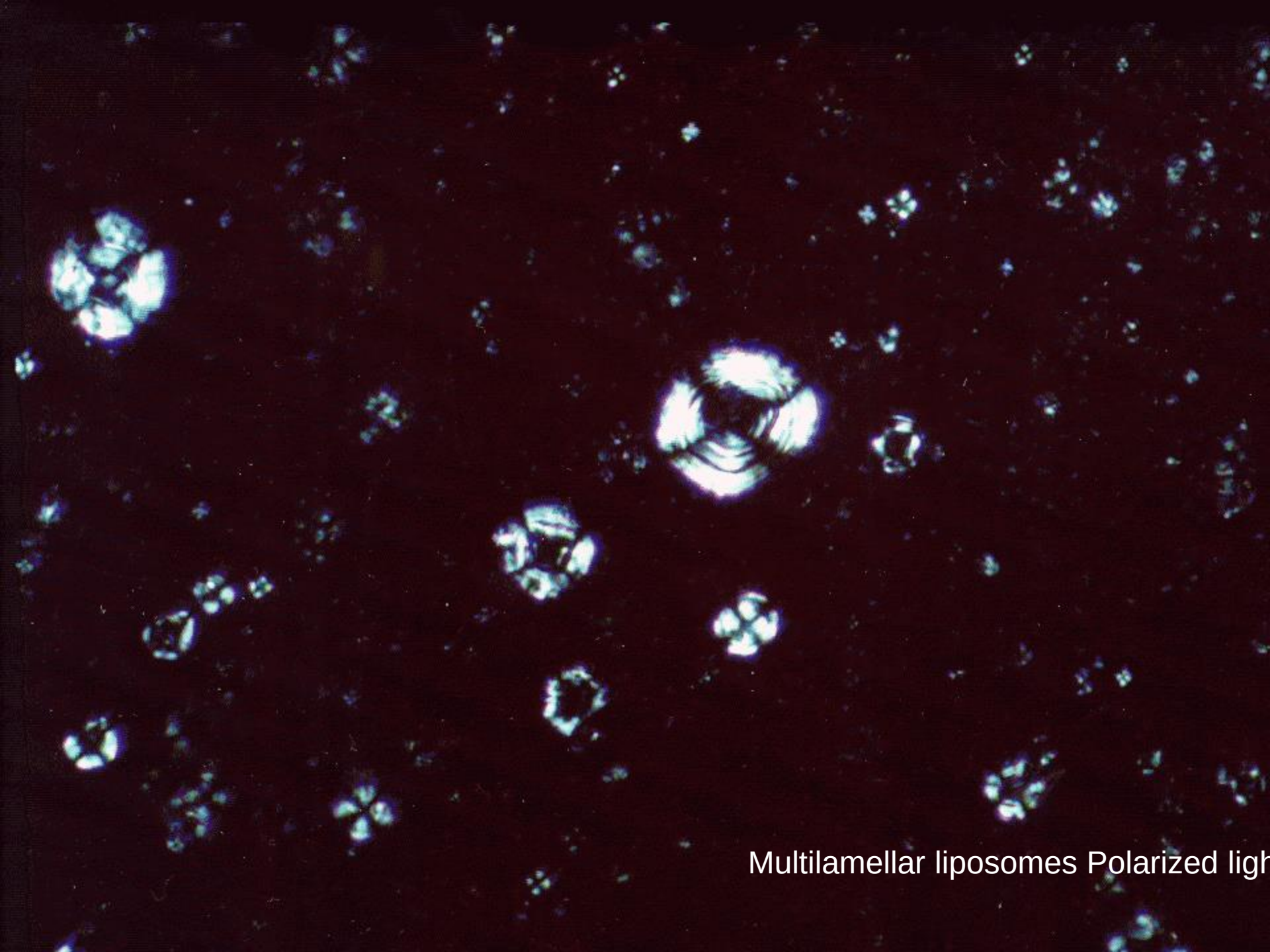
Optical microscope
polarized light



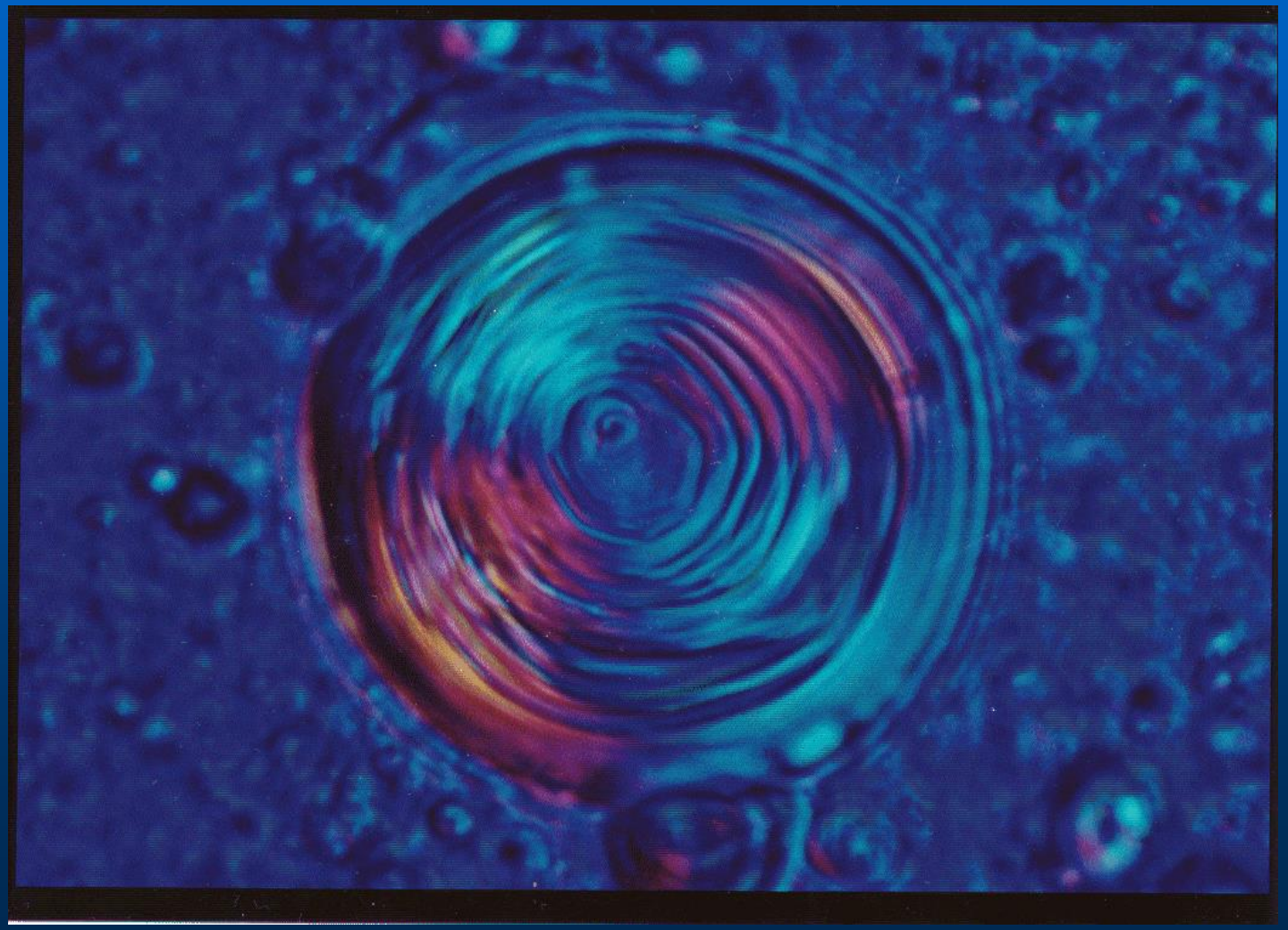
Optical microscope
Nomarski methode

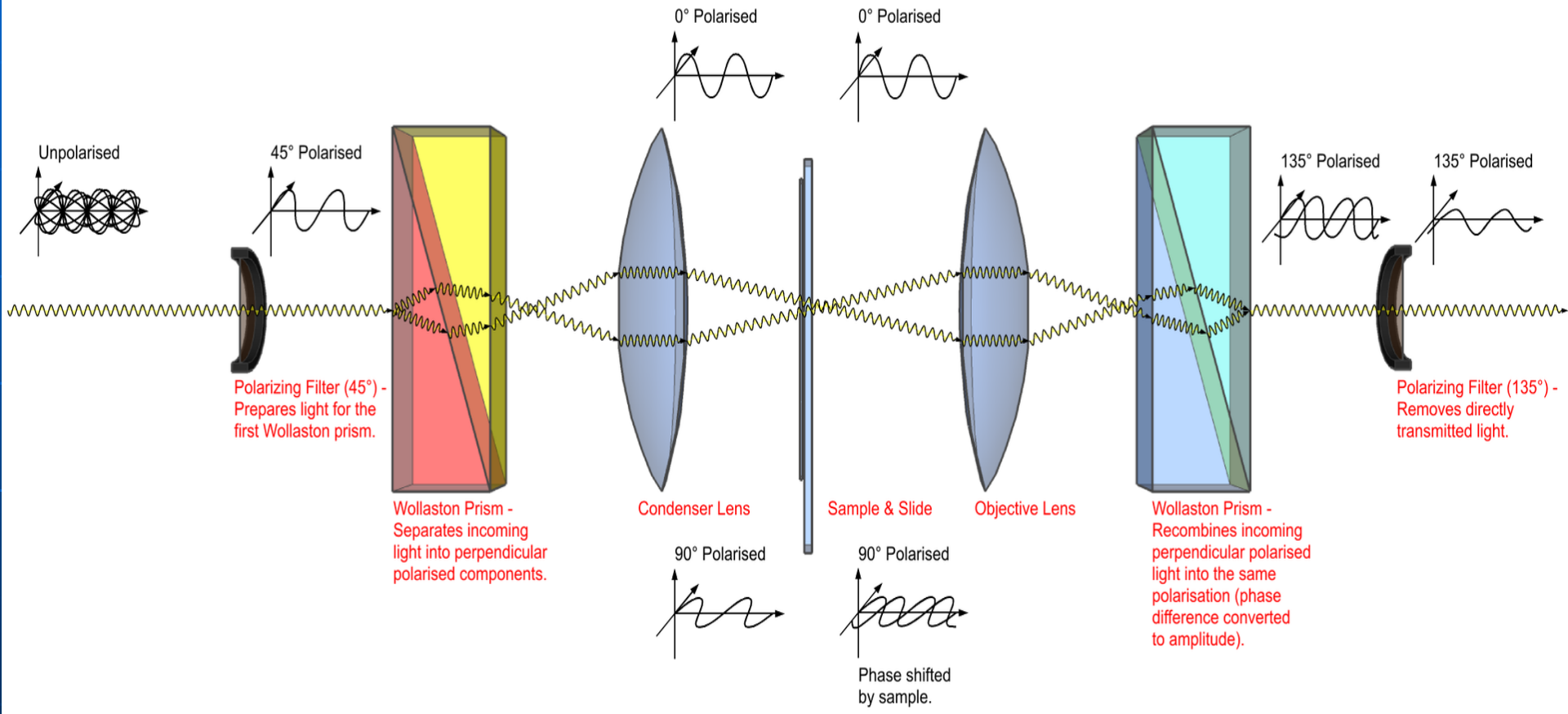


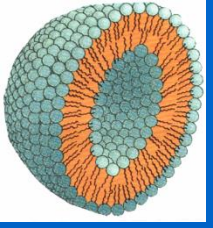
Electron microscope


Multilamellar liposomes Polarized ligh

Nomarski or differential interference contrast (DIC) microscopy (invented by Georges Nomarski in the mid-1950s) is used to image, living or stained specimens, which contain little or no optical contrast when viewed using brightfield illumination.




Unpolarised
45° Polarised
0° Polarised
0° Polarised
135° Polarised
135° Polarised
Polarizing Filter (45°) - Prepares light for the first Wollaston prism.
Wollaston Prism - Separates incoming light into perpendicular polarised components.
Condenser Lens
Sample & Slide
Objective Lens
Wollaston Prism - Recombines incoming perpendicular polarised light into the same polarisation (phase difference converted to amplitude).
Polarizing Filter (135°) - Removes directly transmitted light.
90° Polarised
90° Polarised
Phase shifted by sample.

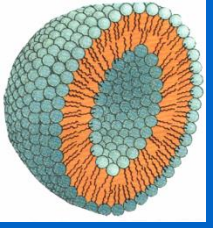


Chemical Characterization

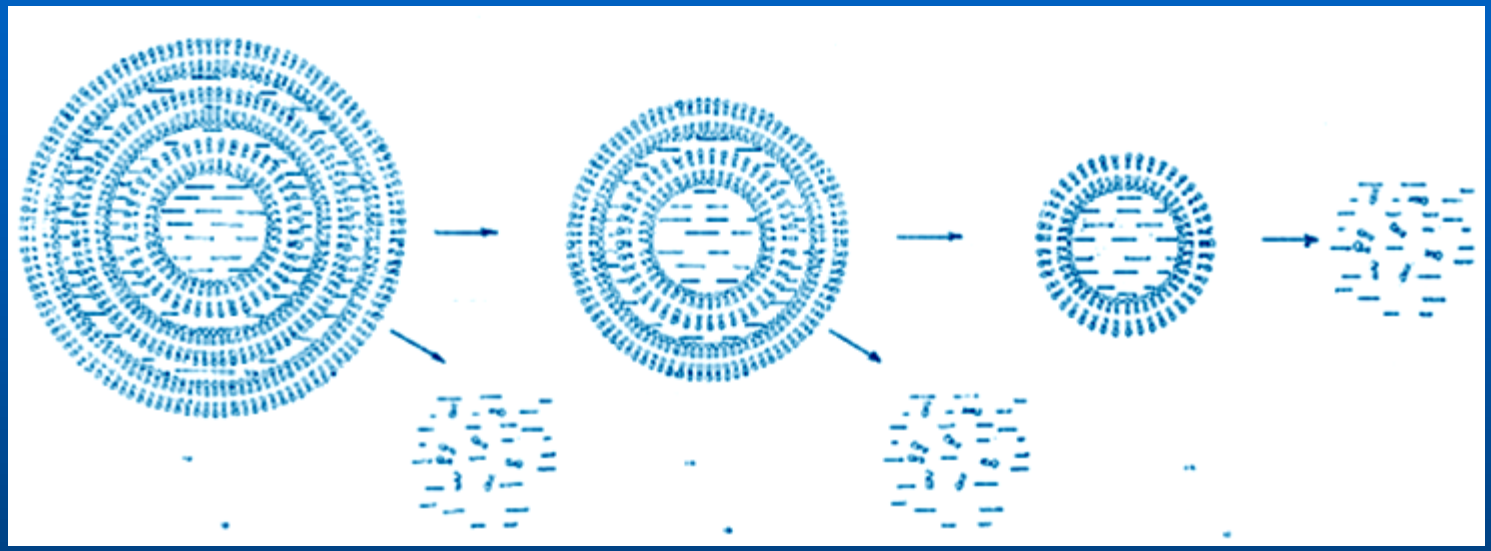
Characterization parameters	**Analytical methods**
Phospholipid concentration	Lipid phosphorous content using Barlett assay,HPLC
Cholesterol concentration	Cholesterol oxidase assay and HPLC
Drug concentration	Appropriate method given in monograph
Phospholipid peroxidation	UV absorbance, TBA,indometric and GLC
Phospholipid hydrolysis	HPLC and TLC
Cholesterol auto-oxidation	HPLC and TLC
Anti-oxidant degradation	HPLC and TLC
pH	pH meter
osmolarity	Osmometer

Biological Characterization

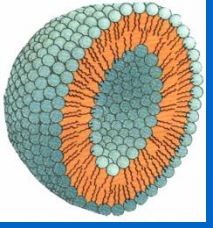
Characterization parameters	**Analytical methods**
sterility	Aerobic or anaerobic cultures
pyrogenicity	Limulus amebocyte lysate (LAL) test
Animal toxicity	Monitoring survival rates, histology and pathology



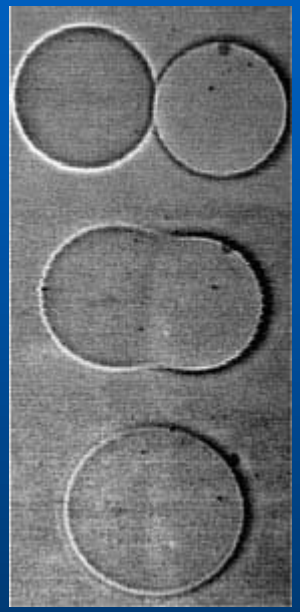
Stability of liposomes

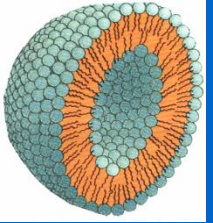


Structure changes during drug release.

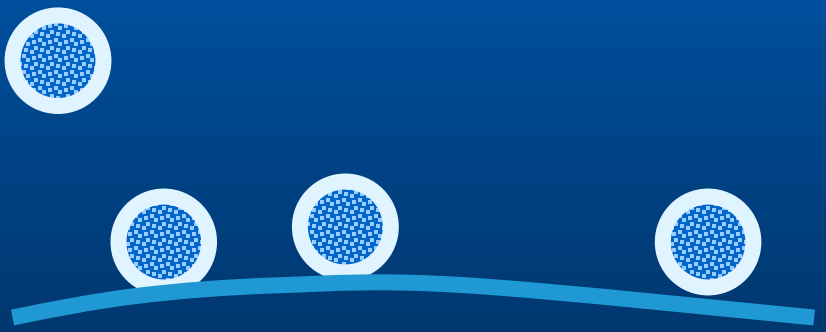


Liposomes examination

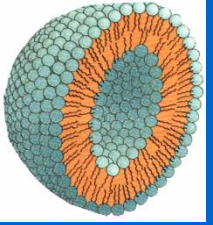




Adsorption



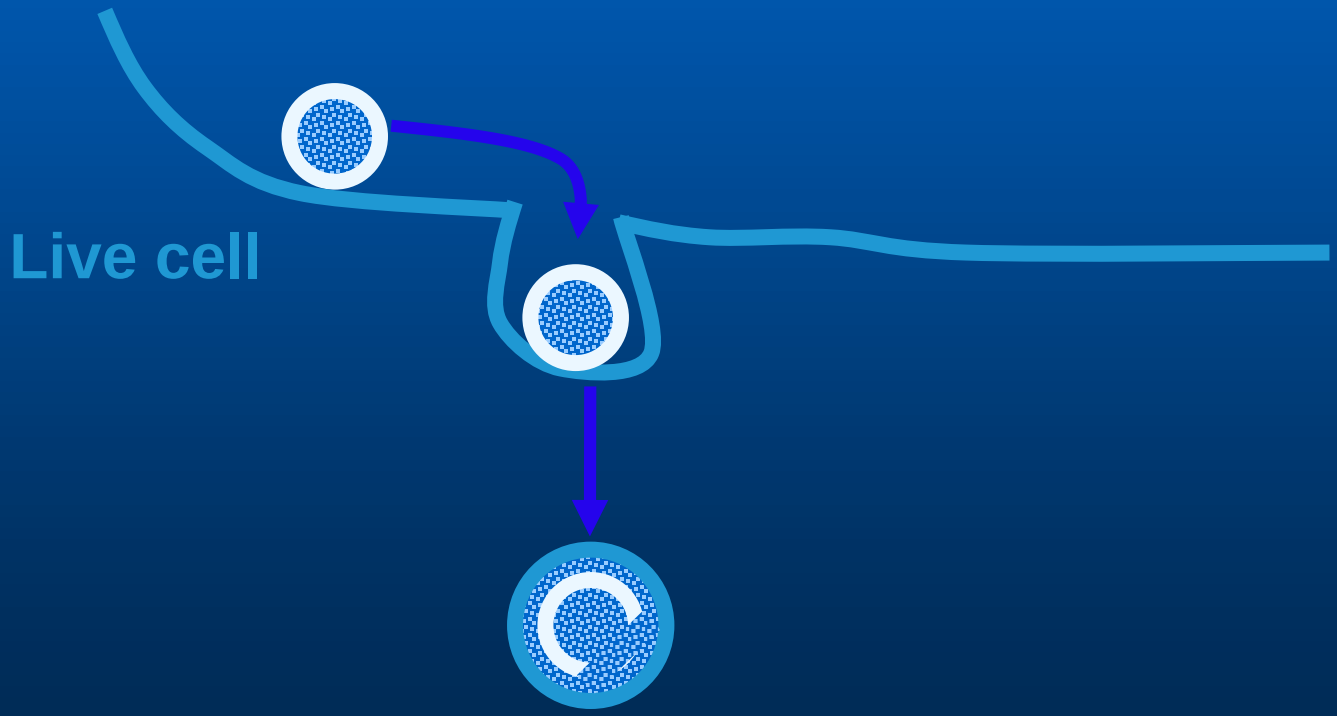
Cell membrane

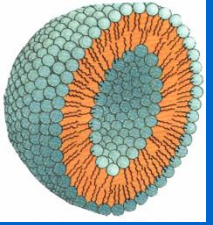


Liposomes Cell membrane interaction

endocytosis

liposomes get into the lysosomal vesicles and their contents are released there





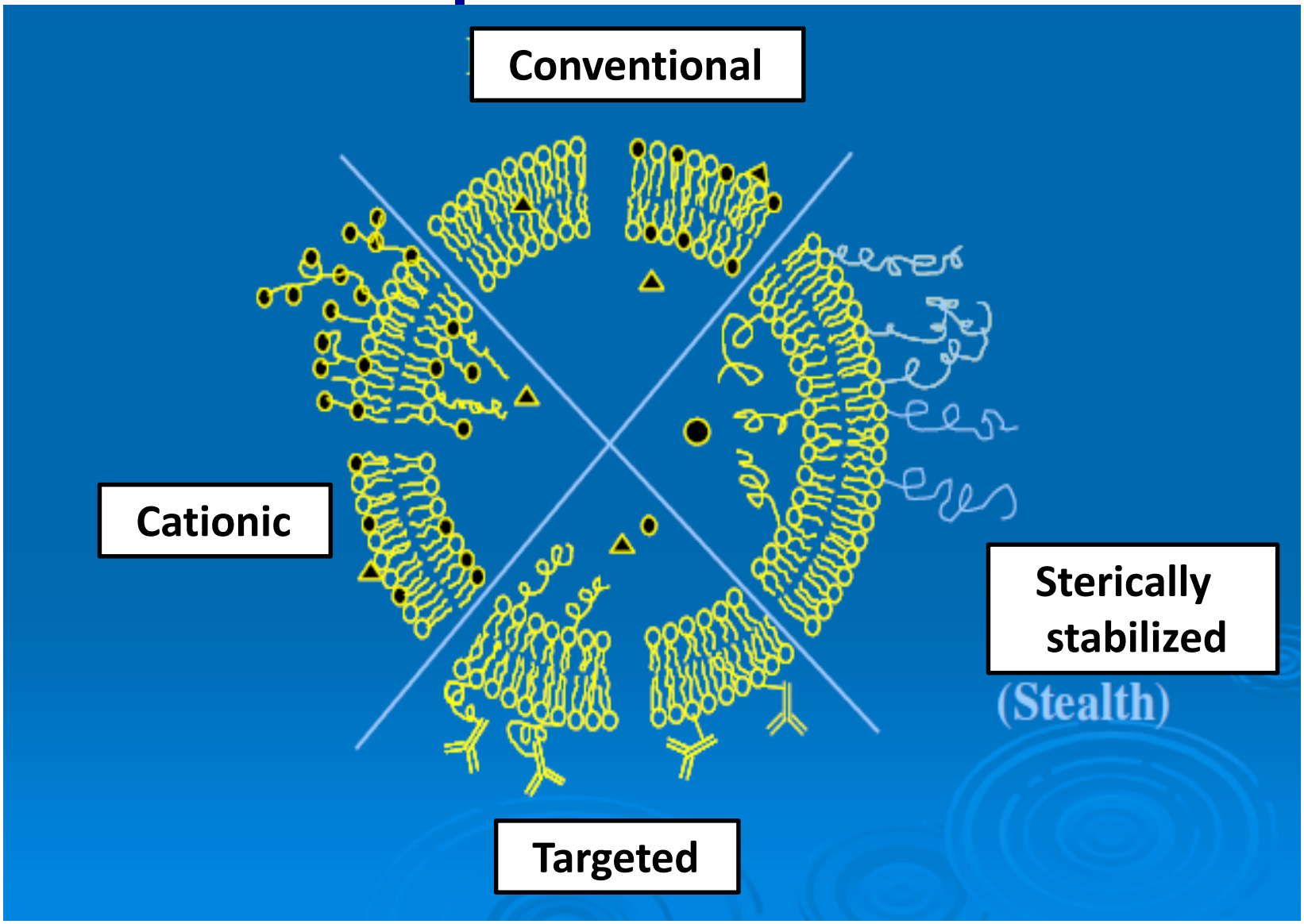
Liposomes Cell membrane interaction

Membrane fussion

the liposome membrane merges with the cell membrane and the drug is directly introduced into the cytoplasm

Live cell

4 basic type of liposomes used in pharmacutics



Liposomes

applications

Various liposomal product in dermatology and cosmetics (launched or investigated)

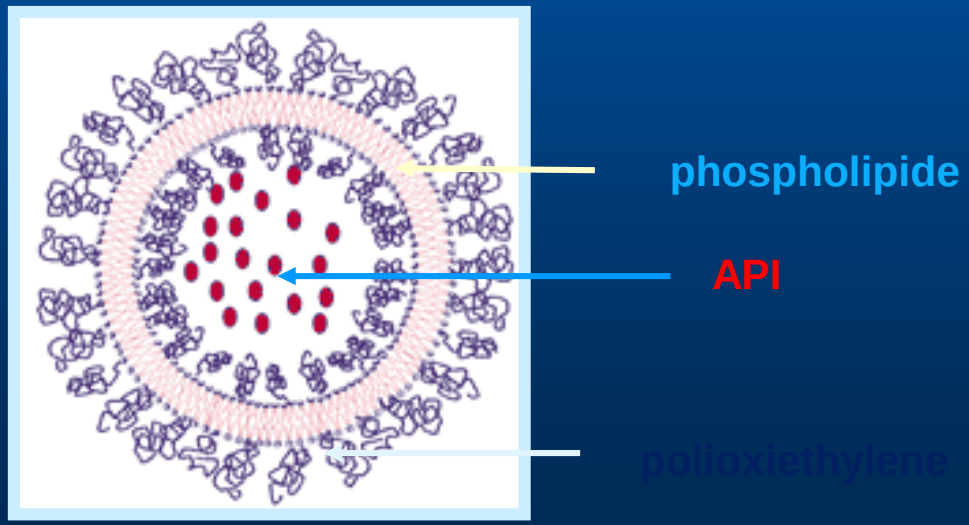
Vesicular system	Marketed by	Liposomes and ingredients
Capture™	Christian Dior	Liposomes in gel with ingredients
Plentitude™	L'Oreal	Tanning agent in liposomes
Dermosome™	Microfluidics	Skin care, loaded liposomes
Penta™	Pentapharm	Humectant pentavitin R in liposomes
Coatsome NC™	Nichiya liposomes Co	Liposomes with humectant

Liposomes application

The rapid clearance of liposomes should be prevented

- liposomes primarily the liver and spleen take up quickly
- It can inhibit the rapid elimination empty liposomes simultaneous addition
- Materials incorporating the liposome wall

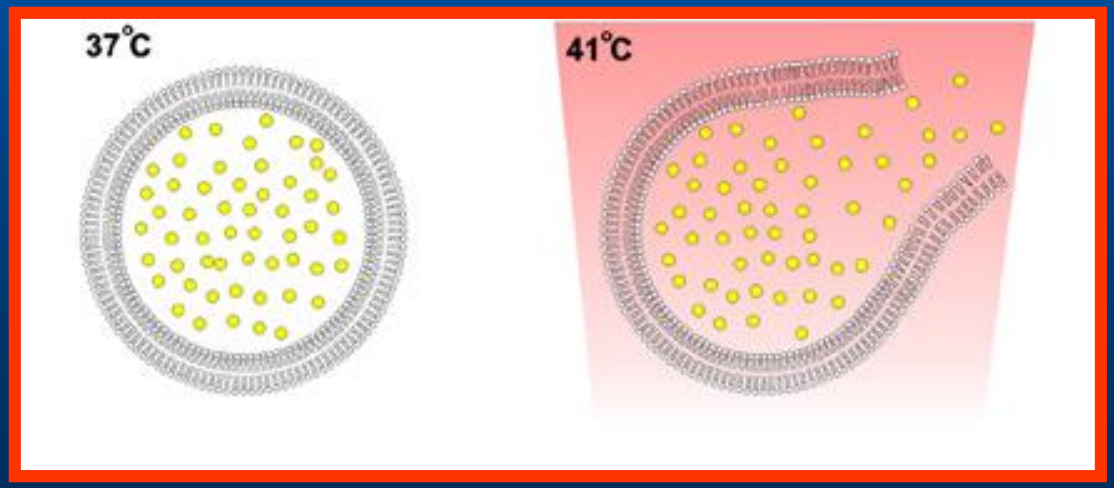
•polyoxyethylene
•cholesterol
•PVP
•polyacrylamided



Liposomes application

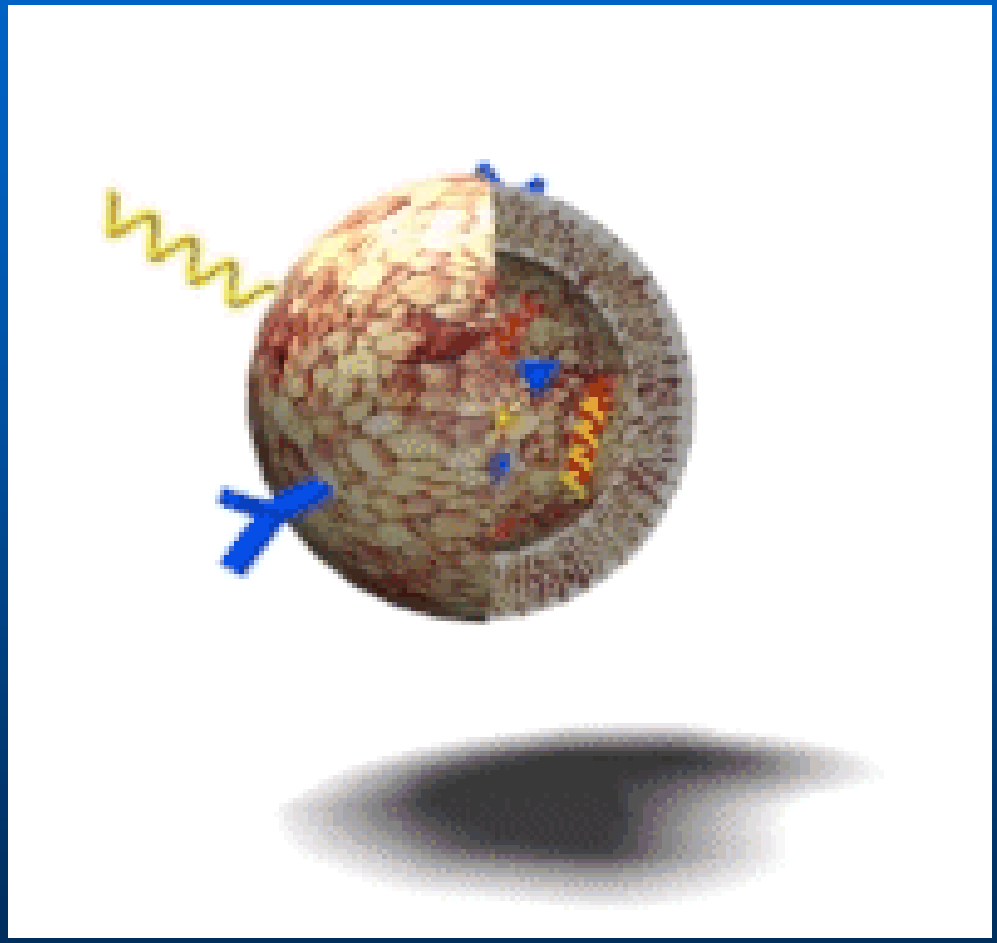
Passive targetting

temperature sensitive liposomes



Liposomes application

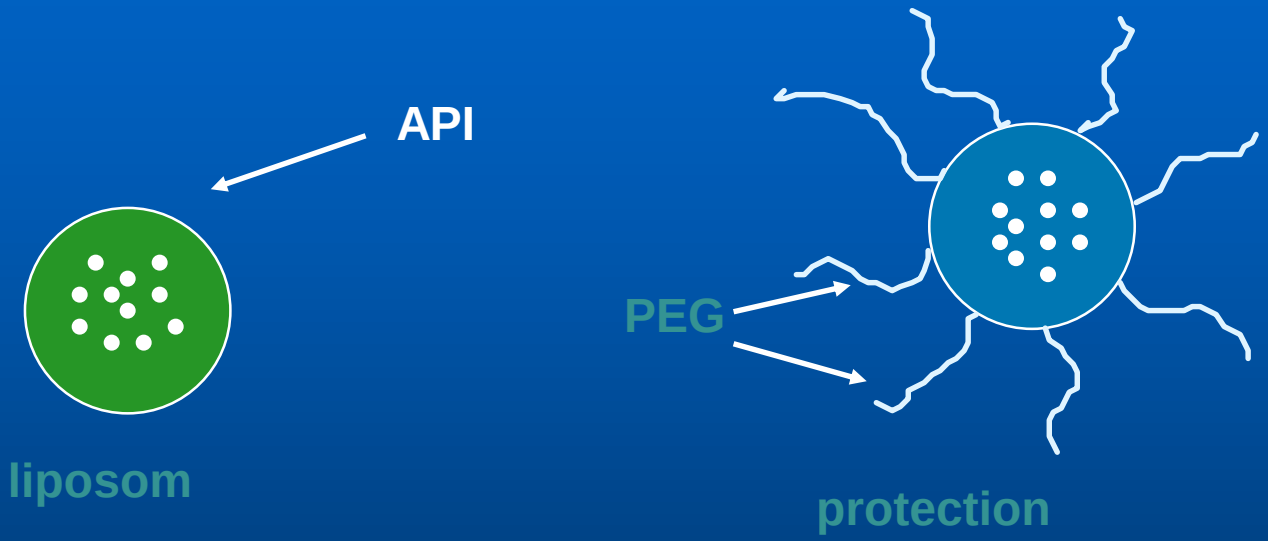
Active targeting

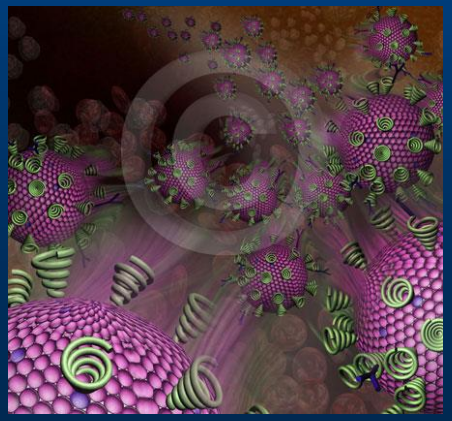


- membrane functionalization

Liposomes application

Doxorubicin liposomal AIDS treatment of Kaposi sarcoma

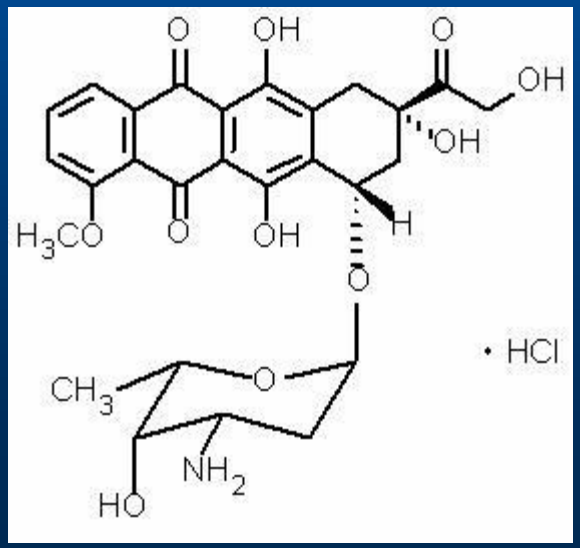




Liposomes application

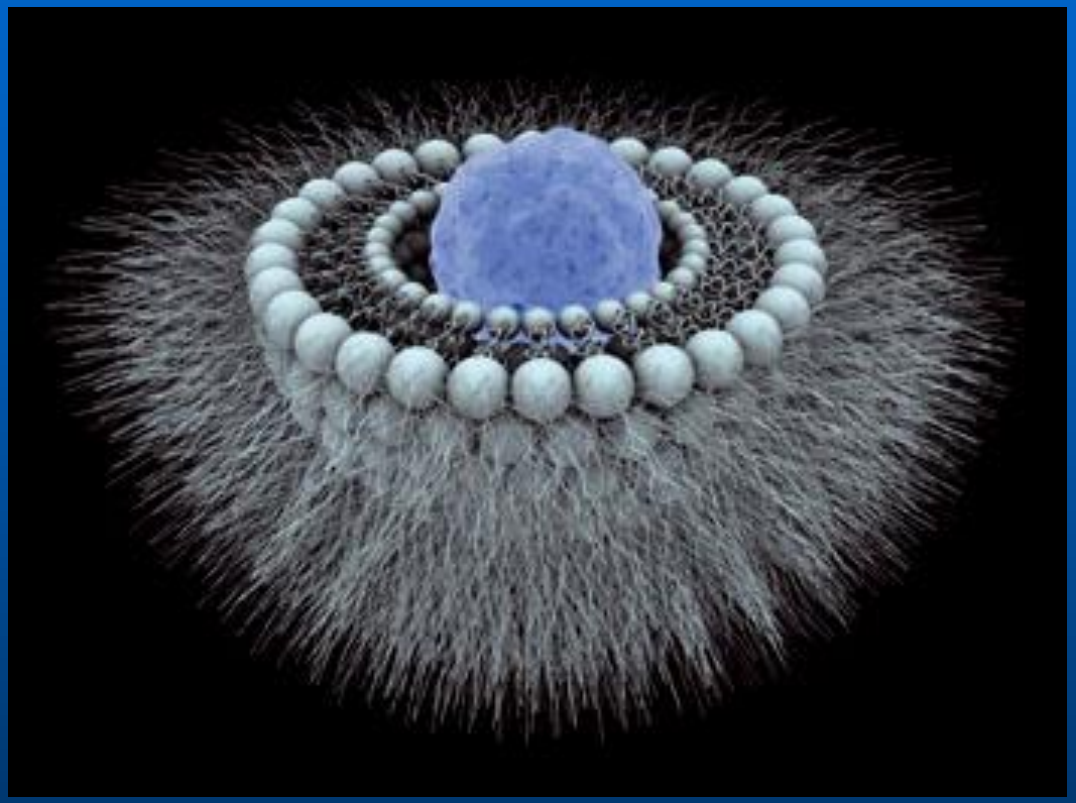
Doxil®, Caelyx pegylated doxorubicin hydrochloride (PLDH). Ovarian cancer, lung cancer, Kaposi's sarcoma related to AIDS infection, myeloma treatment

Myocet®, lung cancer in combination with cyclophosphamide



doxorubicin hidrochloride

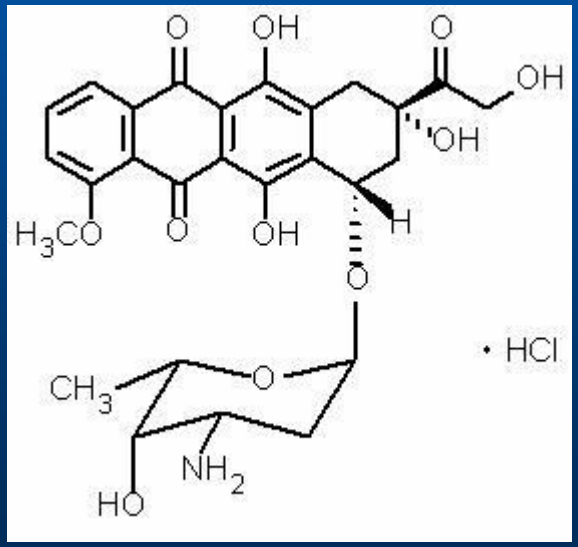
Liposomes application



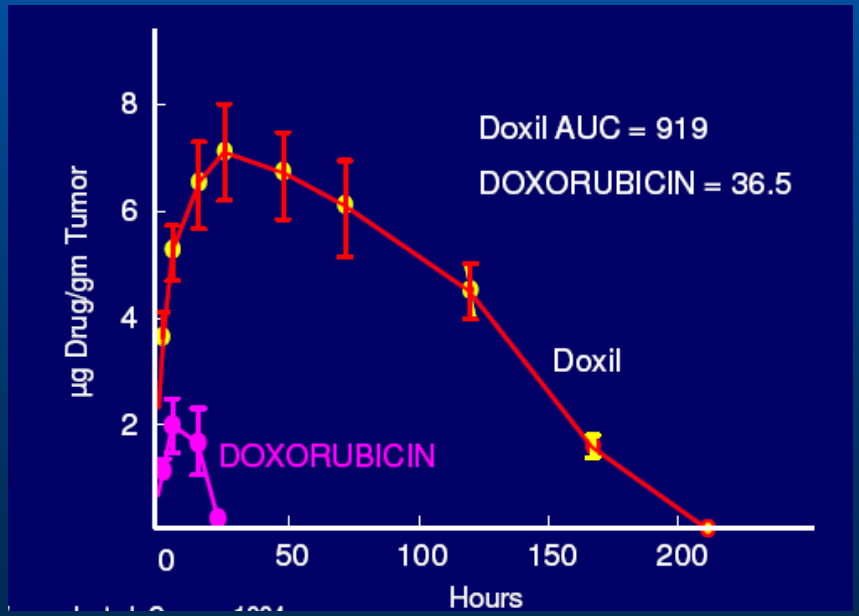
Pegilated liposomes

Liposomes application

Doxorubicin avoiding the cell, chromatin is primarily tied to the stock. Doxorubicin form inhibits DNA and RNA synthesis replicative cycle of "S" phase by intercalation between DNA base pairs and the complex. Unfortunately, in itself a significant cardiotoxic side has.
Liposome significantly reduced side effects, provide rapid cell penetration, improved bioavailability.



doxorubicin HCl

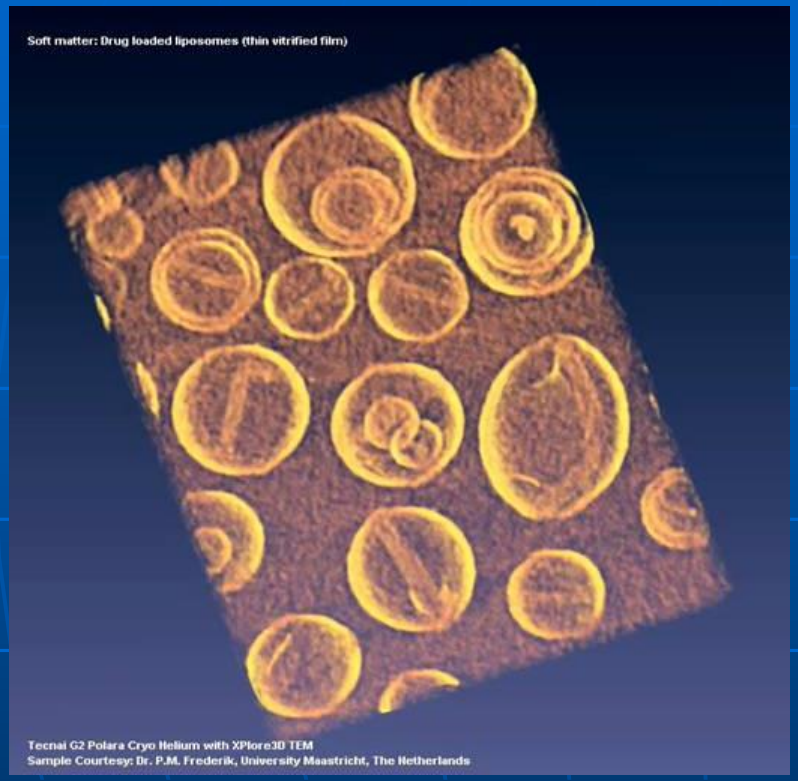


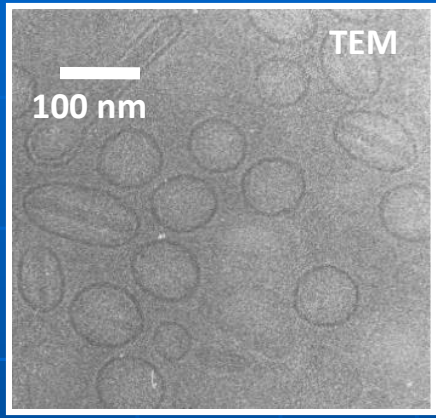
Liposomes application

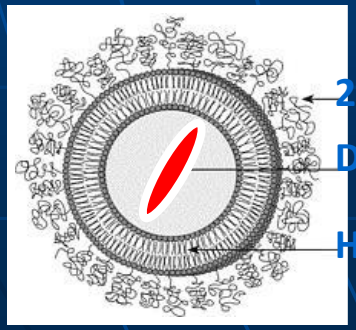
Doxil ® 2 mg / ml concentrate for infusion (Caelyx in Europe As in circulation), in which the so-called drug doxorubicin. It is in the form Stealth® ("stealth") liposomes.

This proprietary name developed by ALZA Corporation, indicating non-PEGylated liposomes, which implies that the vesicles in the bloodstream may lurk machrphages monocyte-front, and approx. Thanks to 55-hour half-life of the active ingredient for tumor capillaries Once in the larger pores is mainly provided through the pathological tissues.

Liposomes application







Séma

First, the accumulation in tumor tissue, thereby not damaging the heart.

nano medicine brought the first on the market with generic versions already on the market

Liposomes application

Name	API	Application	Year
Doxil, Caelyx	Doxorubicin	Ovarian cancer, breast cancer, Kaposi's sarcoma	1995
Abelcet	**Amphotericin B**	Systemic fungal infections	**1995**
DaunoXome	Daunorubicin	Solid tumors	1996
Ambisome	**Amphotericin B**	Fungal infections	**1997**
Epaxal-Berna	Hepatitis A antigen	Hepatitis vaccine	1997
DepoCyt	Cytarabin	Tumors	1999
Amphotec	**Amphotericin B**	Systemic fungal infections	2000
Myocet	Doxorubicin	Fungal infections	2000
Visudyne	Verteporfin	Macular degeneration, ocular histoplasmosis	2000

Liposomes application

	Encapsulated drug	indication
1	all-trans retionoic acid	T cell lymphoma
2	amikacin	bacterial infections
3	ampicillin	listeria infection
4	annamycin	breast cancer, leukemia
5	antisense oligo	pancreatic cancer
6	camptosar	colon cancer
11	cyclosporin	immunosuppression
12	doxorubicin	breast cancer
13	gangciclovir	cytomegalovirus infection
14	interleukin-2	immunostimulation
15	lipid A	immunostimulation
16	methotrexate	various cancers
17	mitoxantrone	prostate cancer