Liposomes

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What are liposomes?

They 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 were first described in 1964 by A.D. Bangham and his colleague R.W. Thorne after examining and analysing a dispersion of phospholipids in water under an electron microscope (Betageri et al., 1993).

They found that the phospholipids automatically arranged themselves to form structures that they referred to as "bag-like".

A close colleague, Gerald Weissman, suggested the structures be called liposomes, which he then defined as "microscopic vesicles composed of one or more lipid bilayers".

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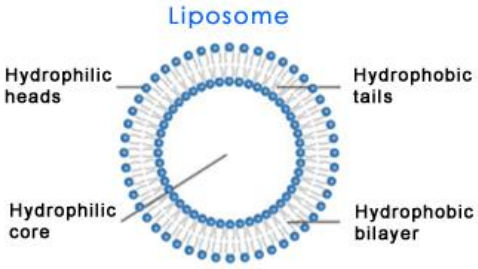

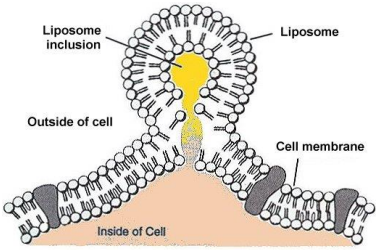

Acceptance of liposome into cell

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Advantages of liposomes

Provides selective passive targeting to tumor tissues

Liposomes increased efficacy and therapeutic index of drug (actinomycin-D)

Increased stability via encapsulation

Liposomes reduce the toxicity of the encapsulated agent (amphotericin B, Taxol)

Improved pharmacokinetics (reduced eliminations, increased circulation life time)

Flexibility to couple with site specific ligands to achieve active targeting

Liposomes are non-toxic, flexible, biocompatible, completely biodegradable, and non-immunogenic for systemic and non-systemic administrations

Site avoidance mechanism - Liposomes do not dispose in certain organs, such as heart, kidneys, brain, and nervous system and this reduces cardio-, nephro-, and neuro-toxicity. Typical examples are reduced nephrotoxicity of Amphotericin B, and reduced cardiotoxicity of Doxorubicin liposomes

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Benefits of drug load in liposome	Examples
1. Improved solubility of lipophilic and amphiphilic drugs	Amphotericin B, porphyrins, minoxidil, some peptides, and anthracyclines, respectively; hydrophilic drugs, such as anticancer agent doxorubicin or acyclovir
2. Passive targeting to the cells of the immune system, especially cells of the mononuclear phagocytic system	Antimonials, amphotericin B, porphyrins, vaccines, immunomodulators
3. Sustained release system of systemically or locally administered liposomes	Doxorubicin, cytosine arabinoside, cortisones, biological proteins or peptides such as vasopressin
4. Site-avoidance mechanism	Doxorubicin andamphotericin B
5. Site-specific targeting	Anti-inflammatory drugs, anti-cancer, anti-infection
6. Improved transfer of hydrophilic, charged molecules	Antibiotics, chelators, plasmids, and genes
7. Improved penetration into tissues	Corticosteroids, anesthetics, and insulin

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Disadvantages of liposomes

Expensive - The cost is high because of high costs associated with the raw materials used in lipid excipients as well as expensive equipment needed to increase manufacturing

In most cases liposomal formulations are non toxic, but certain formulations such as the cationic formulations tend to be cytotoxic

Liposomes are sensitive to heat and radiation hence difficult to sterilise

They have a short half life and have stability issues - Sometimes phospholipid undergoes oxidation and hydrolysis-like reaction

Encapsulation efficiency is low

Rapid clearance from the blood stream by phagocytic cells of the mononuclear phagocyte system (MPS), which is also referred to as the reticulo-endothelial system (RES)

Leakage and fusion of encapsulated drug/molecules

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Components of liposome structure

- Liposomes are versatile in that the entire membrane of the liposome can be composed of either natural or man-made phospholipids.
- The properties of the liposomes can be changed entirely depending on the phospholipids used.
- The basic components of liposomes are phospholipids which are stabilised by cholesterol, with other stabilisers sometimes added to the mixture depending on the specific use of the liposome.

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Phospholipids

The structure of the phospholipids are as follows: on the one end of the molecule are 2 hydrophobic fatty acid chains containing 10-24 carbon atoms and 0-6 double bonds in each chain

The other end of the molecule, is made up of phosphoric acid and is hydrophilic.

This molecule is not as such water soluble but rather, the molecules aggregate and align automatically in a planar bilayer form

In this way the hydrophobic parts of the molecule are kept from water and the hydrophilic part of the molecule can interact

The double fatty acid chains interaction with one another are also thought to help create the round shape which these molecules form naturally

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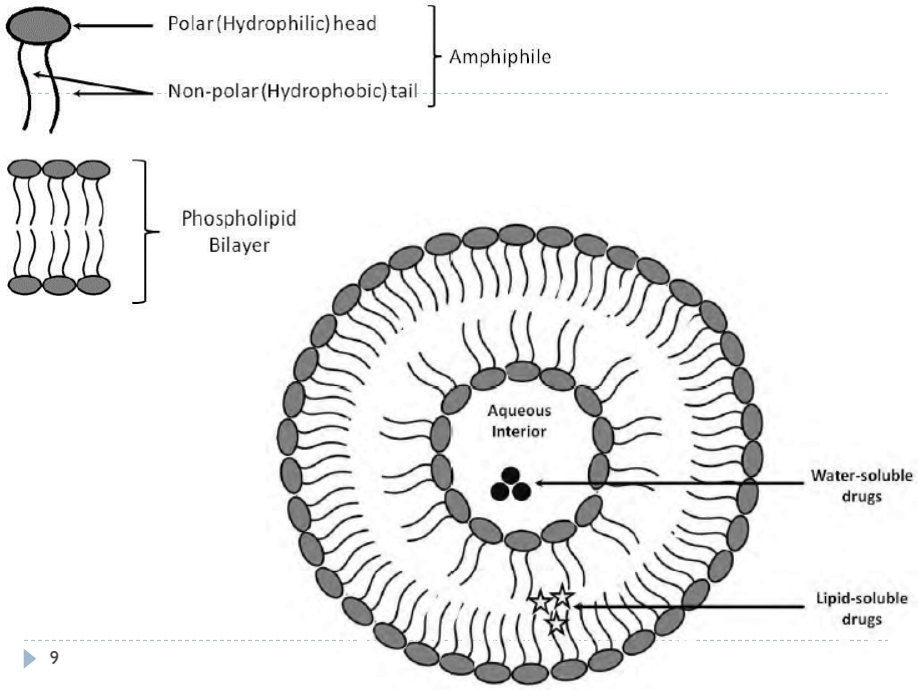

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Lipids all have a temperature at which their fluidity changes.

This temperature is also known as transition temperature (T_C). The T_C is directly proportional to the length of the acyl chain; the longer the chain, the higher the T_C and the more rigid the membrane.

The rigidity of the membrane is also responsible for better stability.

More rigid membranes keep entrapped drugs inside, or in other words, prevent leakage.

The T_C is very important, as it can affect the way the membrane reacts to fusing with other liposomes, aggregation, stability, permeability as well as contributing to the way the liposomes react in the presence of biological systems

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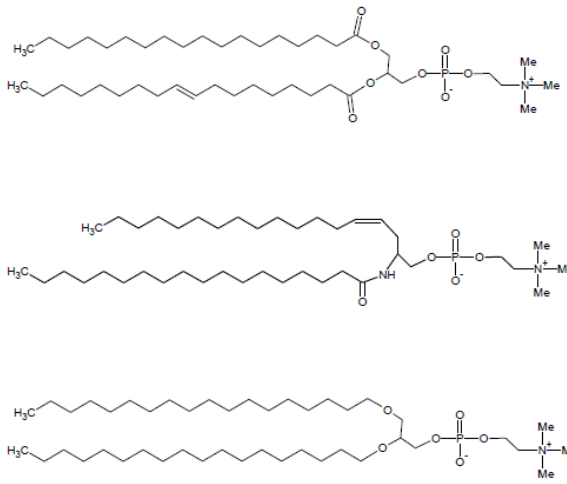

Phosphatidyl choline, also known as lecithin
1,2-diacyl-sn-glycerol-3-phosphoryl choline

Spingomyelin
N-acyl-trans-4sphingenine-1-phosphoryl choline

Phosphatidyl choline linked by ether
1,2 dialkyl-sn-glycerol-3-phosphoryl choline

Figure 2.3: The main classes of phospholipids that contain choline. As adapted from New (1990).

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Phosphatidylcholines

Phospholipids containing the choline group are one of the most abundant lipids in nature.

The phospholipid most often used for liposomes is the phospholipid known as phosphatidylcholine (PC or lecithin).

This phospholipid is very popular because of its relative low cost and general tendency to be neutral

PC is procured from natural sources, plants with soybeans as an example, and mammalian sources such as bovine heart, spinal column or in some cases from egg yolk.

PC gives the membrane rigidity.

The structure of the specific lipid provides the fluidity as well as bilayer strength.

These factors are dependent on the amount of saturation as well as the length of the hydrocarbon chain

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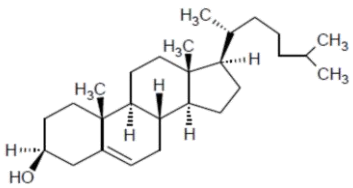
Cholesterol

One of the other components normally included into the membrane of liposomes is cholesterol

Cholesterol on its own does not in fact create the specific recognisable bilayer structure.

When it is added into the mixture the it stabilises the liposomes, or in other words, it increases the T_C of the membrane.

It decreases the permeability of the bilayer, thus helping to keep the liposome stable and to keep the intended drug entrapped



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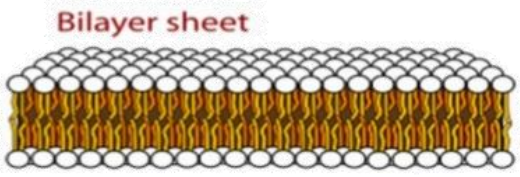
Mechanism of liposome formation

- In aqueous media PL as they are not soluble align themselves closely in planar bilayer sheets or lipid cakes which are thermodynamically stable
- Polar head groups face outwards into the aqueous medium and the lipidic chains turns inwards to avoid the water phase giving rise to double layer or bilayer



- This structure is also known as LAMELLA

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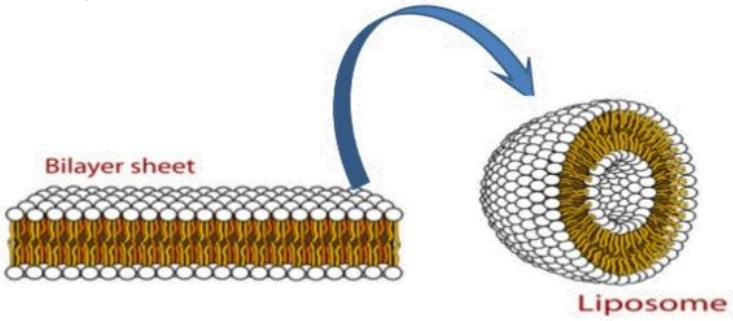
- For the liposomes to be formed, upon further hydration, the lipid cake (lamella) swells eventually they curve to form a closed vesicle in the form of sphere
- These spheres are known as liposomes



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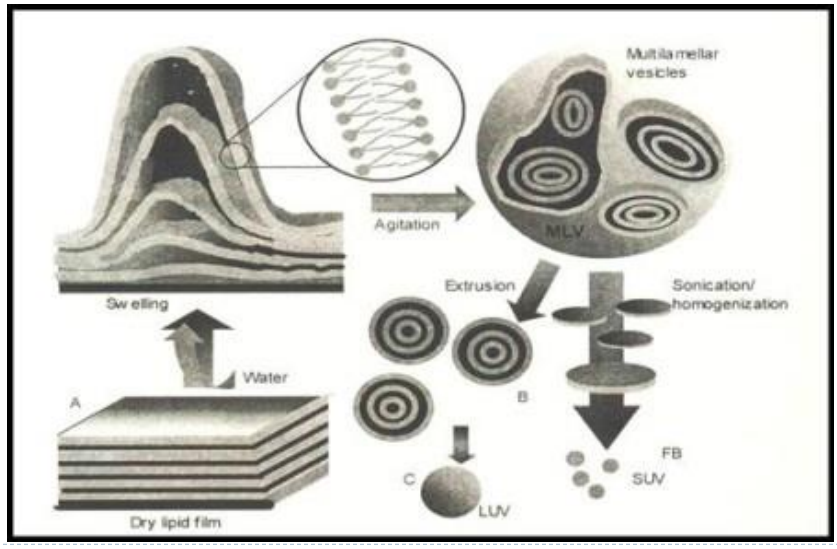

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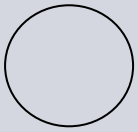
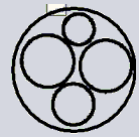
Classification of liposomes

- Based on structural parameters
- Based on method of preparation
- Based on composition and application

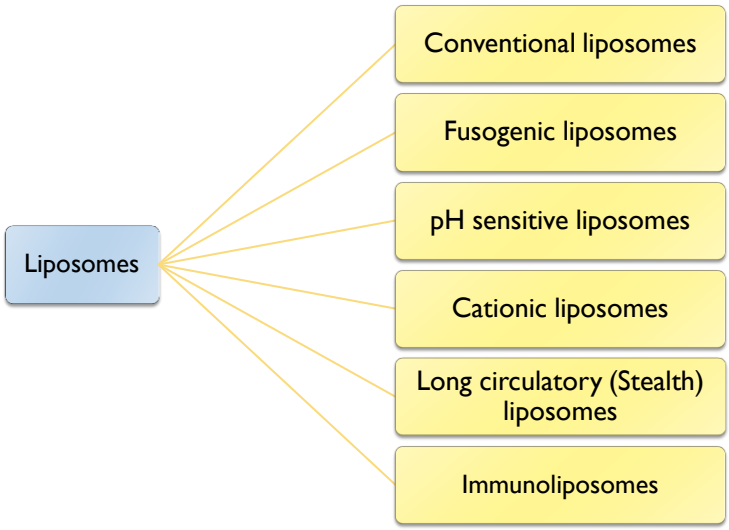
Sr. No.	Vesicle type	Abbreviation	Diameter size	Number of lipid bilayers	Diagrammatic representation
1	Small unilamellar vesicles	SUV	20 – 100 nm	One	
2	Large unilamellar vesicles	LUV	>100 nm	One	
3	Giant unilamellar vesicles	GUV	>1 µm	One	

Sr. No.	Vesicle type	Abbreviation	Diameter size	Number of lipid bilayers	Diagrammatic representation
4	Multilamellar vesicles	MLV	>0.5µm	5 – 20	
5	Oligolamellar vesicles	OLV	0.1 - 1 µm	5	
6	Multivesicular vesicles	MVV	>1 µm	Multicompartmental structure	

Based on method of preparation

Sr. no.	Method of preparation	Type of liposome
1	Single or oligolamellar vesicles made by reverse phase evaporation	SUV or OLV
2	Multilamellar vesicles made by reverse phase methods	MLV-REV
3	Stable plurilamellar vesicles	SPLV
4	Frozen and thawed MLV	FAT-MLV
5	Vesicles prepared by extrusion method	VET
6	Vesicles prepared by French press	FPV
7	Vesicles prepared by fusion	FUV
8	Dehydration-rehydration vesicles	DRV
9	Bubblesomes	BSV

Based on composition and application



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Methods of liposome preparation and drug loading

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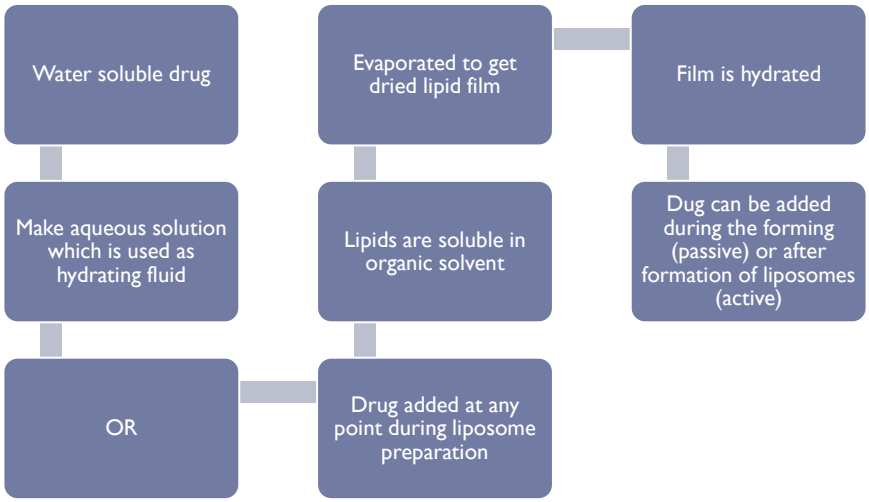

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Methods of passive loading

1. Mechanical dispersion methods
2. Solvent dispersion methods
3. Detergent solubilisation

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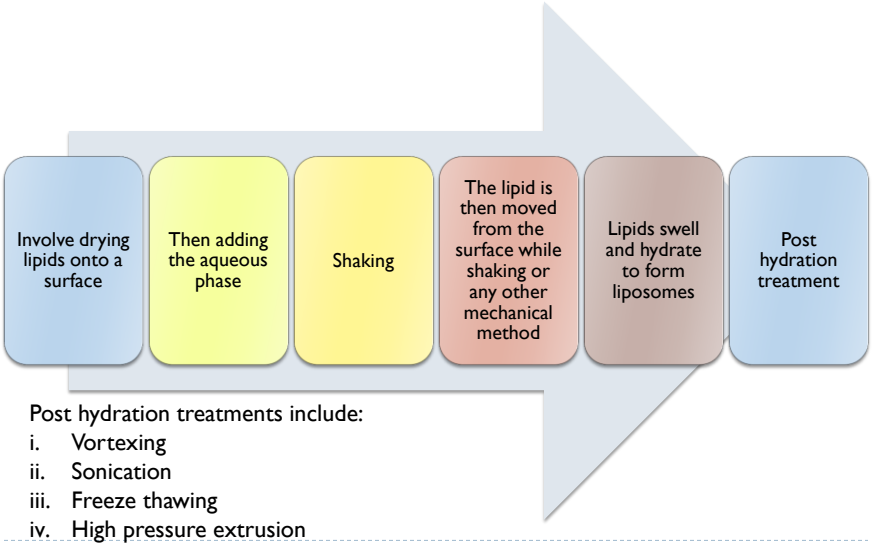
Mechanical dispersion methods



Post hydration treatments include:

i. Vortexing
ii. Sonication
iii. Freeze thawing
iv. High pressure extrusion

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Methods in this class are the following:

- Hand-shaken multilamellar vesicles (MLVs).
- Non-shaken vesicles.
- Pro-liposomes.
- Freeze drying.
- Processing of lipids hydrated by physical means.
- Micro-emulsification liposomes (MEL).
- Sonicated vesicles.
- French pressure cell liposomes.
- Membrane extrusion liposomes.
- Dried reconstituted vesicles (DRVs).
- Freeze-thaw sonication (FTS) method.
- pH-induced vesiculation.
- Calcium-induced fusion to produce large Unilamellar vesicles

Study any ONE

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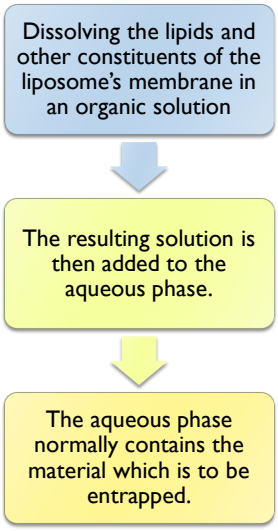
Solvent dispersion methods



Methods in this category are the following:

1. Ethanol injection.
2. Ether injection.
3. Rapid solvent exchange vesicles
4. De-emulsification methods
5. Double emulsion vesicles
6. Reverse phase evaporation vesicles
7. Stable plurilamellar vesicles

Study any ONE

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Detergent depletion methods

- Production methods in this class involve using an intermediary detergent when adding the phospholipids to the aqueous phase.
- The intermediary detergent helps to bring the phospholipids in close contact with the aqueous phase, but still protects the hydrophilic part of the phospholipid.
- These intermediaries are often soluble in both aqueous and organic solutions.
- This method then creates micelles
- They include
  - Bile salt preparation
  - Alkyl glycoside dialysis
  - Triton X-100 solubilised Sendai virus particles

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- It includes

1. Dialysis
2. Column chromatography
3. Detergent adsorption on biobeads

Study any ONE

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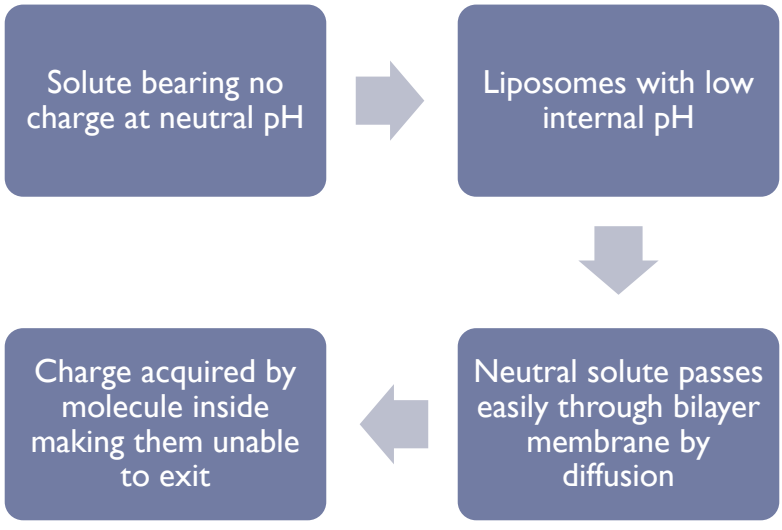
Remote (active) loading



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Advantages of active loading over passive loading

A high encapsulation efficiency and capacity
A reduced leakage of the encapsulated compounds
"bed side" loading of drugs thus limiting loss of retention of drugs by diffusion, or chemical degradation during storage
Flexibility for the use of constitutive lipids as drug is loaded after the formation of carrier units
Avoidance of biological active substance during preparation steps in dispersion thus reducing safety hazards
The transmembrane pH gradient can be developed using various methods depending upon the nature of the drug to be encapsulated
For amphiphatic weak bases by remote loading procedures such as using a proton gradient or ammonium sulphate gradient
For amphiphatic weak acids by remote loading procedures using a calcium acetate gradient

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Types of drugs with site of their entrapment

Type of drug	Log p	Site of entrapment	Examples
Lipophilic	> 5	Lipid bilayer	Cyclosporine
Hydrophilic	< -0.3	Aqueous domain	CDP choline
Amphiphilic	1.7 – 4	Both aqueous and lipid phases	Actinomycin D
Biphasic insoluble	--	--	--

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Characterization of liposomes

Sr. no	Test	Equipment/ method
1	Vescicle shape and lamellarity	Freeze fracture electron microscopy ^{31}P NMR
2	Vesicle size and size distribution	Light microscopy Fluorescent microscopy Electron microscopy Laser light scattering Photon correlation spectroscopy Field flow fractionation Gel permeation and gel extrusion analysis
3	Surface charge	Free flow electrophoresis Zeta potential measurement
4	Encapsulation efficiency	Mini-column centrifugation method Protamine aggregation method

For extended table refer notes

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Interactions of liposomes with cells

- The interactions of liposomes with cells are important as this behaviour can often help predict why liposomes react in certain ways in vitro as well as in vivo.
- The ways in which the liposomes interact with cells are as follow:
  1. Intermembrane transfer
  2. Contact release
  3. Adsorption
  4. Fusion
  5. Phagocytosis or endocytosis

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Intermembrane transfer	• This type of interaction occurs when the lipid components of liposomes interact with cell membranes. • The components such as the PC, cholesterol and PE can exchange freely from one membrane to the other without disrupting the liposome integrity
Contact release	• When the liposome comes into the close proximity of cells, the interaction starts and the permeability of the liposomal membrane increases drastically. • The increased permeability leads to the release of the aqueous interior, or in other words, the liposome's content. • This causes a very high dosage of the drug in the cells vicinity
Adsorption	• Cell adsorption occurs when the liposome attaches to the surface of a cell • It is the first step to endocytosis
Fusion	• The liposomes come into close proximity of the cells, from where the fusion can then take place. • The liposome content is completely introduced into the cytoplasm of the cell
Phagocytosis or endocytosis	• Engulf the liposome through the cell membrane and into a sub-cellular vacuole • Lysosomes then attach to this internalised vacuole which contains the liposome. • The lysosomes introduce lysosomal enzymes that break down the lipids of the liposomes to fatty acids and in so doing, release the solutes contained in the liposome

Therapeutics applications of liposomes

- Liposomes as drug/ protein delivery vehicles
  - Controlled and sustained drug release in situ
  - Enhanced drug solubiliation
  - Altered pharmacokinetics and biodistribution
  - Enzyme replacement therapy and lysosomal storage disorders
- Liposomes in anti-microbial, anti-fungal (lung therapeutics) and anti-viral (anti HIV) therapy
- Liposomes in tumor therapy
  - Carrier of small cytotoxic molecule
  - Vehicle for macromolecules as cytokines or genes

- Liposomes in gene delivery
  - Gene and antisense therapy
  - Genetic (DNA) vaccination
- Liposomes in immunology
  - Immunoadjuvant
  - Immunomodulator
  - Immunodiagnosis
- Liposomes as artificial blood surrogates
- Liposomes as radiopharmaceutical and radiodiagnostic carriers
- Liposomes in cosmetics and dermatology
- Liposomes in enzyme immobilisation and bio-reactor technology

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Commercial products containing liposomes

Anti-fungal

- Amphotericin B – highly toxic
- Abelcet™, AmBisome™ and Amphocil™

Tumor

- Targeting
- Doxil™ and DuanoXome™

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Nanoparticles

- They are sub-nanosized colloidal structures composed of synthetic or semi synthetic polymers
- Nanoparticle loaded bioactives could not only deliver drugs to specific organs within the body but delivery rate could be controlled
- Nanocarriers can be:
  - Nanospheres: solid core spherical particulates which are nanometric in size. They contain drug within the matrix or adsorbed on its surface
  - Nanocapsules: vesicular systems in which drug is encapsulated within the central volume surrounded by an embryonic continuous polymeric sheath
  - Nanocrystals/ nanoparticluates – drug particles reduced to nano sizes

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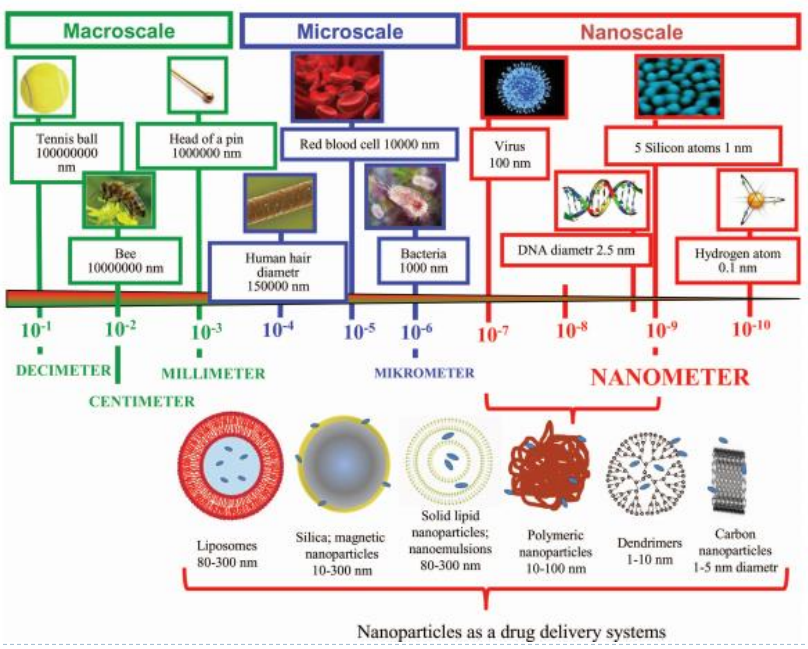

Nanoparticles as a drug delivery systems

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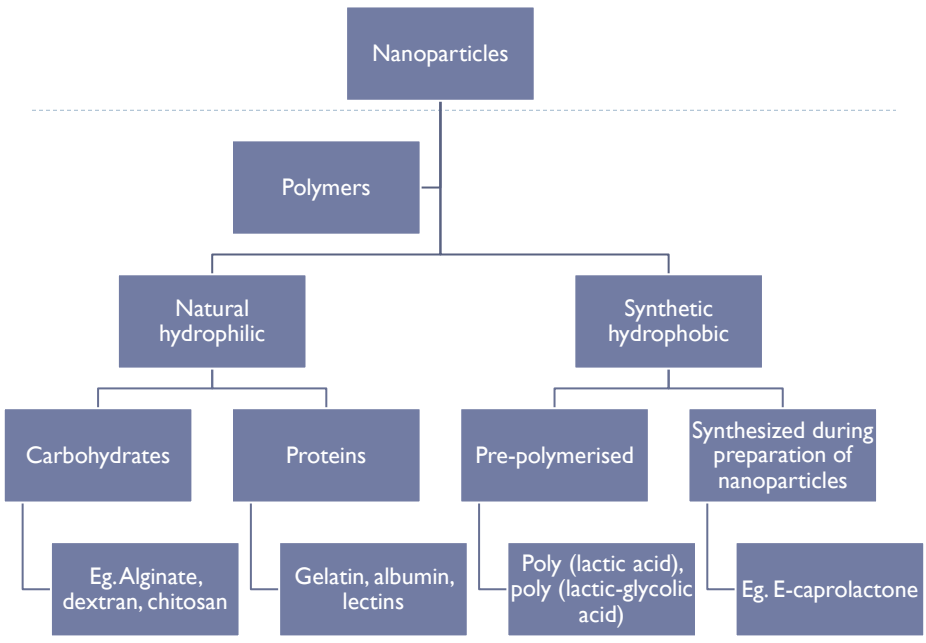

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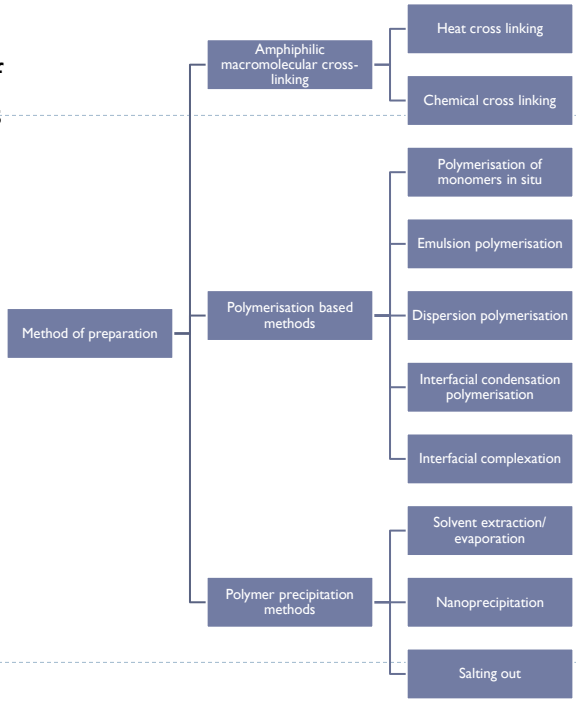
Preparation techniques of nanoparticles



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Characterization of nanoparticles

Parameter	Characterization method(s)
Particle size and size distribution	Photon correlation spectroscopy Laser defractometry TEM, SEM Atomic force microscopy Mercury porositometry
Charge determination	Laser doppler anemometry, Zeta potential
Surface hydrophobicity	Water contact angle measurements Rose bengal (dye) binding Hydrophobic interaction chromatography X-ray photoelectron spectroscopy
Chemical analysis of surface	Static secondary ion mass spectroscopy, Sorptometer
Carrier-drug interaction	Differential scanning calorimetry
Nanoparticle dispersion stability	Critical flocculation temperature
Release profile	Dissolution apparatus/ dialysis
Drug stability	Chemical analysis of drug

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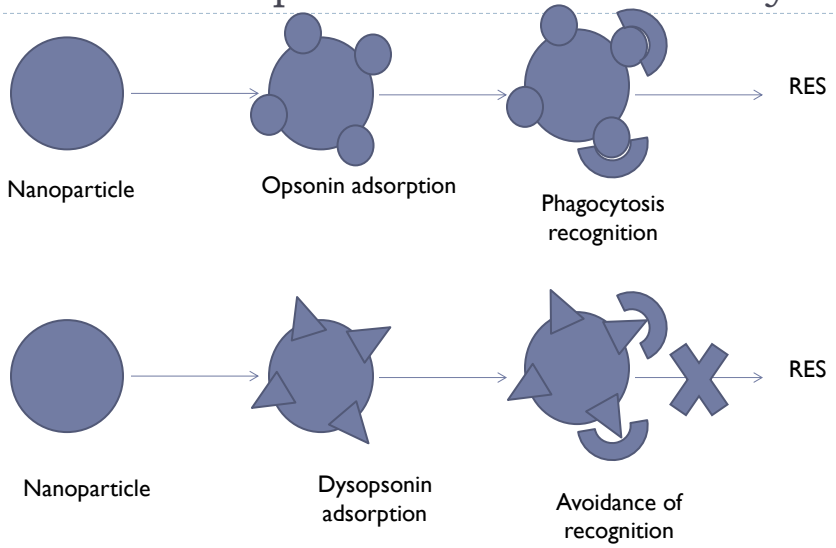
Fate of nanoparticles within the body



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Surface engineering of nanoparticles

Steric stabilised (stealth) nanoparticles	• Deters them from opsonisation • Coating with polyethylene oxide and polyethylene glycol
Antibody coated nanoparticles	• Site specific delivery • Monoclonal antibodies are used
Magnetically guided nanoparticles	• Minimises RES uptake • Fe3O4 particles are loaded and NPs are guided externally using a magnet
Bioadhesive nanoparticles	• Mucoadhesive polymer - lectins

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Therapeutic applications of nanoparticles

Application	Purpose
Cancer therapy	Targeting, reduced toxicity, enhanced uptake of antitumor agents, improved in vivo and in vitro stability
Intracellular targeting	Target RES organs for intracellular infections
Prolonged systemic circulation	Avoid RES
Vaccine adjuvant	Enhances immune response, alternate acceptable adjuvant
Peroral absorption	Enhanced BA, protection from GI enzymes
Ocular absorption	Improved retention
DNA delivery	Enhanced delivery
Oligonucleotide delivery	Enhanced delivery
Other applications	Cross BBB, enzyme immunoassays, radioimaging

Advantages of nanoparticles

Localisation

- Controlled and sustained release of the drug during the transportation and at the site of localization, altering organ distribution of drug and subsequence clearance of the drug so as to achieve increase in drug therapeutic efficiency and reduction in side effects.

Stability of drug

- Drug can be incorporated in to the system without any chemical reaction; this is an important factor for preserving the drug.

Control release

- Controlled release and drug degradation characteristics can be readily modulated.

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Improved efficacy	• There is no wastage of drug and thus enhanced bioavailability of drug at specific site in right proportion for prolonged period of time
Solubilisation	• It improve the solubility of poorly water soluble drugs, prolong half life of drug systemic circulation by reducing immunogenicity, release drug at sustained rate and lower the frequency of administration
Patient compliance	• It provides comfort and compliance to the patient and yet improves the therapeutic performance of the drug over conventional systems

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Disadvantages

- Rapid clearance of targeted systems.
- Immune reactions against intravenous administered carrier systems.
- Insufficient localization of targeted systems into tumour cells.
- Diffusion and redistribution of released drugs.
- Requires highly sophisticated technology for the formulation.
- Requires skill for manufacturing storage, administration.
- Drug deposition at the target site may produce toxicity symptoms.
- Difficult to maintain stability of dosage form. E.g.: Resealed erythrocytes have to be stored at 40 C.
- Drug loading is usually low. E.g. As in micelles. Therefore it is difficult to predict /fix the dosage regimen.

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Microemulsion

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Microemulsion

- Word coined by Schulman and co-workers in 1959
- It is a clear fluid system of an ordinary milky emulsion (macroemulsion) obtained by titration with medium-chain alcohol such as pentanol, hexanol to the point of clarity.
- Definition: It is a thermodynamically stable, translucent dispersions of oil and water stabilised by an interfacial film of surfactant molecules

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Alternate names

- Transparent emulsion
- Swollen micelle
- Micellar solution
- Solubilized oil

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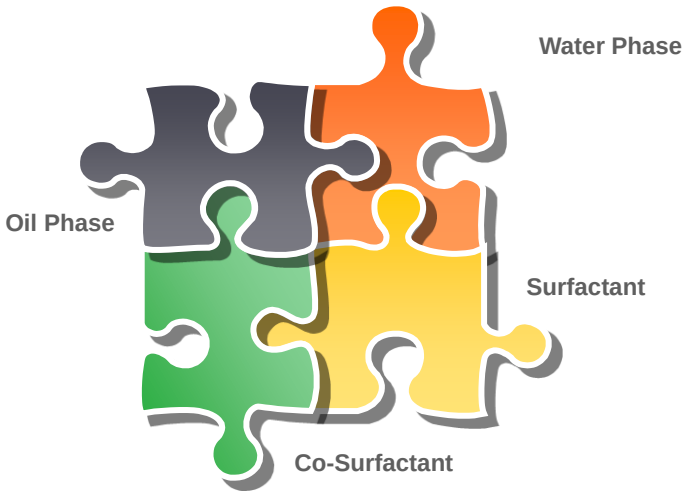

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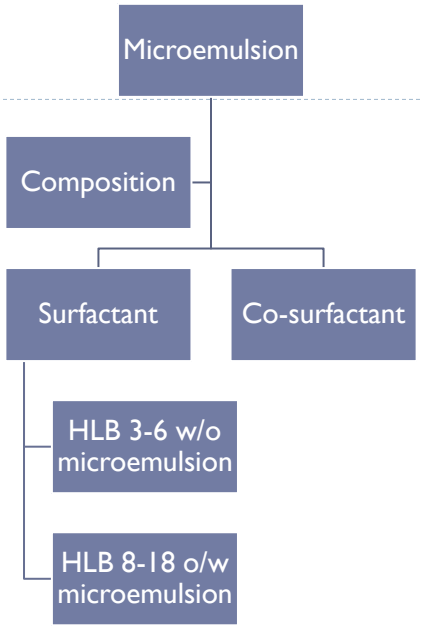

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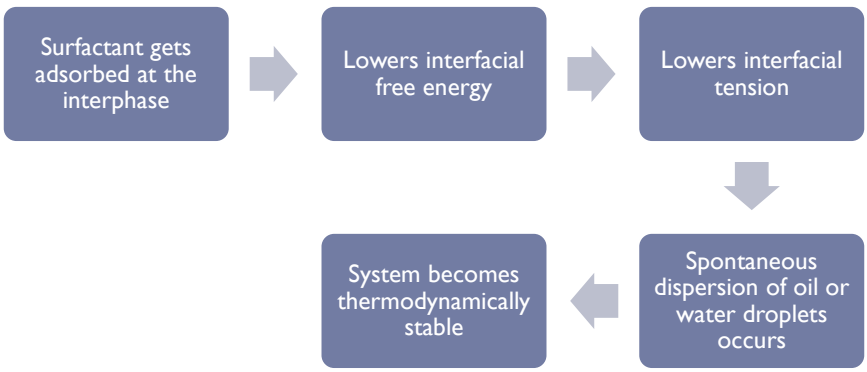
Surfactant



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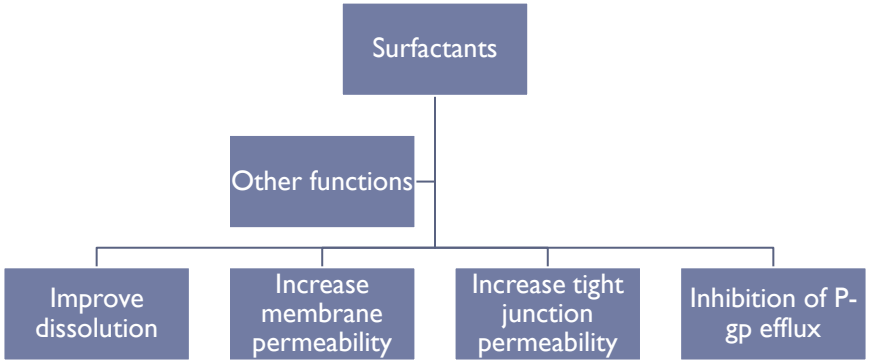

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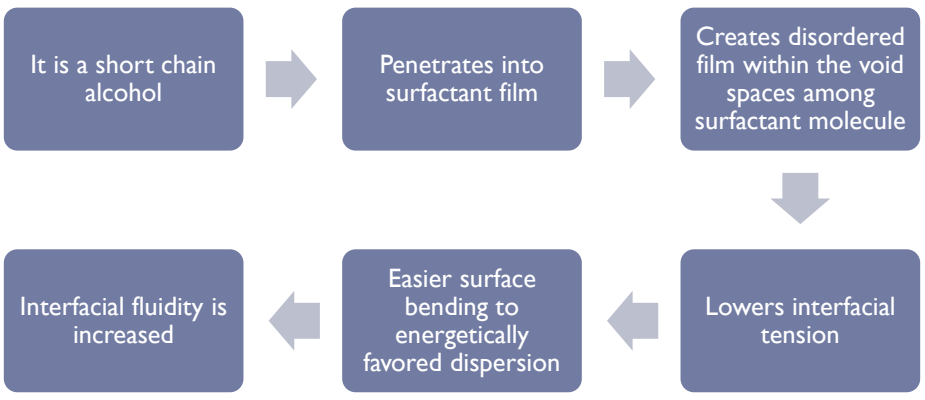
Co-surfactant



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Co surfactants

- They allow the interfacial film sufficient flexible to take up different curvatures required to form microemulsion over a wide range of composition.

1. Short to medium chain length alcohols (C3-C8) reduce the interfacial tension and increase the fluidity of the interface.
2. Surfactant having HLB greater than 20 often require the presence of cosurfactant to reduce their effective HLB to a value within the range required for microemulsion formulation.

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• Co-surfactants act by:

• By reducing the interfacial tension

• By increasing the flexibility and fluidity of the interface by positioning itself between the surfactant tails which alters the solvent properties of both the dispersed and continuous microemulsion phases;

• By lowering overall viscosity.

• By being often soluble in both organic and aqueous phases, co-surfactants help solubilise poorly soluble compounds (*e.g., peptides, vitamins*

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Constituents of Microemulsion

Oil phase :-
Isopropyl Myristate
Oleic acid
Olive oil
Mineral oil
Medium chain triglyceride
Soybean oil
Captex 355
Isopropyl palmitate
Sunflower Oil
Safflower Oil

Surfactants :-
Tween 80
Tween 40
Labrafil M1944CS
Polyoxyethylene-35-ricinoleate
Brij 58
Span 80
Cremophor EL
Labrasol
Cremophor RH
Lecithin

Cosurfactant/Stabilizer :-
Propylene glycol
Ethylene glycol
Ethanol
1-butanol
Isopropyl alcohol
PEG 600
Glycerol
PEG 400

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Emulsion

- Thermodynamically unstable
- Milky appearance
- Prepared by energy input
- Droplet size – upto few micrometer
- Low surface area 15 cm^2/g
- Surfactant conc – low
- Co-surfactant not needed

Microemulsion

- Thermodynamically stable
- Translucent, isotropic
- Self-microemulsifying
- Droplet size < 200 nm
- High surface area 200 cm^2/g
- Surfactant conc high
- Co-surfactant added

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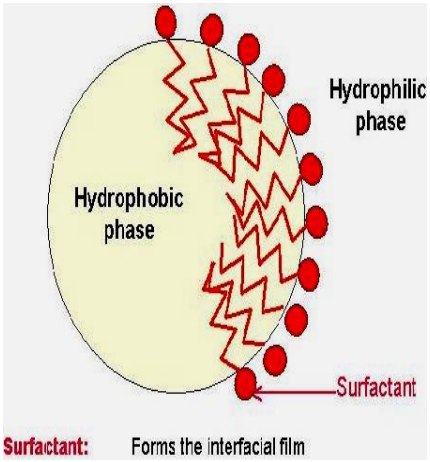
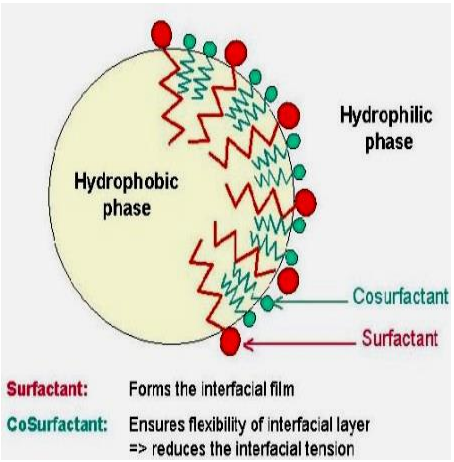


Macro emulsion



Micro emulsion

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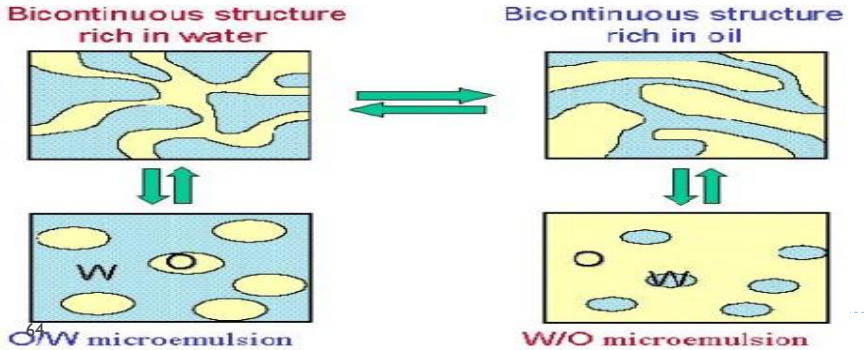
Types of micro emulsion

- O/W Microemulsion
- W/O Microemulsion
- Bi continuous Microemulsion



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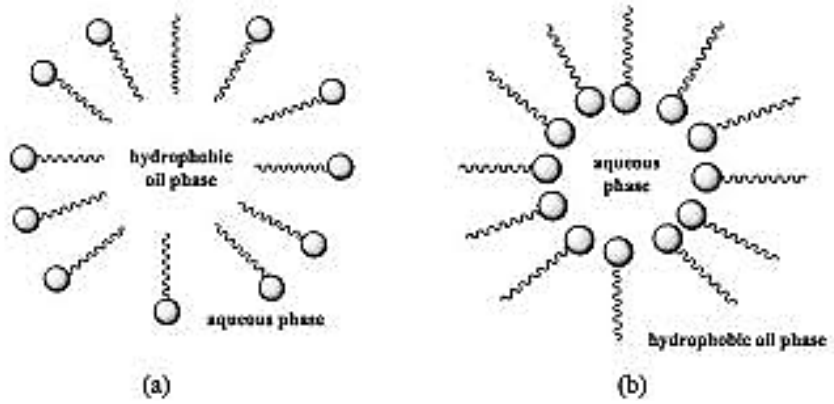
Structure of microemulsion



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THEORIES OF MICRO EMULSION

- Interfacial or mixed film theory
- Solubilization Theory
- Thermodynamic theory

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Interfacial/Mixed Film Theories:

- They considered that the spontaneous formation of microemulsion droplets was due to the formation of a complex film at the oil-water interface by the surfactant and co-surfactant.
- This caused a reduction in oil-water interfacial tension to very low values (from close to zero to negative)
- equation. $\gamma_i = \gamma_{o/w} - \pi_i$

Where,

$\gamma_{o/w}$ = Oil-water interfacial tension without the film present

π_i = Spreading pressure

γ_i =Interfacial tension

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Solubilization Theories:-

- The surfactants and co-surfactants soluble in one phase slowly solubilise the 2nd phase

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Thermodynamic theory

- The process of formation of oil droplets from a bulk oil phase is accompanied by an increase in the interfacial area ΔA, and hence an interfacial energy ΔG .
- The entropy of dispersion of the droplets is equal to $T \Delta S$ and hence the free energy of formation of the system is given by the expression.

$$\Delta G = \gamma \Delta A - T \Delta S$$

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- When the interfacial tension is made sufficiently low that the interfacial energy becomes comparable to or even lower than the entropy of dispersion.
- The free energy of formation of the system becomes zero or negative. This explains the thermodynamic stability of micro emulsions.
- The co-surfactant along with surfactant lower the interfacial tension to a very small even transient negative value
- At this value, interface would expand to form fine dispersed droplets.
- Adsorb more surfactant and co-surfactant until their bulk condition is depleted enough to make interfacial tension positive again
- This process is known as "Spontaneous Emulsification" which forms the micro emulsion.

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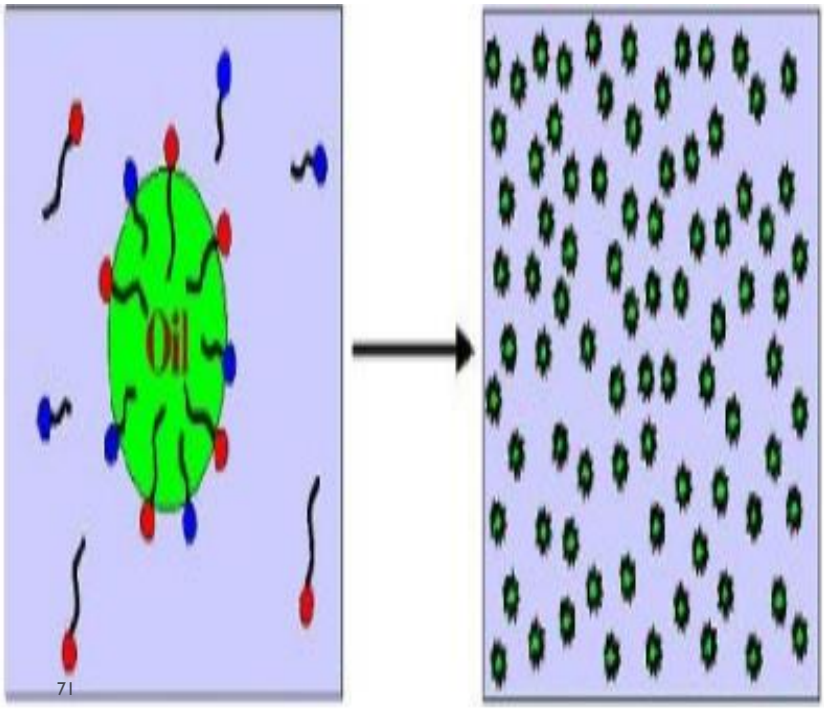

PHASE DIAGRAM

- Phase diagram is a plot showing the condition of pressure and temperature under which two or more physical states can exist together in a state of dynamic equilibrium.
- Micro emulsion consist of three component namely:
  - Oil
  - Water
  - Surfactant and co surfactant
- The concentration of co surfactant is varied.
- So a ternary diagram can be formed

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- The surfactant concentration in the electrolyte solution can be kept constant and the co-surfactant concentration can be the third variable.
- This enables us to utilize the conventional triangular phase diagram plot.
- Temperature variations are then represented by slices across a parallel-sided triangular prism.

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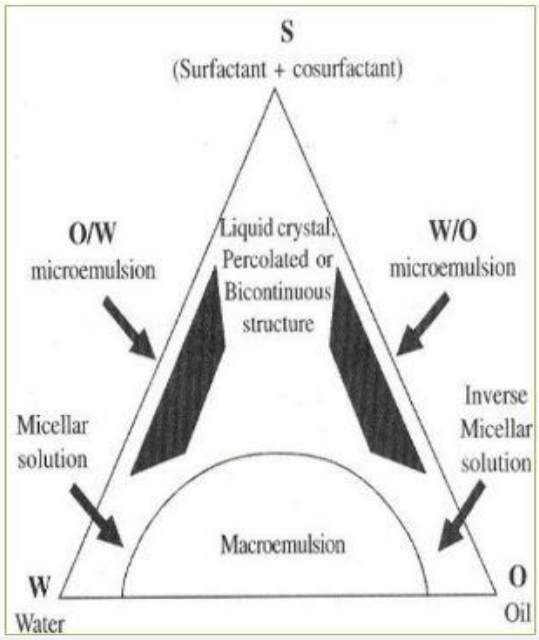

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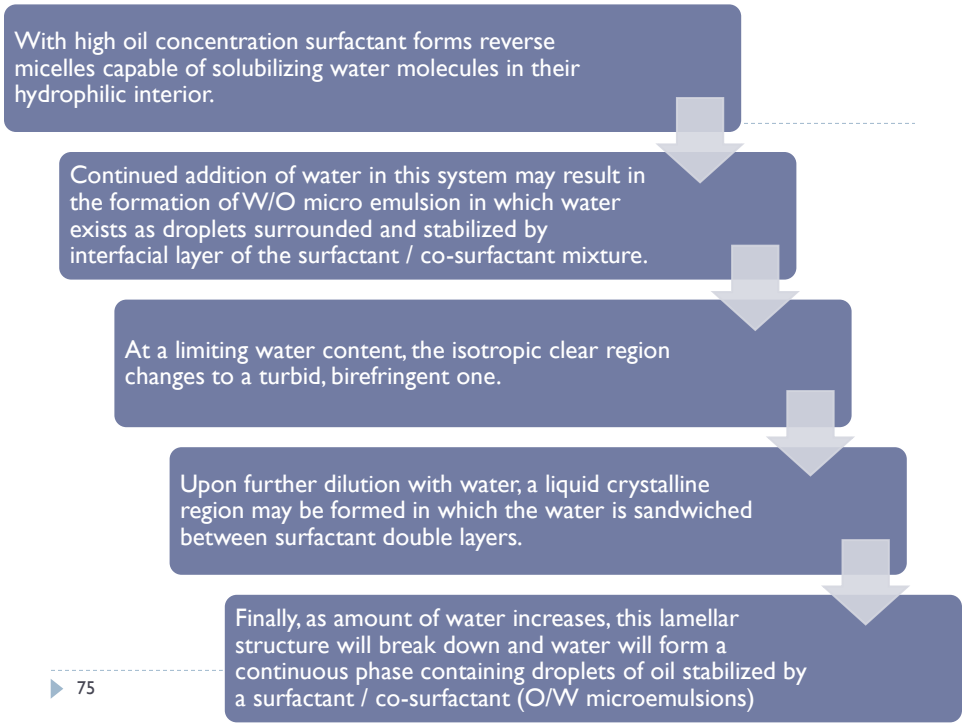

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- The composition of the three-component systems are shown as the intersections of the broken lines.
- The ends of the 'tie-lines' give the compositions of the two phases, while the 'lever rule' gives the amounts of each.
- Any change to the system which changes the hydrophobic balance of the surfactant may enable us to move from one type of system to another.

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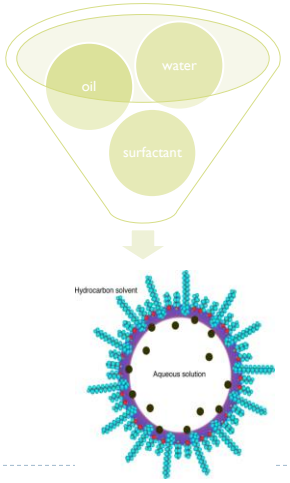
Formation of Microemulsion

- Micro emulsion is formed when
  - the interfacial tension at the O/W interphase are brought very low level.
  - The interfacial film is kept highly flexible and fluid.



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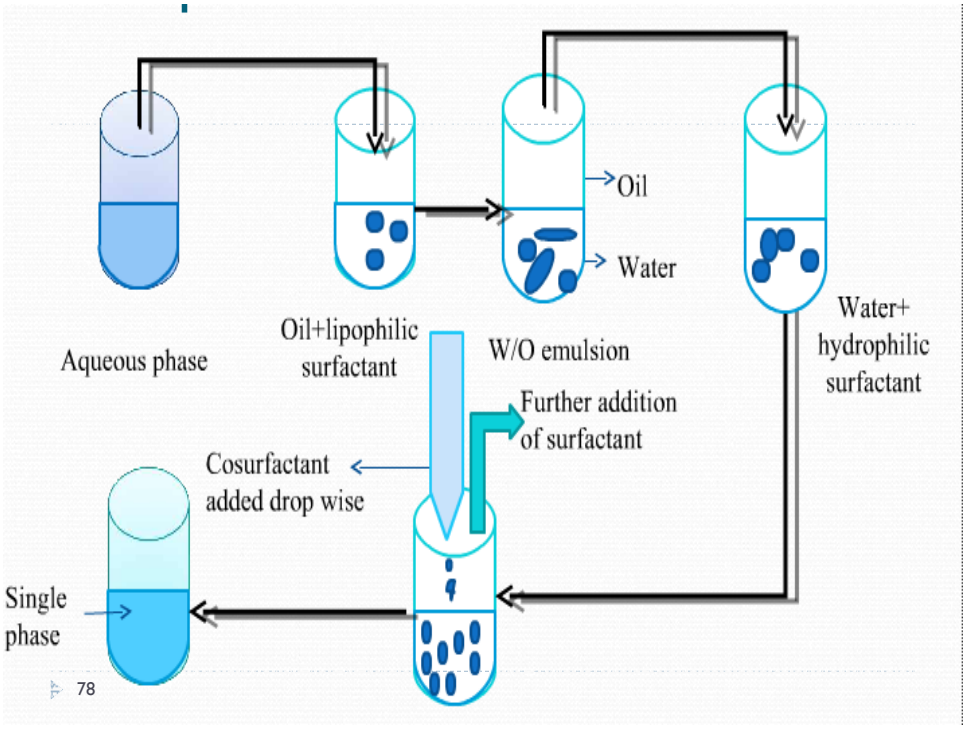

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Preparation of Microemulsion

- Following are the different methods are used for the preparation of microemulsion:

1. Phase titration method
2. Phase inversion method

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Phase titration method

- *1)*dilution of an oil-surfactant mixture with water.(w/o)
- 2) dilution of a water-surfactant mixture with oil.(o/w)
- 3) mixing all components at once.

• In some systems, the order of ingredient addition may determine whether a microemulsion forms or not.

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Phase inversion method :

Phase Inversion Temperature (PIT), *i.e., the* temperature range in which an o/w microemulsion inverts to a w/o type or *vice versa.*

• **using non-ionic surfactants,** polyoxyethylene are very susceptible to temperature since surfactant solubility (in oil or water) strongly depends on temperature.

With increasing temperature, the polyoxyethylene group becomes dehydrated, altering the critical packing parameter which results in phase inversion.

• **For ionic surfactants,** increasing temperatures increase the electrostatic repulsion between the surfactant headgroups thus causing reversal of film curvature.

Hence the effect of temperature is opposite to the effect seen with non-ionic surfactants.

EVALUATION

Parameters Studied	Techniques Used
Phase Behaviour	Phase contrast microscopy and freeze fracture TEM
Size and Shape	Transmission Electron Microscopy (TEM), SEM,DLS
Rheology	Brookfield Viscometer
Conductivity	Conductivity Meter
Zeta Potential	Zetasizer
pH	pH Meter
Drug Release Studies	Franz Diffusion Cells
Physical Stability Study	Ultracentrifuge

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- Oral drug delivery
- Ocular drug delivery
- Pulmonary drug delivery
- Transdermal drug delivery
- Parenteral drug delivery
- For solubilization of drug
- In biotechnology
- Others

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In Vitro Drug Permeation Studies

- Excised human cadaver skin from the abdomen can be obtained from dead who have undergone postmortem not more than 5 days ago in the hospital. The skin is stored at 4°C and the epidermis separated.
- The skin is first immersed in purified water at 60°C for 2 min and the epidermis then peeled off.
- Dried skin samples can be kept at -20°C for later use.
- Alternatively the full thickness dorsal skin of male hairless mice may be used.
- The skin shall be excised, washed with normal saline and used.

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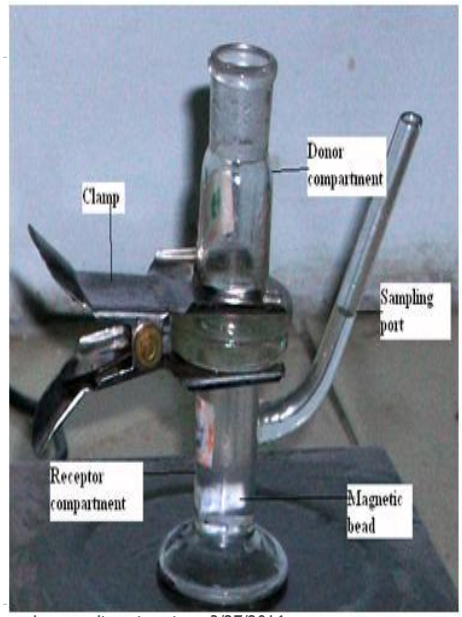
- The passive permeability of lipophilic drug through the skin is investigated using Franz diffusion cells with known effective diffusional area.
- The hydrated skin samples are used. The receiver compartment may contain a complexing agent like cyclodextrin in the receiver phase, which shall increase the solubility and allows the maintenance of sink conditions in the experiments.
- Samples are withdrawn at regular interval and analyzed for amount of drug released.

Fig. Franz diffusion cell



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Stability Studies

- The physical stability of the microemulsion must be determined under different storage conditions (4, 25 and 40 °C) during 12 months.
- Depending on different regulatory agency requirement it'll vary according to them.

Application of microemulsion in delivery of drug

Oral delivery

- Microemulsions have the potential to enhance the solubilization of **poorly soluble drugs (particularly BCS class II or class IV)** and **overcome the dissolution related bioavailability problems**.
- These systems have been **protecting the incorporated drugs against** oxidation, enzymatic degradation and enhance membrane permeability.
- **Presently**, Sandimmune Neoral(R) (Cyclosporine A), Fortovase(R) (Saquinavir), Norvir(R) (Ritonavir) etc. are the commercially available microemulsion formulations.
- Microemulsion formulation can be potentially useful to improve the oral bioavailability of poorly water soluble drugs by enhancing their solubility in gastrointestinal fluid.

- **Parenteral delivery**
  - The formulation of parenteral dosage form of lipophilic and hydrophilic drugs has proven to be difficult.
  - O/w microemulsions are beneficial in the parenteral delivery of sparingly soluble drugs where the administration of suspension is not required.
  - They provide a means of obtaining relatively high concentration of these drugs which usually requires frequent administration.
  - Other advantages are that they exhibit a higher physical stability in plasma than liposome's or other vehicles and the internal oil phase is more resistant against drug leaching.
  - Several sparingly soluble drugs have been formulated into o/w microemulsion for parenteral delivery.

- **Topical delivery**
  - Topical administration of drugs can have advantages over other methods for several reasons, one of which is the avoidance of hepatic first-pass metabolism of the drug and related toxicity effects.
  - Another is the direct delivery and targetability of the drug to affected areas of the skin or eyes.
  - Now a day, there have been a number of studies in the area of drug penetration into the skin.
  - They are able to incorporate both hydrophilic (5-flurouracil, apomorphine hydrochloride, diphenhydramine hydrochloride, tetracaine hydrochloride, methotrexate) and lipophilic drugs (estradiol, finasteride, ketoprofen, meloxicam, felodipine, triptolide) and enhance their permeation.

- **Ophthalmic delivery**
  - Low corneal bioavailability and lack of efficiency in the posterior segment of ocular tissue are some of the serious problem of conventional systems.
  - Recent research has been focused on the development of new and more effective delivery systems.
  - Microemulsions have emerged as a promising dosage form for ocular use.
  - Chloramphenicol, an antibiotic used in the treatment of trachoma and keratitis, in the common eye drops hydrolyzes easily.
  - Fialho *et al. studied microemulsion based* dexamethasone eye drops which showed better tolerability and higher bioavailability. The formulation showed greater penetration in the eye which allowed the possibility of decreasing dosing frequency and thereby improve patient compliance.

- ***Nasal delivery***
  - Recently, microemulsions are being studied as a delivery system to enhance uptake of drug through nasal mucosa.
  - In addition with mucoadhesive polymer helps in prolonging residence time on the mucosa.
  - Lianly et al. investigated the effect of diazepam on the emergency treatment of status epilepticus.
  - They found that the nasal absorption of diazepam fairly rapid at 2 mg kg-1 dose with maximum drug plasma concentration reached within 2-3 min

- ***Drug targeting***
  - Drug targeting to the different tissues has evolved asthe most desirable goal of drug delivery.
  - By altering pharmacokinetics and biodistribution of drugs and restricting their action to the targeted tissue increased drug efficacy with concomitant reduction of their toxic effects can be achieved.
  - Shiokawa *et al. reported a* novel microemulsion formulation for tumor targeting of lipophilic antitumor antibiotic aclainomycin A (ACM).
  - They reported that a folate-linked microemulsion is feasible for tumour targeted ACM delivery.
  - They also reported that folate modification with a sufficiently long PEG chain on emulsions is an effective way of targeting emulsion to tumour cells.

Advantages

- Increase the rate of absorption
- Eliminates variability in absorption
- Helps solublize lipophilic drug
- Provides a aqueous dosage form for water insoluble drugs
- Increases bioavailability
- Various routes like tropical, oral and intravenous can be used to deliver the product
- Rapid and efficient penetration of the drug moiety
- Helpful in taste masking
- Provides protection from hydrolysis and oxidation as drug in oil phase in O/W microemulsion is not exposed to attack by water and air.
- Liquid dosage form increases patient compliance.
- Less amount of energy requirement.

Disadvantages

- Use of a large concentration of surfactant and cosurfactant necessary for stabilizing nano droplets.
- Limited solubilizing capacity for high-melting substances.
- The surfactant must be nontoxic for using pharmaceutical applications.
- Microemulsion stability is influenced by environmental parameters such as temperature and pH. These parameters change upon microemulsion delivery to patients