NOVEL DRUG DELIVERY SYSTEM

TARGETED DRUG DELIVERY SYSTEM (TDDS)

The aim of any drug delivery system is to modulate the pharmacokinetics and dynamic of the drug in a beneficial way.

A delivery system that delivered the drug to a specific organ or tissue or near its vicinity.

TDDS may provide maximum therapeutic activity by preventing drug degradation or inactivation during transit to the target site.

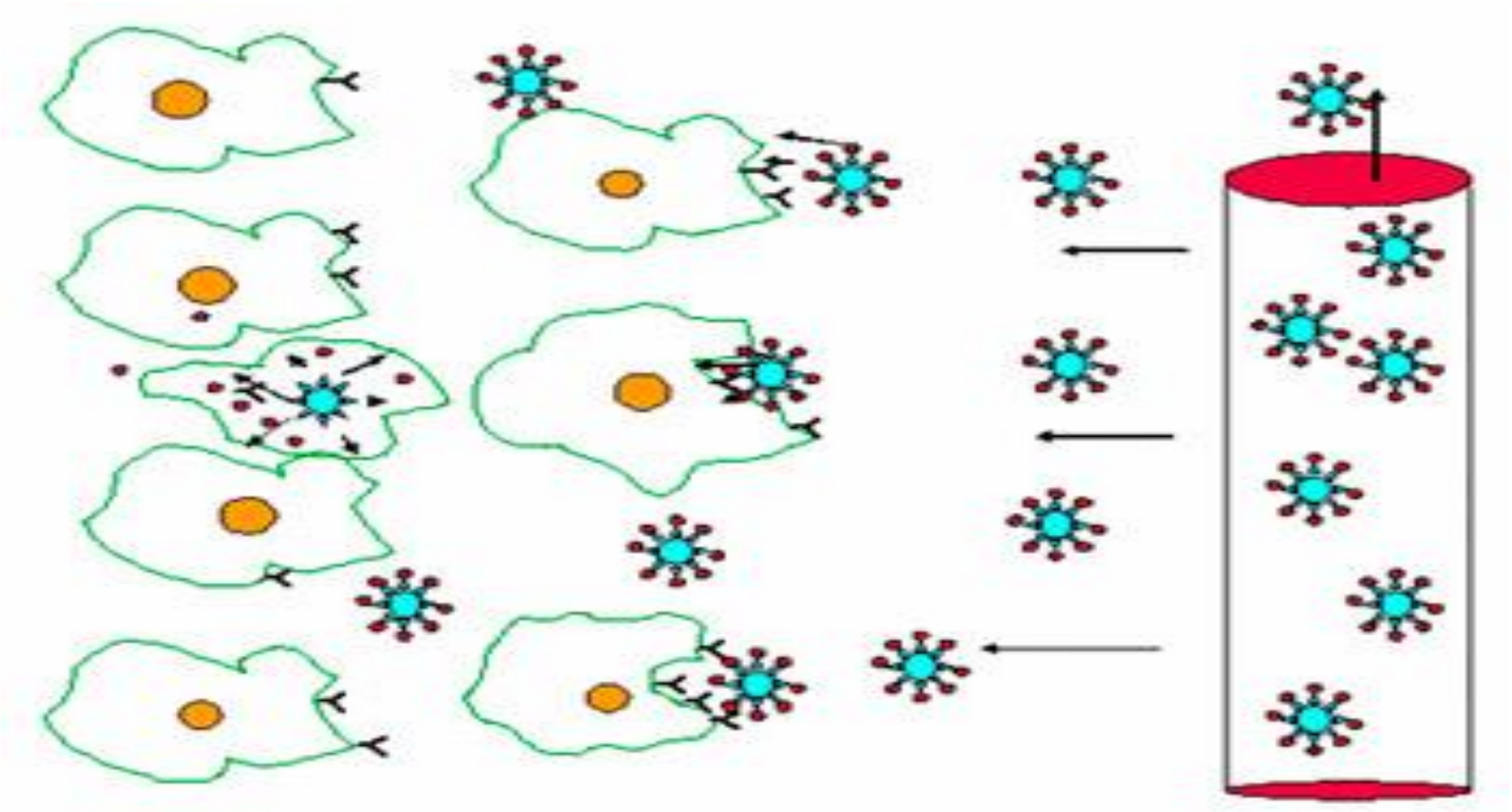
It can protect body from adverse effects and minimize the toxicity of potent drug by reducing dose.

An ideal targeted delivery system should be;

- Non toxic
- Biocompatible
- Biodegradable
- Physicpochemically stable

The preparation of the delivery system must be reasonably simple, reproducible and cost effective.

Targeted Drug Delivery



Importance of TDDS

- Drug delivery formulations involve low cost research compared that for discovery of new molecule,
- Minimizing the drug use would significantly reduce the effective cost of drug which would give financial relief to the patients,
- Delivery systems increase commercial opportunity by distinguishing a drug from competitive threats posed by “me too” drugs and
- Novel means of delivery particularly using can allow branded drugs to be rescued from abyss of generic competition (may be called “resurrection of drug”).

DRUG CARRIERS

Substances that facilitate time-controlled delivery, organ-specific targeting, protection, prolonged in vivo function, and decrease of toxicity of drugs.

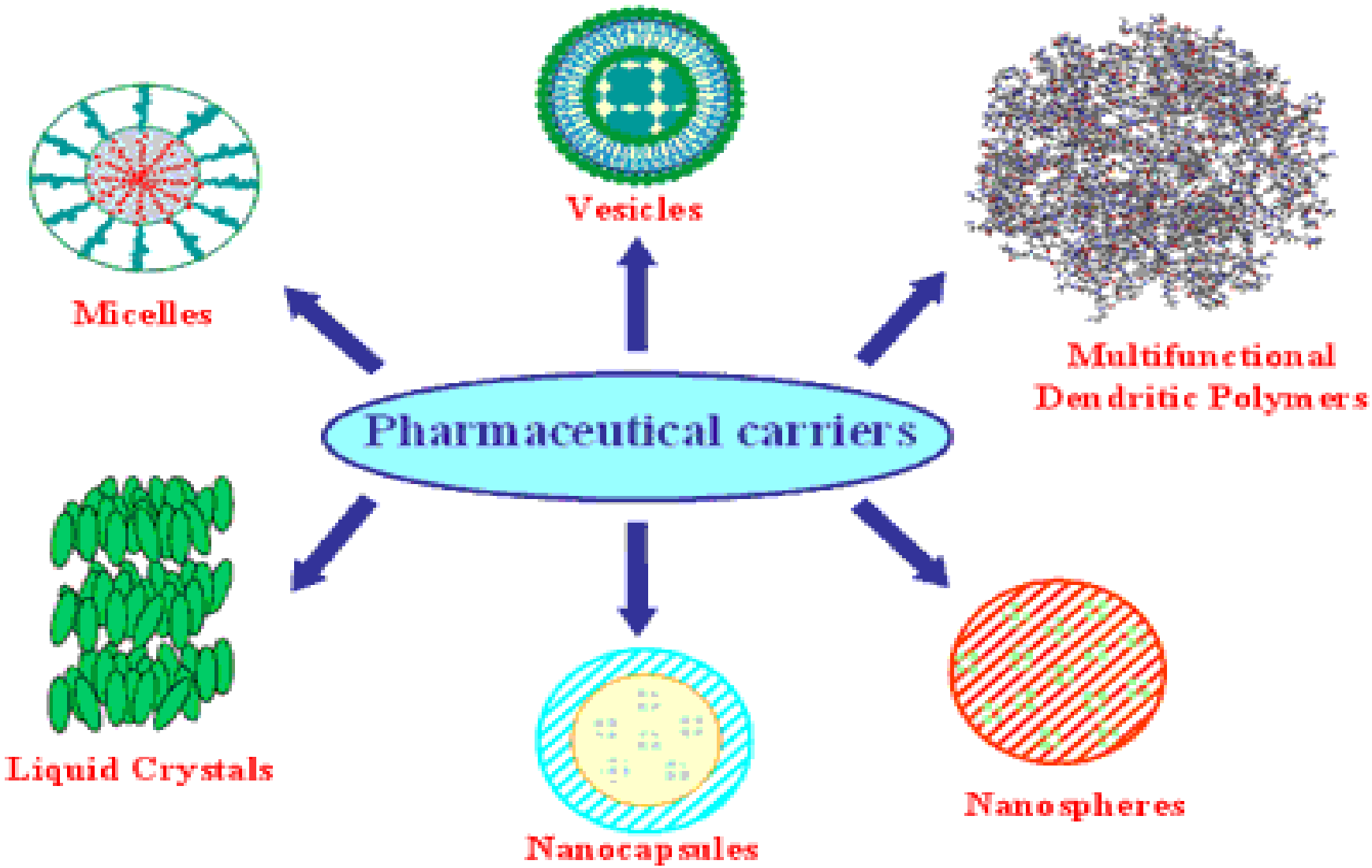
Drug Carriers are used in drug delivery systems such as:

- ✓ **Controlled-release technology to prolong in vivo drug actions,**
- ✓ **Decrease drug metabolism,**
- ✓ **Reduce drug toxicity.**
- ✓ **Ligand mediated Targeting**
- ✓ **Bioadhesives**
- ✓ **Prodrugs**

Drug Carriers

1. Colloidal Drug Particles i.e; (Liposomes, Niosomes, Nanoparticles)
2. Resealed Erythrocytes
3. Microspheres
4. Biodegradable polymers

Drug Delivery Carriers

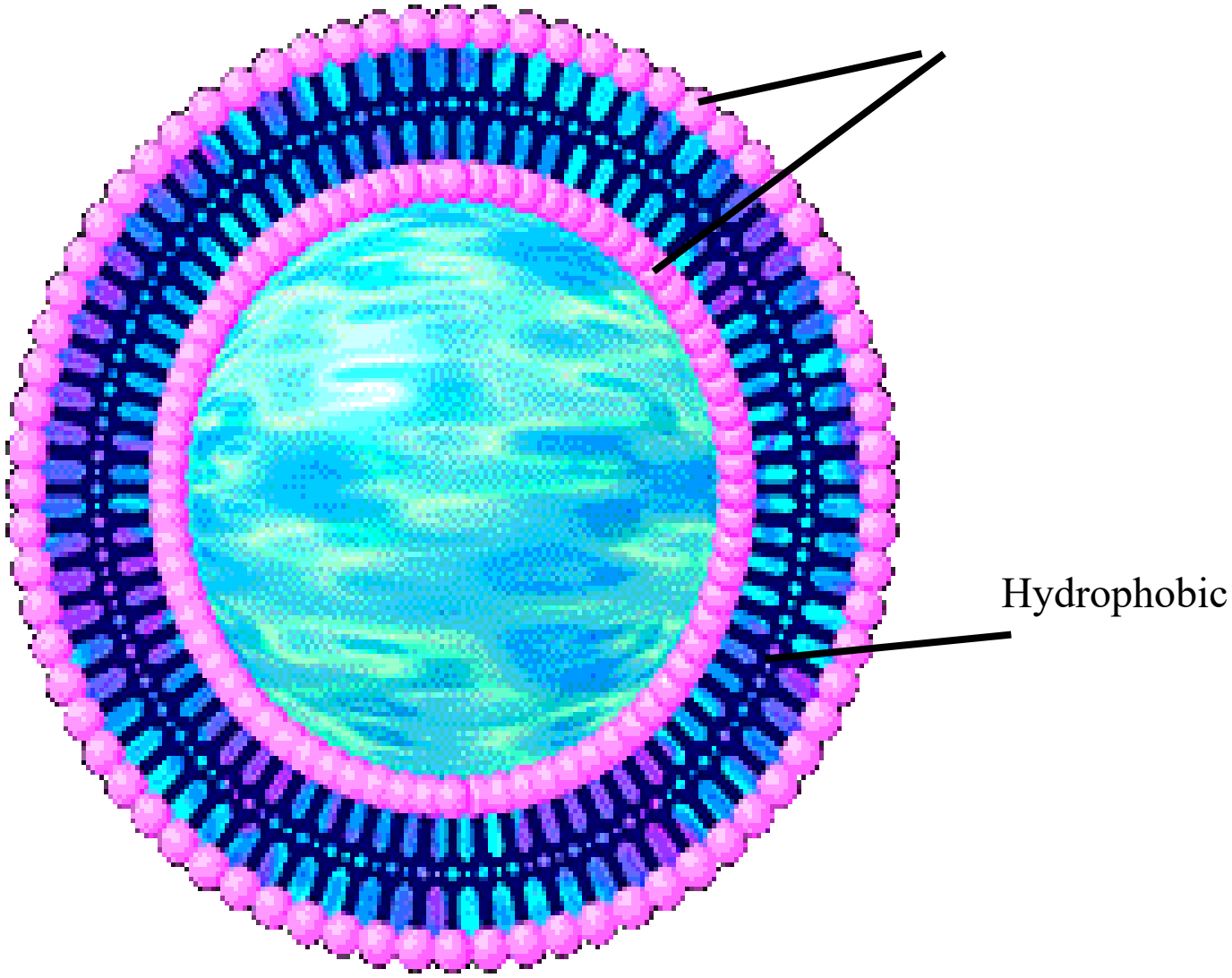


LIPOSOME

A spherical vesicle composed of a bi-layer membrane, of phospholipids and cholesterol bi-layer.

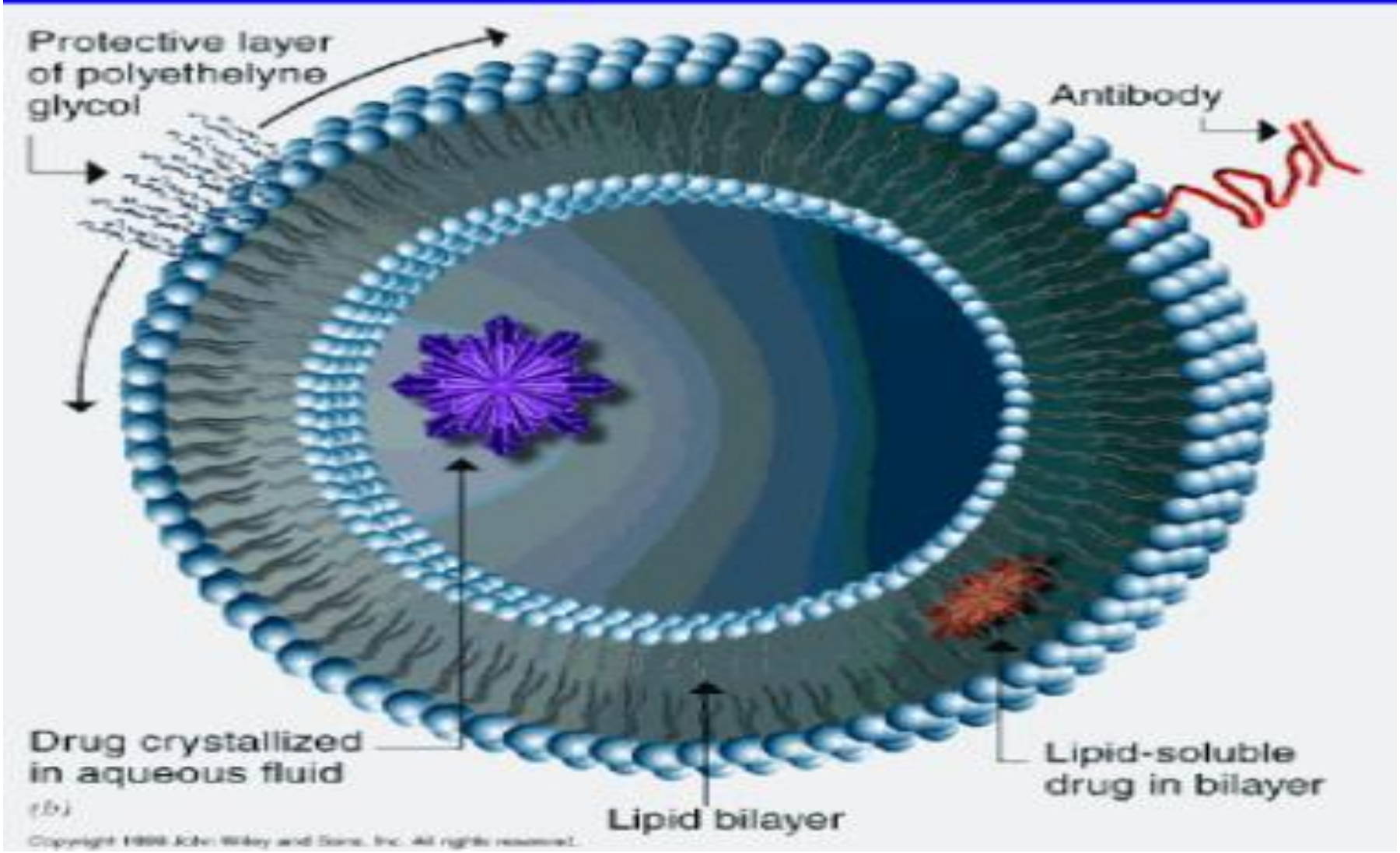
Liposomes can be composed of naturally-derived phospholipids with mixed lipid chains.

Liposomes are used as drug carriers and loaded with a great variety of molecules, such as small drug molecules, proteins, nucleotides and even plasmids.


Hydrophilic
Hydrophobic

- Their exterior lipid bi-layer is chemically reactive, thereby providing a means to conveniently couple "tags" on a covalent basis.
- Such "tags" can be antibodies, antigens, cell receptors, nucleic acid probes, etc.
- This provides significant versatility in assay formats (i.e., immunoassay, receptor-based, nucleic acid probe, etc.) possible.
- With diameters ranging in size from approximately 50 nm to 800 nm, their aqueous core encapsulates up to millions of molecules of signal generating "markers" that can be detected in a variety of different way.
- A variety of different encapsulants are possible including visually detectable dyes (since the lipid bilayer is transparent), optically and fluorometrically detectable dyes, enzymes, and electroactive compounds.
- This provides significant versatility in the detection schemes possible.

Liposomes



DIRECTION

Liposomes can target a drug to the intended site of action in the body, thus enhancing its therapeutic efficacy (drug targeting, site-specific delivery). Liposomes may also direct a drug away from those body sites that are particularly sensitive to the toxic action of it (site-avoidance delivery).

DURATION

Liposomes can act as a depot from which the entrapped compound is slowly released over time. Such a sustained release process can be exploited to maintain therapeutic (but nontoxic) drug levels in the bloodstream or at the local administration site for prolonged periods of time. Thus, an increased duration of action and a decreased frequency of administration are beneficial consequences.

PROTECTION

Drugs incorporated in liposomes, in particular those entrapped in the aqueous interior, are protected against the action of detrimental factors (e.g. degradative enzymes) present in the host.

INTERNALIZATION

Liposomes can interact with target cells in various ways and are therefore able to promote the intracellular delivery of drug molecules that in their ‘free' form (i.e. non-encapsulated) would not be able to enter the cellular interior due to unfavorable physicochemical characteristics (e.g. DNA molecules).

AMPLIFICATION

If the drug is an antigen, liposomes can act as immunological adjuvant in vaccine formulations.

Properties of liposomes

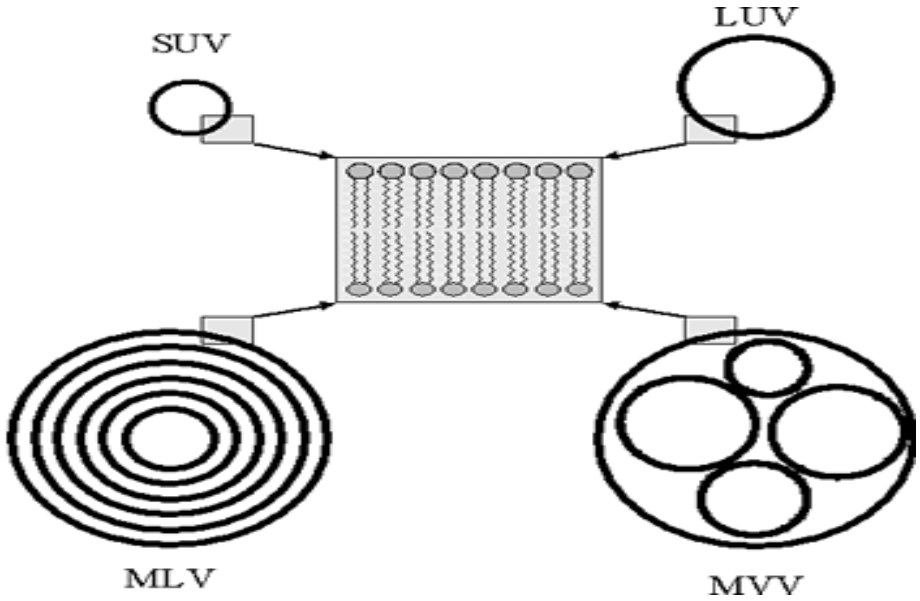
For medical applications as drug carriers the liposomes can be injected intravenously and when they are modified with lipids which render their surface more hydrophilic, their circulation time in the bloodstream can be increased significantly. "Stealth" liposomes are especially being used as carriers for hydrophilic (water soluble) anticancer drugs like doxorubicin, mitoxantrone and others.

To further improve the specific binding properties of a drug-carrying liposome to a target cell, - such as a tumour cell -, specific molecules (antibodies, proteins, peptides etc.) are attached on their surface.

CLASSIFICATION OF LIPOSOMES:

1. SIZE AND LAMELLARITY:

- Small Unilameller Vesicles (SUV) 25 to 100 nm that consist of a single lipid bi-layer.
- Large Unilameller Vesicles (LUV) 100 to 400 nm that consist of a single lipid bi-layer.
- Multilameller Vesicles (MLV) 200 nm to several µm, consist of two or more concentric lipid bi-layers.
- Giant Vesicles above 1 µm.

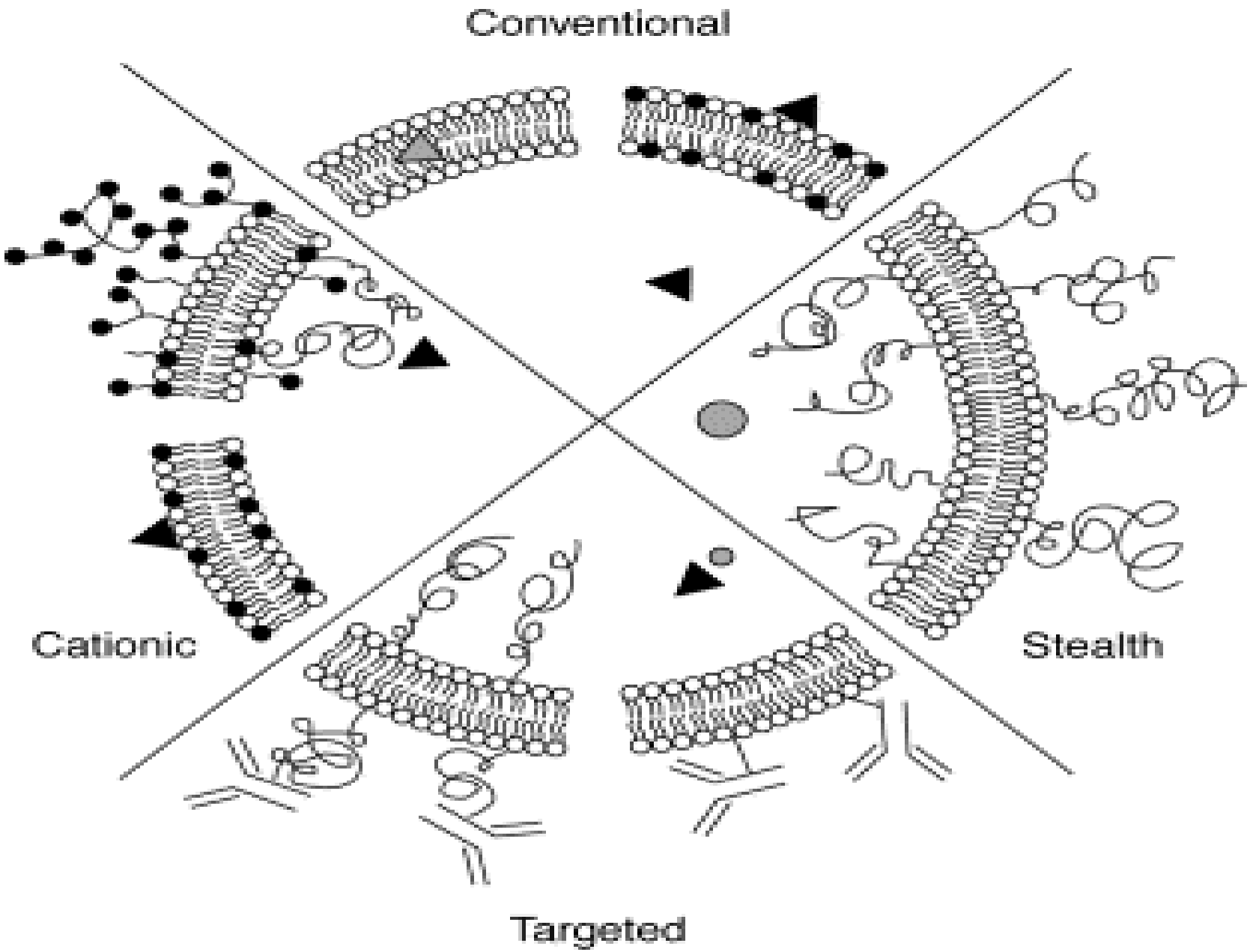


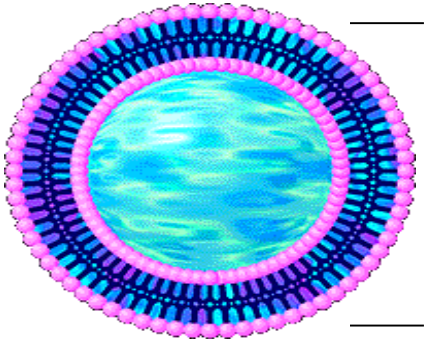
2. MONONUCLEAR-PHAGOCYTIC SYSTEM (MPS) UPTAKE AND COATING

- Mononucler-Phagocytic System
- Reticuloendothielial System

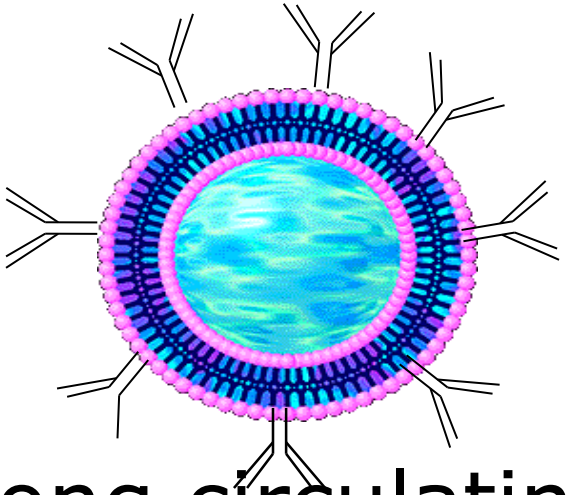
3.

S.#	Liposome type	Major application
1	Conventional liposomes	Macrophage targeting Local depot Vaccination
2	Long circulating liposomes	Selective targeting to pathological areas Circulating microreservoir
3	Immunoliposomes	Specific targeting
4	Cationic liposomes	Gene delivery

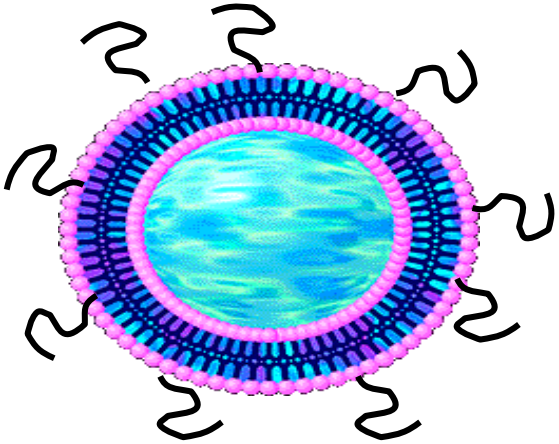

Conventional
Cationic
Stealth
Targeted



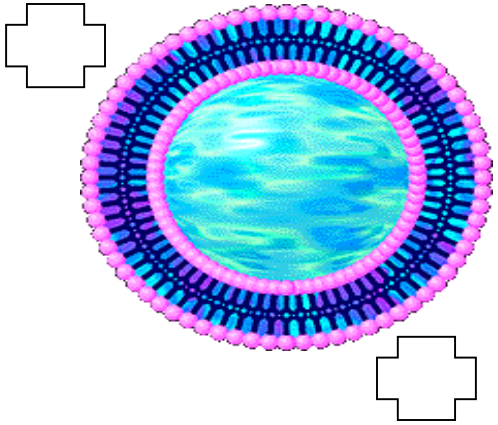
Conventional



Long circulating



Immuno



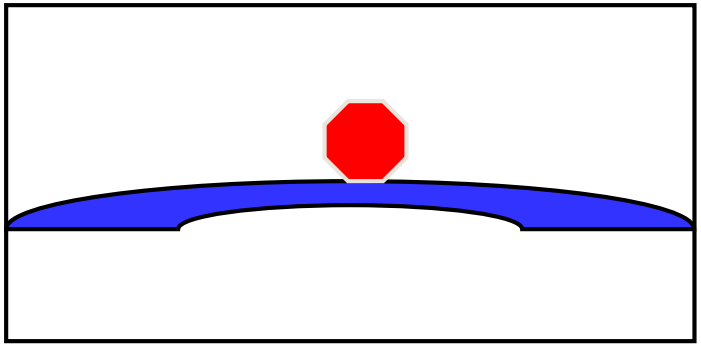
Cationic

INTERACTION MECHANISM

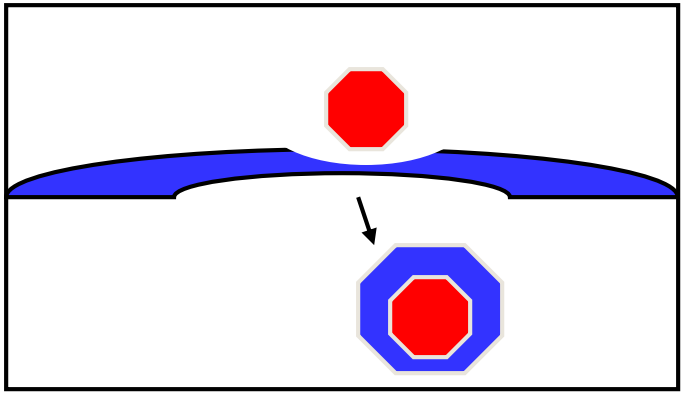
Liposome can interact with cells by four different mechanisms.

1. Endocytosis by phagocytic cells of the RES.
2. Adsorption to the cell surface either by nonspecific hydrophobic or electrostatic forces or by specific interactions with cell surface components.
3. Fusion with the plasma cell membrane by insertion of lipid bi-layer of the liposome into the plasma membrane, with simultaneous release of liposomal content into the cytoplasm.
4. Transfer of liposomal lipids to cellular or sub-cellular membranes or vice versa, without any association of liposome content.

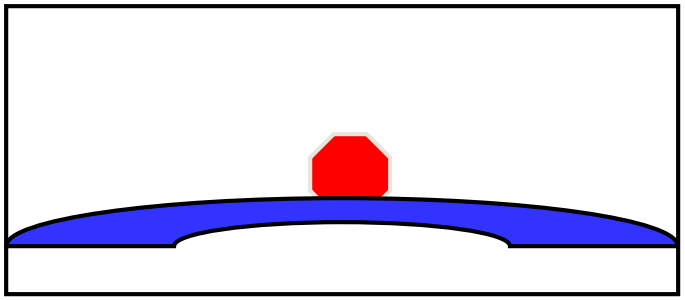
Adsorption



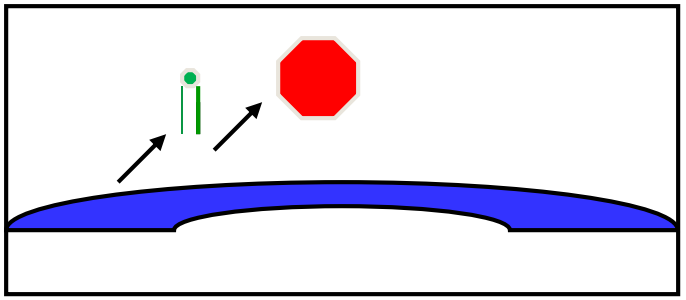
Endocytosis



Fusion



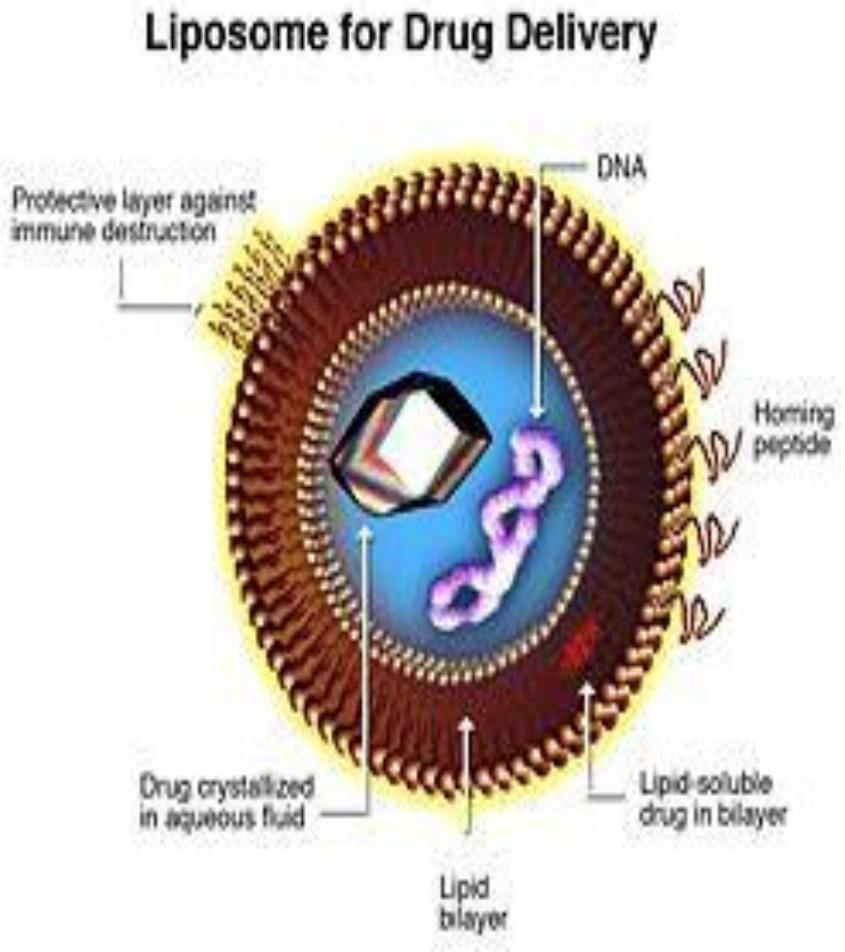
Lipid Transfer



APPLICATION

A liposome encapsulates aqueous solution inside a hydrophobic membrane; dissolved hydrophilic solutes which can not readily pass through the lipids.

Hydrophobic chemicals can be dissolved into the membrane, and in this way liposome can carry both hydrophobic and hydrophilic molecules. To deliver the molecules to sites of action, the lipid bi-layer can fuse with other bi-layers such as the cell membrane, thus delivering the liposome contents.



1. Liposomes as drug/protein delivery vesicles.
2. Controlled and sustained drug release.
3. Altered pharmacokinetics and bio-distribution.
4. Enzyme replacement therapy.
5. Used in antimicrobial, antifungal and antiviral therapy.
6. Used in tumour therapy.
7. Carriers for small cytotoxic molecules.
8. Vehicles for large molecules as cytokines and genes.
9. Used for immunological products.
10. Used in Radiopharmaceuticals.
11. Used in cosmetics and dermatologic products.
12. Used for enzyme immobilization.

ASSAY AND CHARACTERIZATION:

Assay	Methodology / Analytical Target
Characterization	
pH	pH meter
Osmolarity	Osmometer
Phospholipid concentration	Lipid phosphorus content / HPLC
Phospholipid composition	TLC and HPLC
Cholesterol concentration	Cholesterol oxidase assay and HPLC
Drug concentration	Appropriate method
Chemical stability	
pH	pH meter
Phospholipid peroxidation	Conjugated dienes, lipid peroxides and FA composition (GLC)

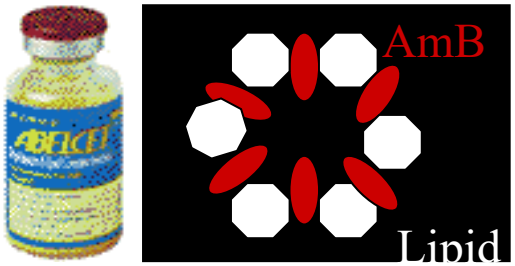
ASSAY AND CHARACTERIZATION:

Assay	Methodology / Analytical Target
Phospholipid hydrolysis	HPLC, TLC and FA concentration
Cholesterol auto-oxidation	HPLC, TLC
Antioxidant degradation	HPLC, TLC
Physical stability	
Vesicle size distribution: submicron range	DLS
micron range	Coulter Counter Light Microscopy, Laser Diffraction and GEC
Electrical surface potential and surface pH	Zeta-potential Measurements and pH Sensitive Probes
Numbers of bi-layers	SAXS, NMR

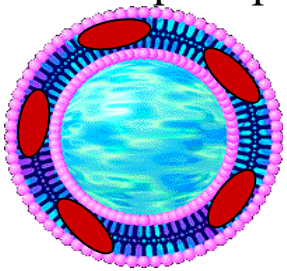
SAXS = Small Angle X-Ray Scattering; DLS, = Dynamic Light Scattering; FA = Fatty Acid

Liposomal Formulation of AmB

AmBisome®
(amphotericin B) liposome for injection



Phospholipid : AmB ratio



Cholesterol - only few %moles

Exact Mechanism of liposomes not understood

- Diffusion
- Lipid transfer

Decrease in toxicity

No decrease in effectiveness of drug against fungi

ASSAY AND CHARACTERIZATION (Continue):

Assay	Methodology / Analytical Target
Percentage of free drug	GEC, IEC and Protamine Precipitation
Dilution-dependent drug release	Retention loss on dilution
Relevant body fluid induced leakage	GEC / TLC and protaminc precipitation
Biological characterization	
Sterility	Aerobic and anaerobic cultures
Pyrogenicity	Rabbit or LAL test
Animal toxicity	Monitor survival. histology and pathology

GEC = Gel Exclusion Chromatography: GLC = Gas-Liquid Chromatography; IEC = Ion Exchange Chromatography; LAL = Limulus Amoebocyte Lysate;

Problems with Liposomal Preparations of Drugs

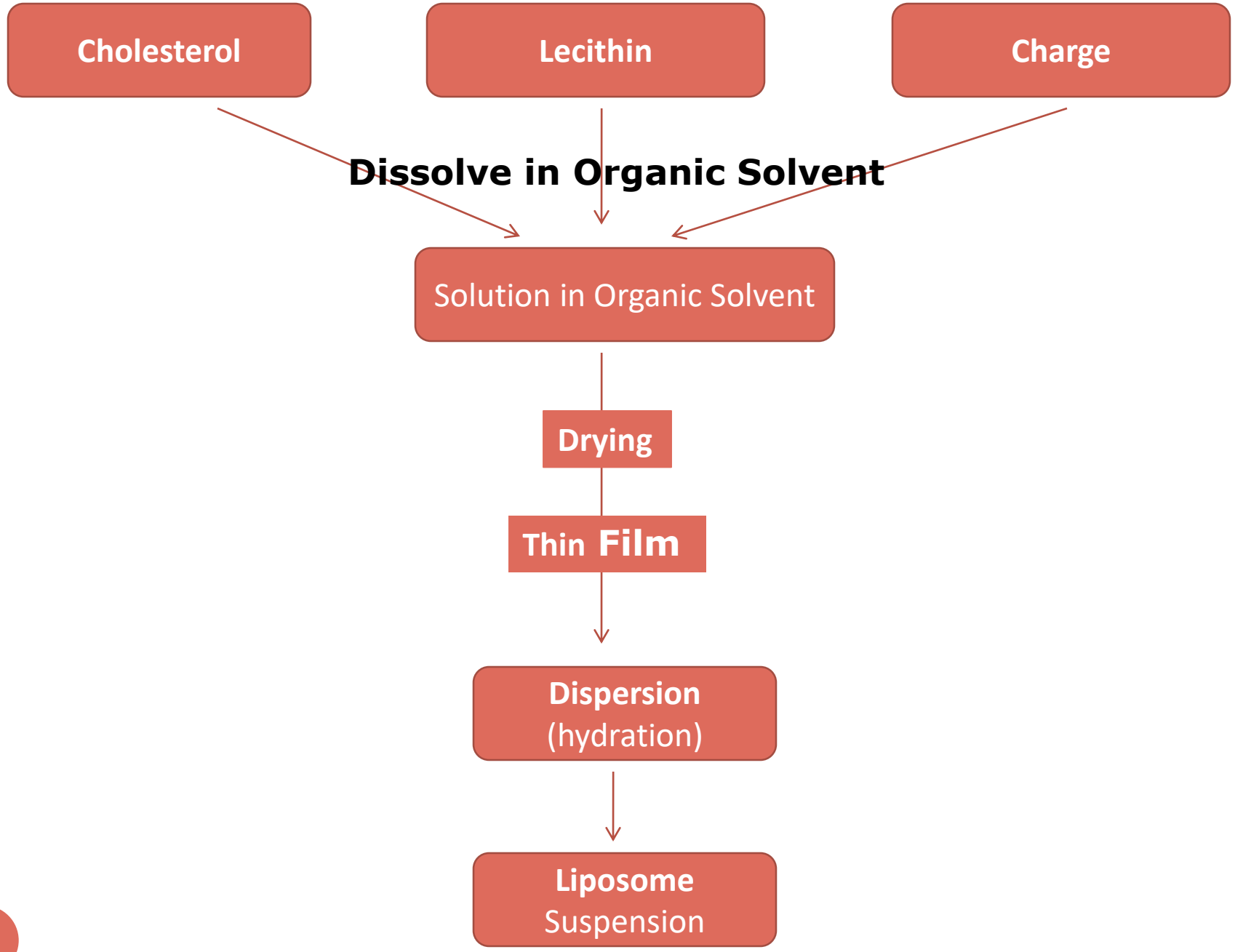
- **Expensive $$$$**
  - Fungizone $40.58 Amphotec $2334
  - Doxil $1200 per treatment, twice the cost of normal protocol of chemotherapy and drugs
- **Lack long term stability (short shelf life)**
- **Physical and chemical instability**
- **Freeze dry and pH adjustment**
- **Low "Pay Load" - poor encapsulation**
- **Polar drugs and drugs without opposite charge**
- **Modifications**
- **Possibility of new side effects e.g. Doxil "hand and foot syndrome**
- **Efficacy e.g.**

  Deliver cDNA of Cystic Fibrosis Trans-membrane Conductance Regulator (CFTR) to epithelial tissue of respiratory system through Cationic Liposome which Fuse to cell membrane and incorporate cDNA into cell Clinical trials - no significant change in symptoms

PREPARATION

Three or four basic stages

1. Drying down lipids from organic solvents.
2. Dispersion of lipids in aqueous medium
3. Purification of resultant liposome
4. Analysis of the final product


Cholesterol
Lecithin
Charge
Dissolve in Organic Solvent
Solution in Organic Solvent
Drying
Thin Film
Dispersion
(hydration)
Liposome
Suspension

A) Multilamellar Liposomes (MLV)

(i) Lipid Hydration Method

- This is the most widely used method for the preparation of MLV.
- Drying a solution of lipids in organic solvent through rotary evaporator under nitrogen till formation of a thin film at the bottom of round bottom flask.
- Then hydrating the film by adding aqueous buffer and vortexing the dispersion for some time at a temperature above the gel-liquid crystalline transition temperature.
- The compounds to be encapsulated are added either to aqueous buffer or to organic solvent containing lipids depending upon their solubility.
- MLV thus produced are simple and a variety of substances can be encapsulated in these liposomes.

(ii) Solvent Spherule Method:

The process involved dispersion of small spherules of volatile hydrophobic solvent in aqueous solution in which lipids are already being dissolved. MLVs are formed when controlled evaporation of organic solvent occurred in a water bath.

B) Small Uni-lamellar Liposomes (SUV)

(1) Sanitation Method

In this method MLVs are sonicated either with a bath type sonicator or a probe sonicator under an inert atmosphere.

This method produces very low internal volume / encapsulation efficiency liposomes.

(2) French Pressure Cell Method

The method involves the extrusion of MLV at 20,000 psi at 4°C through a small orifice. The method is simple rapid, reproducible and involves gentle handling of unstable materials give small volume preparations i.e. 50 mL.

(3) Deposition of egg phosphatidylcholinc mixed with 1.5 %w/v of cetyltetramethylammonium bromide (a detergent) in chloroform / methanol on various supports for example silica gel powder, zeolite X, zeolite ZSM5.

After the removal of organic phase, the system is re-suspended by shaking or stirring in distilled water or 5 mM NaCl. A homogenous population of vesicle with average diameter of 21.5 nm is obtained.

C) Large Unilamellar Liposomes (LUV)

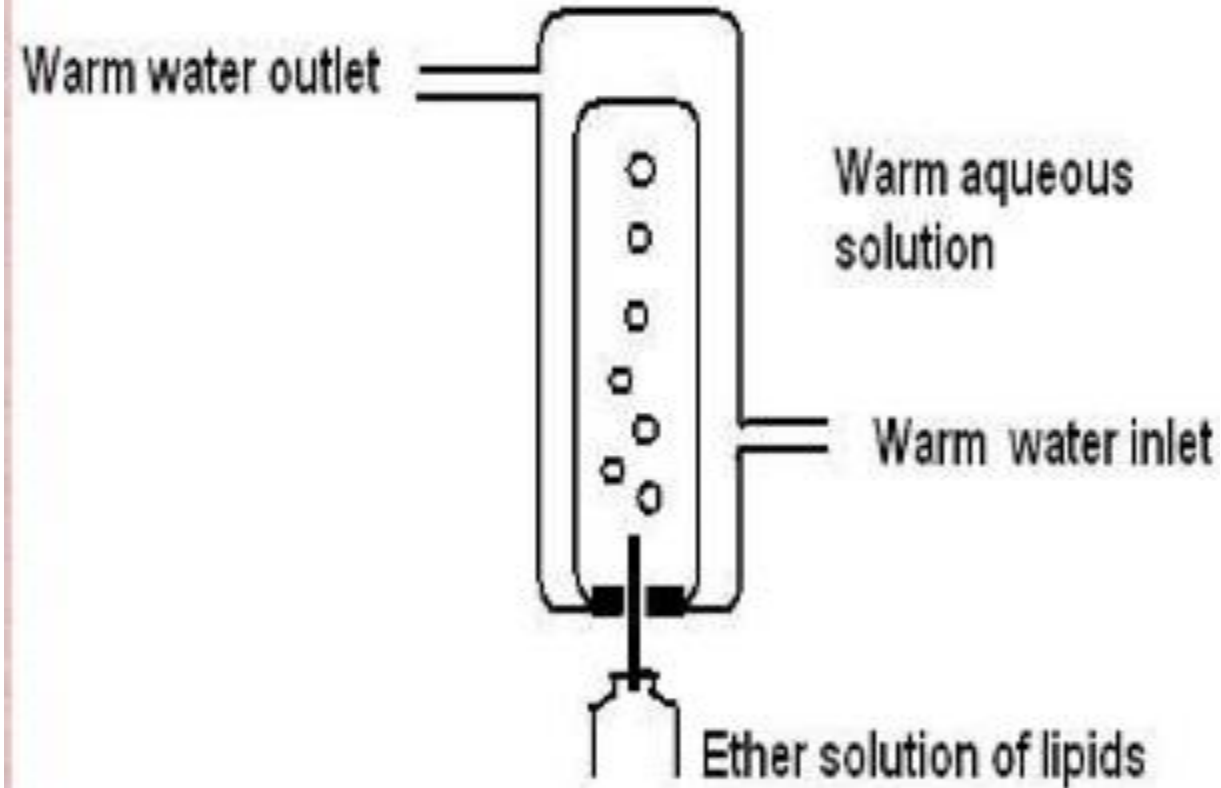
(1) Solvent Injection Methods

(i) Ether Infusion Method

In this process a solution of lipids in diethyl ether or ether/methanol mixture is slowly injected to an aqueous solution of the material to be encapsulated at 55-65°C or under reduced pressure. The subsequent removal of ether under vacuum leads to the formation of heterogeneous liposomes (70-190 nm).

(ii) Ethanol Injection Method

A lipid solution of ethanol is rapidly injected to a vast excess of buffer. The LUVs are immediately formed.



ETHER INJECTION TECHNIQUE

(2) Detergent Removal Methods

In this method lipids are solubilized with the help of detergents in their critical micelles concentrations. When the detergents are removed, through dialysis, from solution the micelles become progressively richer in phospholipid and finally combine to form LUVs.

The advantages of detergent dialysis method are excellent reproducibility and production of liposome populations which are homogenous in size.

Other techniques have also been used for the removal of detergents:

(a) by using Gel Chromatography involving a column of Sephadex G-25,

(b) by adsorption or binding of Triton X-100 (a detergent) to Bio-Beads SM-2.

(c) by binding of octyl glucoside (a detergent) to Amberlite XAD-2 beads.

(3) Reserves Phase Evaporation Method

First water in oil emulsion is formed by brief sonication of a two phase systems containing phospholipids in organic solvent (diethylether or isopropylether or mixture of isopropyl ether and chloroform) and aqueous buffer.

The organic solvents are removed under reduced pressure, resulting in the formation of a viscous gel.

The liposomes are formed when residual solvent is removed by continued rotary evaporation under reduced pressure.

With this method high encapsulation efficiency up to 65% can be obtained in a medium of low ionic strength for example 0.01 M NaCl. The method has been used to encapsulate small, large and macromolecules.

(4) Calcium-Induced Fusion Method

This method is used to prepare LUV from acidic phospholipids by the addition of calcium and later chelating calcium by EDTA. The main advantage of this method is that macromolecules can be encapsulated under gentle conditions. The resulting liposomes are largely unilamellar, although of a heterogeneous size range.

(5) Microfluldization Method

First MLV are prepared by hydration method and then passed through a Microfluidizer at 40 psi inlet air pressure and recycled. This method gives up to 150-160 nm liposomes after 25 recycles.

In the Microfluidizer, the interaction of fluid streams takes place at high velocities (pressures) in precisely defined micro-channels which are present in an interaction chamber. In the chamber pressure reaches up to 10,000 psi this can be cause partial degradation of lipids.

(6) Extrusion under nitrogen through polycarbonate filters LUV can be prepared by passing MLV under nitrogen through polycarbonate membrane filters. The vesicles produced by this method have narrow size distribution. The extrusion is done under moderate pressures (100-250 psi). A special filter holder is required. Such devices are available commercially under the trade names such as LUVET and EXTRUDER and are equipped with a recirculation mechanism that permits multiple extrusions with little difficulty.

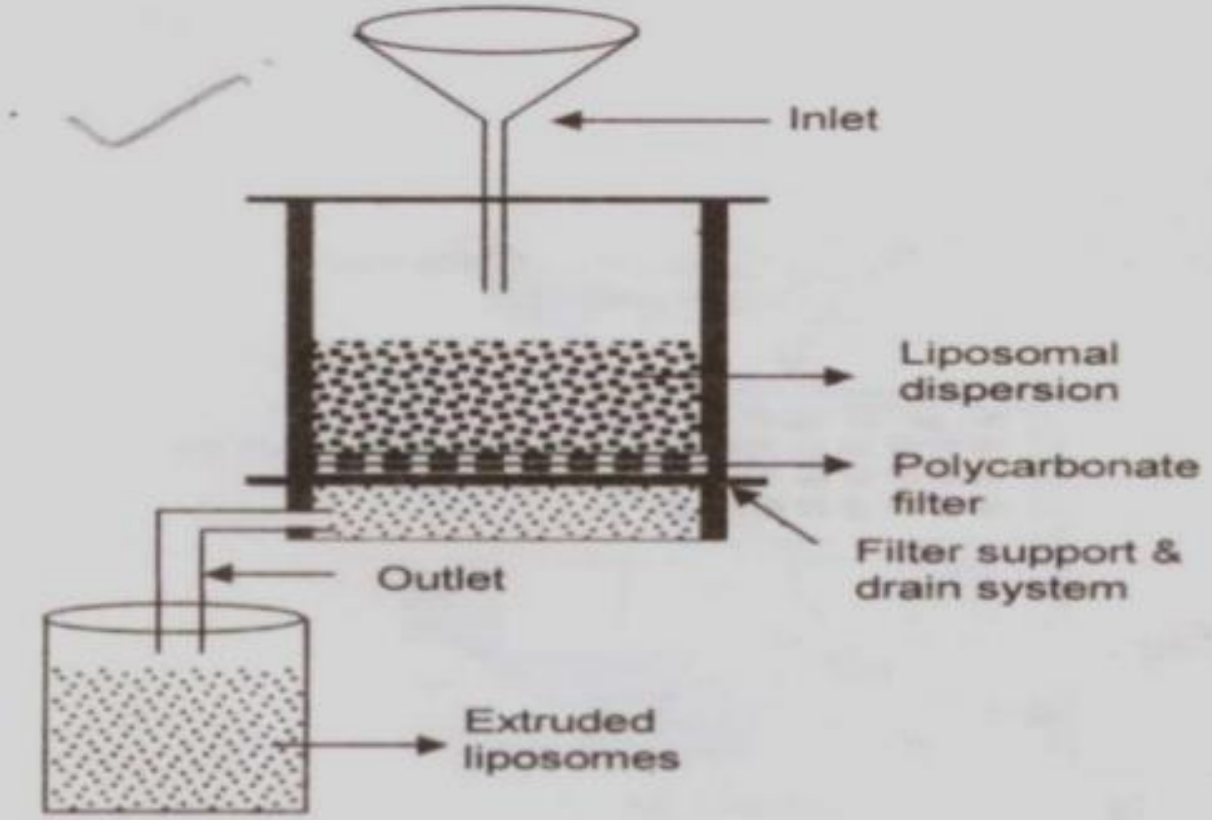


Fig. 5-11. Liposomes Preparation Using Extrusion Technique Based on Polycarbonate Filters

(7) Freeze-Thaw Method

In this method lipid in organic solution is freezed by nitrogen flushing.

Then buffer solution is added and the container is vortexed until a homogenous milky suspension is obtained. The suspension is then sonicated until partial clarification is obtained. The materials which are to be entrapped are added either in solid form or as small aliquot of concentrated solution and then mixed well.

Container is flushed with nitrogen sealed and allowed to freezed by shaking in a bath of liquid nitrogen.

After complete freezing the container is allowed to thaw by standing in the room temperature. Re-sonicated for 30 seconds and the entrapped material is separated by column chromatography or centrifugation.

The encapsulation efficiencies from 20 to 30% were obtained .

(D) Multivesicular Liposomes

The water in oil emulsion is converted to organic solvent spherules by the addition of the emulsion to across solution. The evaporation of organic solvent resulted in the formation of multivesicular vesicles. The diameter of liposomes ranges from 5.6 to 29 pm. The materials which can be encapsulated include glucose, EDTA, human DNA.

These liposomes have very high encapsulation efficiency (up to 89%).

INDUSTRIAL PRODUCTION OF LIPOSOMES

(1) Detergent Dialysis

A pilot plant under the trade name of LIPOPREPR II-CIS is available from Diachema, AG, Switzerland. The production capacity at higher lipid concentration (80 mg/ml) is 30 ml liposomes/minute. But when lipid concentration is 10-20 mg/ml 100 mg/ml then up to many litres of liposomes can be produced. In USA, LIPOPREPR is marketed by Dianorm-Geraete (Maierhofer, 1985).

(ii) Microfluidization

A method based on microfluidization / microemulsiftcation / homogenization was developed for the preparation of liposomes.

MICROFLUIDIZERR is available from Microfludics Corporation, Massachusetts, USA.

A plot plant based on this technology can produce about 20 gallon/minute of liposomes in 50-200 nm size range.

The encapsulation efficiency of such products is up to 75%.

(3) Proliposomes

In proliposomes, lipid and drug are coated onto a soluble carrier to form free-flowing granular material which on hydration forms an isotonic liposomal suspension. The proliposome approach may provide an opportunity for cost-effective large scale manufacture of liposomes containing particularly lipophilic drugs.

The trapped or captured volume is the volume enclosed by a given amount of lipid which is expressed in µL/µmol or µL/mg of the lipid.

Table 1
Internal volume and encapsulation efficiency of liposomes

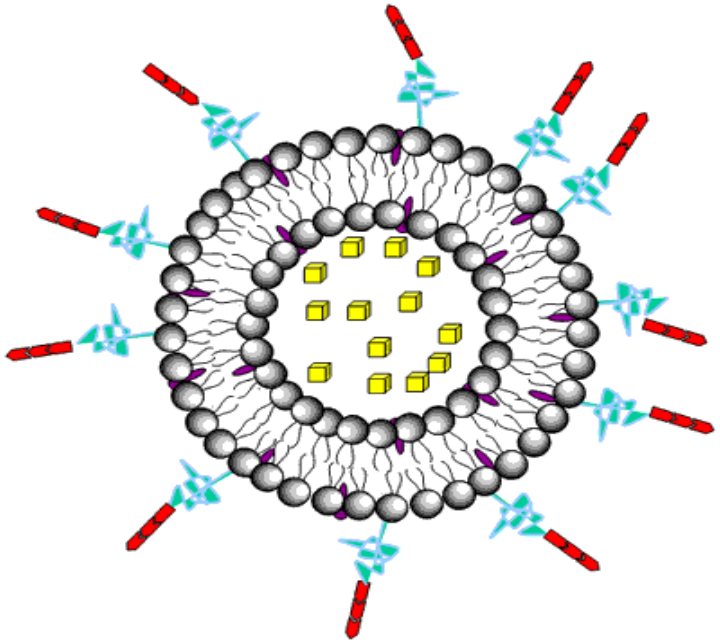
Liposome Type			Internal Volume (µL/ µmole lipid)	(µL/ mg lipid)	Efficiency
MLV	(i)	Hydration Method*	1-4	--	10-25
	(ii)	Solvent Spherule Method	0.3-2.7	0.5-4.0	0.3-1.6
SUV	(i)	Sonication Method	0.02-0.05	--	0.1-1.0
	(ii)	French Pressure Method	0.2-1.5	--	--
Multivesicular Liposome			10-79	15-127	11-89
Giant Liposomes			20	--	--
LUV	(i)	REV Method	--	0.5-15.6	35-65
	(ii)	Modified REV Method	--	--	< 80
	(iii)	Freeze Thaw Method	< 10	--	20-30
	(iv)	Microfluidization Method	0.69-1.03	--	5-75
	(v)	Extrusion through poly-carbonate filters under nitrogen	1.1-2.4	--	--

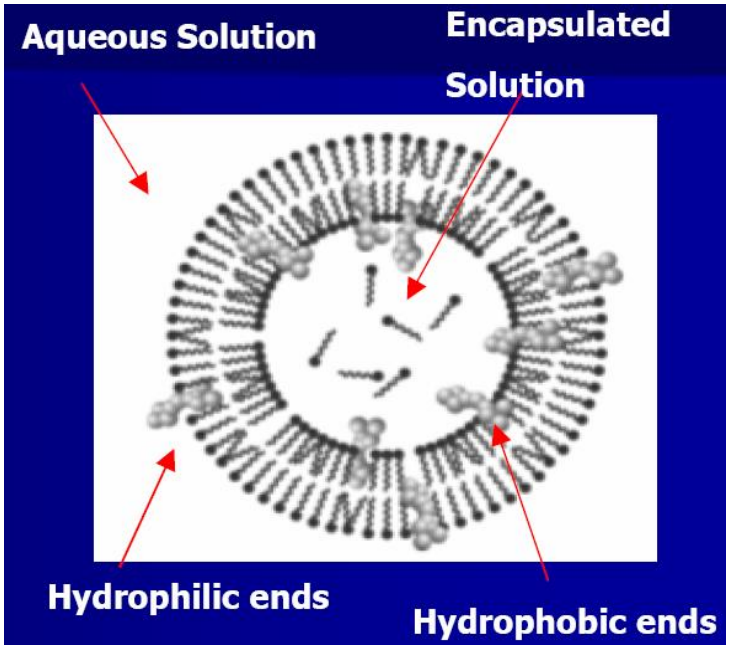
MLV = Multilamellar vesicles
SUV = Small unilamellar vesicles
LUV = Large unilamellar vesicles
REV = Reverse phase evaporation
*Hydration of lipids in the absence of an organic solvent.

NIOSOMES

Bilayers vesicles of non-ionic (amphiphilic) surfactants are called niosomes.

The success achieved with liposomal systems stimulated the search for other vesicle forming amphiphiles and Non-ionic surfactants were among the first alternative materials studied and a large number of surfactants have since been found to self assemble into closed bi-layer vesicles.





Niosomes

- Niosomes, non-ionic surfactant (Tweens & Spans) vesicles, are widely studied as an alternative to liposomes.
- These vesicles appear to be similar to liposomes in terms of their physical properties.
- They are also prepared in the same way and under a variety of conditions, from unilamellar or multilamellar structures.
- Niosomes alleviate the disadvantages associated with liposomes, such as chemical instability, variable purity of phospholipids and high cost.
- They have the potential for controlled and targeted drug delivery. Specially for cutaneous application of 5-alpha dihydrotestosterone, triamicinolone, acetonide, intravenous administration of methotrexate for cancer treatment and sodium stilbogluconate in the treatment of Leishmaniasis etc.
- Niosomes enhanced the penetration of drugs.

- The niosomal encapsulation of methotrexate and doxorubicin increases drug delivery to the tumour and tumoricidal activity as they are not accumulated in liver. Niosomes, possessing a triglycerol when loaded with 200 nm doxorubicin or muramic acid they were not taken up significantly by the liver and thus provided sustain plasma level of these drugs.
- Niosomes and other drugs uptake by the liver and spleen make, niosomes ideal for targeting diseases manifesting in these organs. One such condition is leishmaniasis and a number of studies have shown that niosomal formulations of sodium stibogluconate improve parasite suppression in the liver, spleen and bone marrow.

- Niosomes may also be used as depot systems for short acting peptide drugs on intramuscular administration.
- Niosomes as vaccine adjuvant: Niosomal antigens are potent stimulators of the cellular and humoral immune response. The formulation of antigens as a niosome in water-in-oil emulsion further increases the activity of antigens. The controlled release property of the emulsion formulation is responsible for enhancing the immunological response.
- Niosomes loaded with Vincristine showed that niosomes anticancer drugs were less toxic than free drugs while anticancer drug concentration enhanced significantly at tissue level.

Classification:

1. Small niosomes (SUV) (100 ηm – 200 ηm)
2. Large niosomes (MLV) (800 ηm – 900 ηm)
3. Big niosomes (LUV) (2 – 4 µm)

Method of Preparation:

1. Hydration
2. Reverse Phase Evaporation.

TRANS MEMBRANE PH GRADIENT UPTAKE PROCESS (REMOTE LOADING OR ACTIVE UP TAKE).

This process involve entrapment of drugs in pre-generated niosomes. The drug uptake induced by pH gradient across the lipid bilayers of niosomes.

ADVANTAGE:

- 100 % loading of cationic drugs efficiency.
- Extremely coat effective (decrease by 3 fold)
- Sensitive drug can be loaded even immediately before use.

SOLID NANOPARTICLES

DEFINITION

Nanoparticles are defined as particulate dispersions having a particle size of between 30 and 500 ηm.

Solid nanoparticles are differ from liposomes and niosomes as they are prepared from polymers and do not have an aqueous core but a solid polymer matrix.

Nanoparticles for Drug Delivery

- **Metal-based nanoparticles**
- **Lipid-based nanoparticles**
- **Polymer-based nanoparticles**
- **Biological nanoparticles**

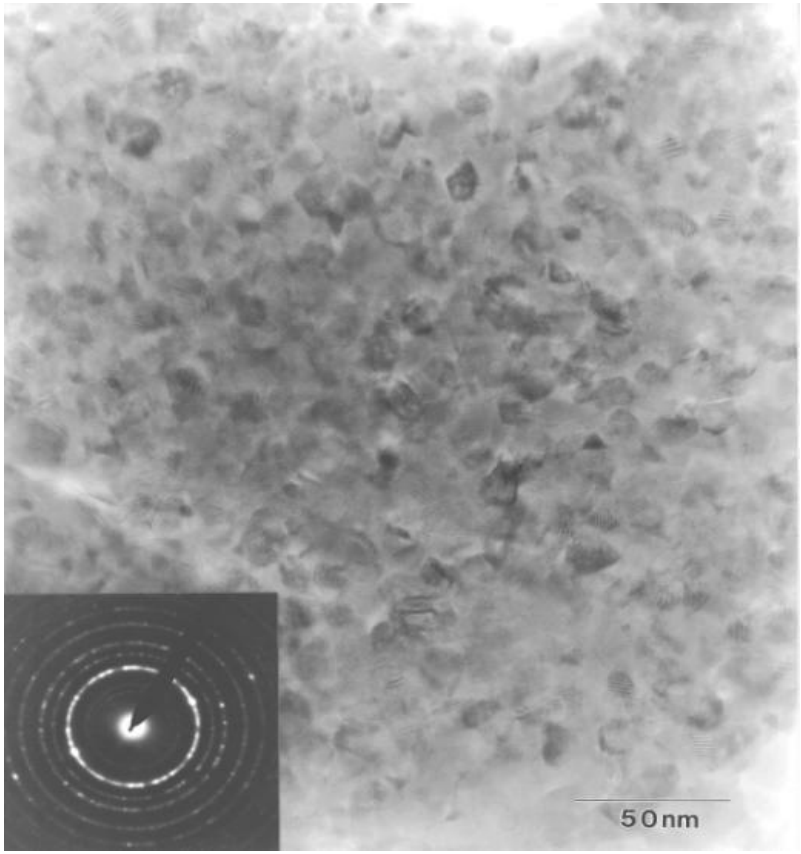
ACHIEVEMENTS

- Development of one dose a day ciprofloxacin using nanotechnology
- Tumor targeted taxol delivery using nanoparticles in Phase 2 clinical trial stage
- Improved ophthalmic delivery formulation using smart hydrogel nanoparticles
- Oral insulin formulation using nanoparticles carriers.
- Liposomal based Amphotericin B formulation

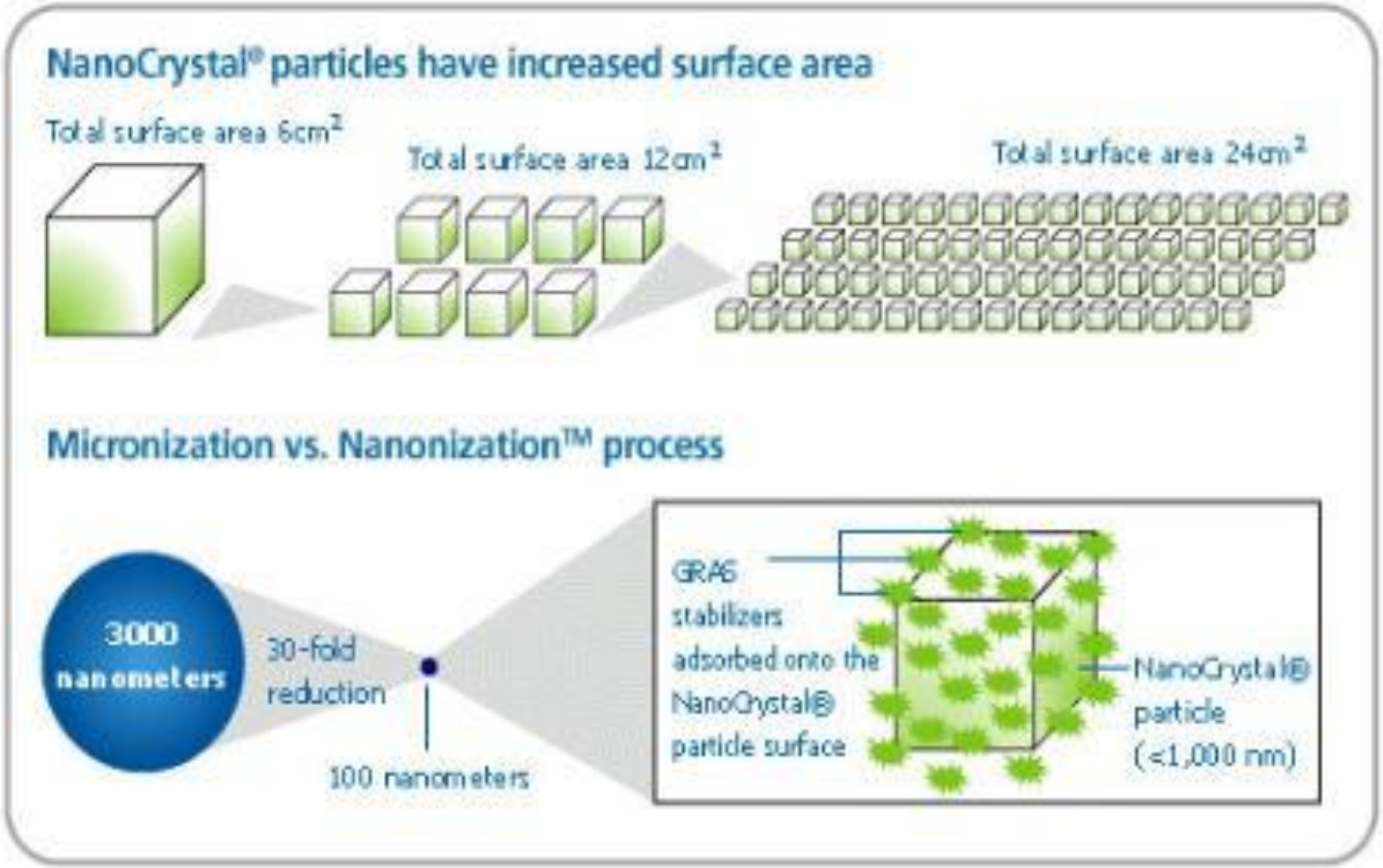
Nanopowder

- Nanopowders are powders composed of nanoparticles, having an average diameter below 50 nanometers (ηm).
- A jar of a true nanopowder when emptied from chest height to toward the floor will disperse into the air before reaching the floor.

Nanopowder



Nanocrystals



Nanocrystals

- When the size of the material is reduced to less than 100 nanometers, the realm of quantum physics takes over and materials begin to demonstrate entirely new properties.
- Nano-design of drugs by various techniques like milling, high pressure homogenization, controlled precipitation etc., are explored to produce, drug nanocrystals, nanoparticles, nanoprecipitates, nanosuspensions (which for ease of understanding commonly mentioned as nanocrystals).
- As decreased size will increase the solubility of drugs hence, this technology is explored to increase oral bioavailability of sparingly water soluble drugs.

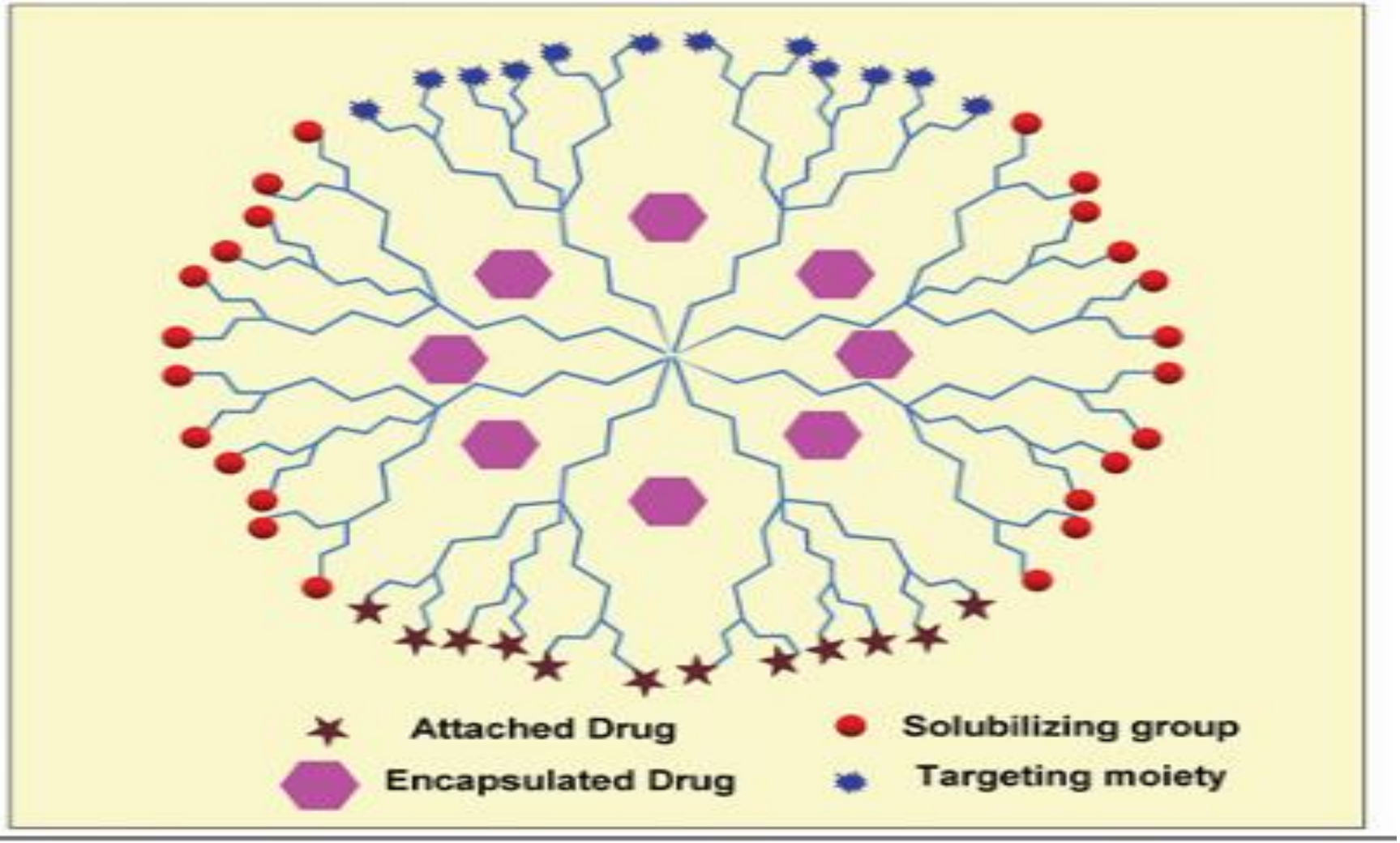
Dendrimers

These branched macromolecules are constructed around a simple core unit.

Dendrimers have a high degree of molecular uniformity, narrow molecular weight distribution, specific size and shape characteristics, and a highly-functionalized terminal surface.

The manufacturing process is a series of repetitive steps starting with a central initiator core. Each subsequent growth step represents a new "generation" of polymer with a larger molecular diameter, twice the number of reactive surface sites, and approximately double the molecular weight of the preceding generation.

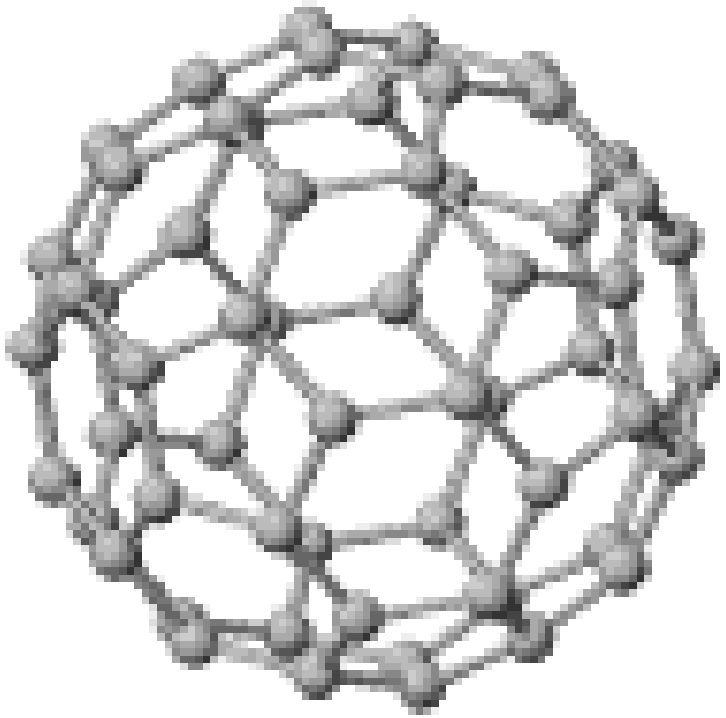
Dendrimers

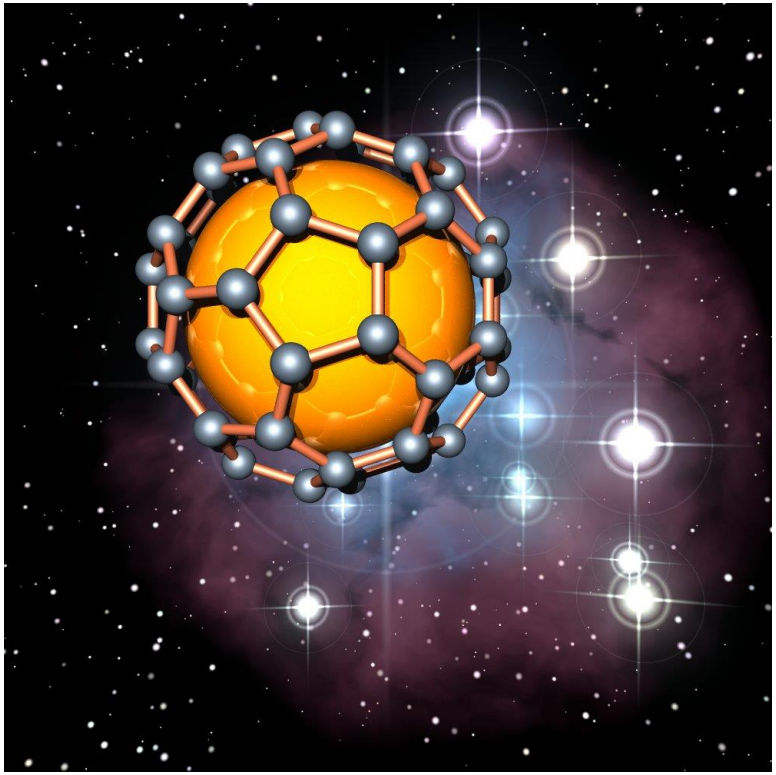

Attached Drug
Encapsulated Drug
Solubilizing group
Targeting moiety

Polymeric Nanoparticles

In recent years, biodegradable polymeric nanoparticles have attracted considerable attention as potential drug delivery devices in view of their applications in drug targeting to particular organs / tissues, as carriers of DNA in gene therapy, and in their ability to deliver proteins, peptides and genes through a per oral route of administration.

Carbon 60





Carbon 60

C60 are spherical molecules about 1nm in diameter, comprising 60 carbon atoms arranged as 20 hexagons and 12 pentagons: the configuration of a football.

Hence they find application as NanoPharmaceuticals with large drug payload in their cage like structure.

On the other hand with development of various chemical substitutes for C60, it is possible to develop functionalized C60 with better drug targeting properties

Carbon Nanotube

Carbon Nanotube

Carbon nanotubes are adept at entering the nuclei of cells and may one day be used to deliver drugs and vaccines.

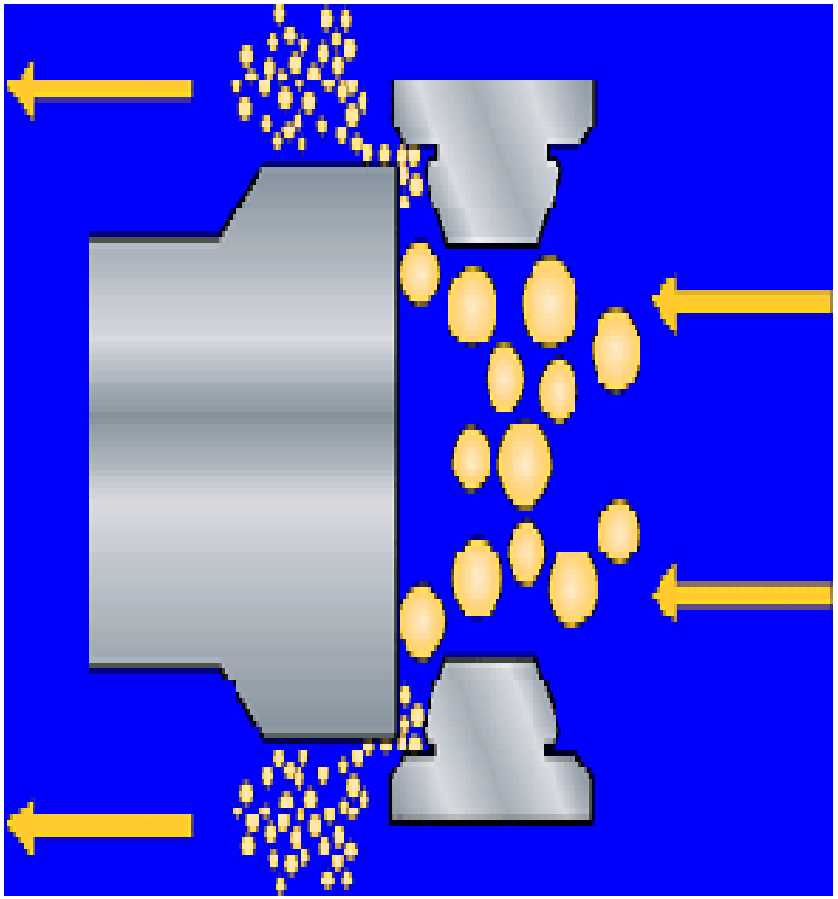
The modified nanotubes have so far only been used to ferry a small peptide into the nuclei of fibroblast cells.

But the researchers are hopeful that the technique may one day form the basis for new anti-cancer treatments, gene therapies and vaccines.

Equipments for Nanoparticles

1. Homogenizer
2. Ultra Sonicator
3. Mills
4. Spray Milling
5. Supercritical Fluid Technology
6. Electrospray
7. Ultracentrifugation
8. Nanofiltration

Homogenizer & Ultra Sonicator



Methods of Drug Delivery

- Oral Delivery

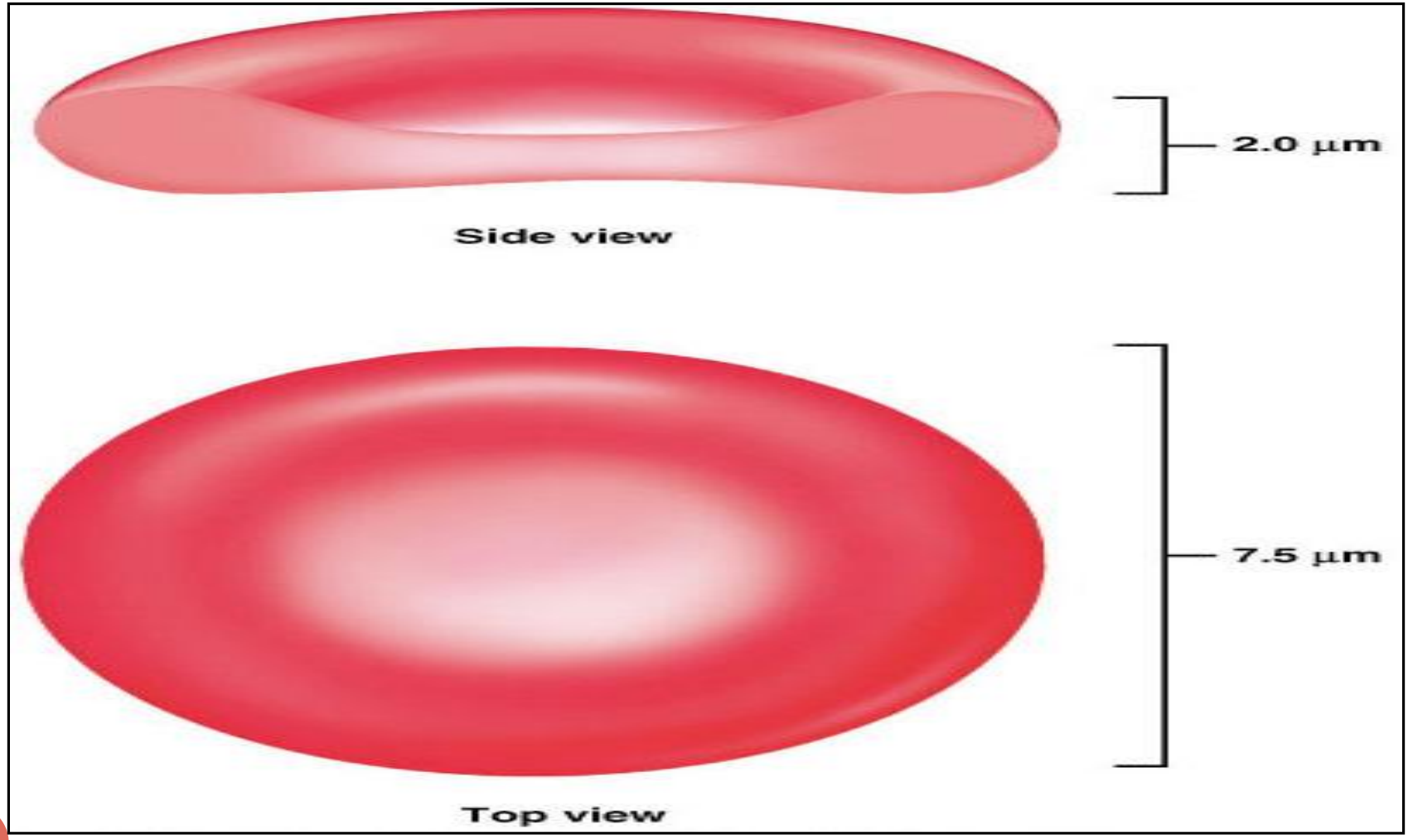
- Inhalation

- Transdermal

- Implantation

- Injection

Resealed Erythrocytes



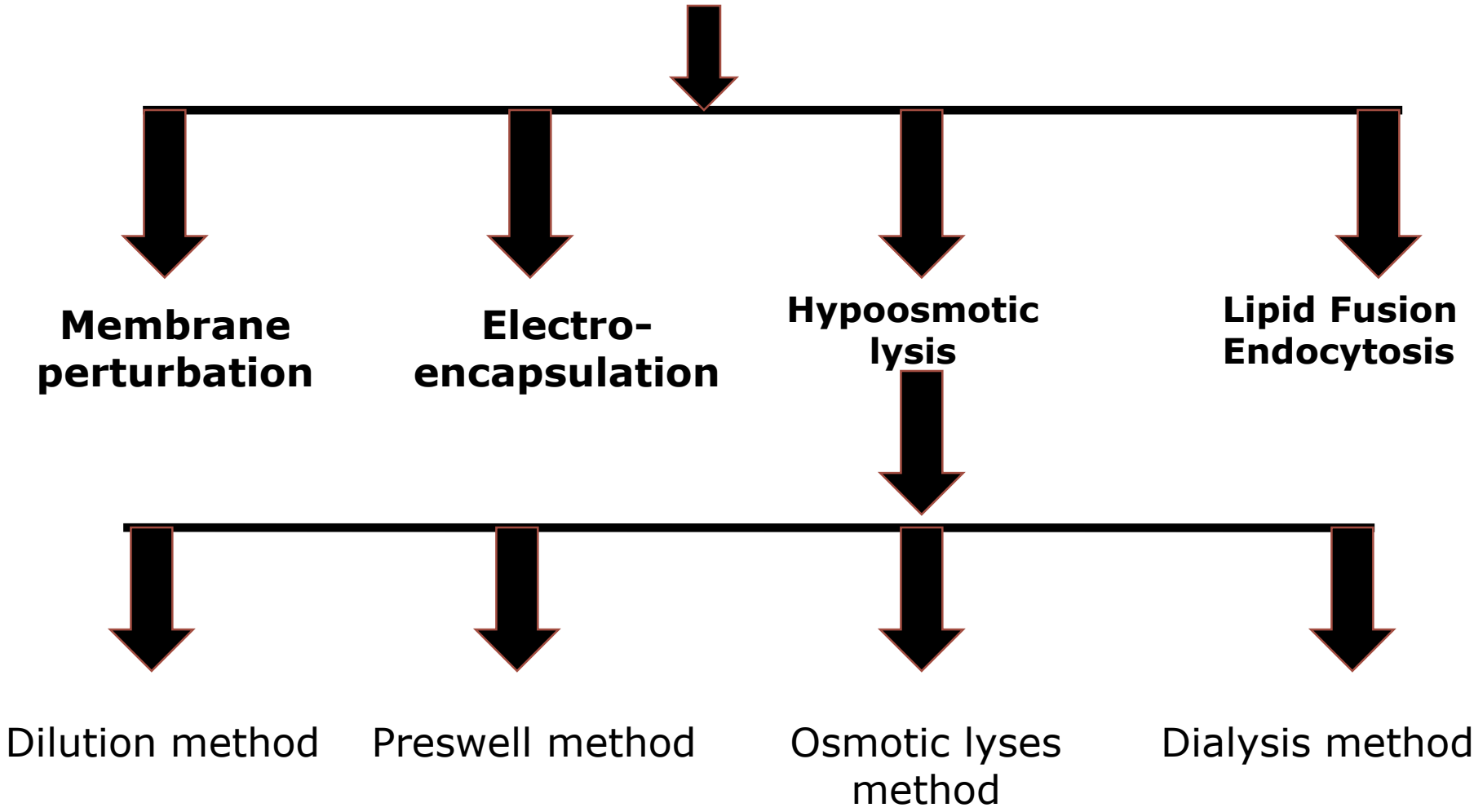
Advantages of erythrocyte as carrier:-

- ✓ Biodegradable
- ✓ Isolation is easy
- ✓ Non immunogenic
- ✓ large volume of drug can be encapsulated in small volume of erythrocytes
- ✓ Prolong systemic activity of drug
- ✓ Protection from premature degradation
- ✓ Prodrug concept (Bioreactor)
- ✓ Reduce Adverse Effect
- ✓ Peptide & Enzyme Delivery

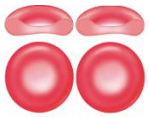
Disadvantages :-

- ❖ Possibility of Leakage of cells & dose dumping
- ❖ Molecule Alter Physiology Of cell

Methods Of Drug Loading



Dilutional Haemolysis



0.4% NaCl

Hypotonic

Membrane ruptured RBC

Drug

Loading buffer

Loaded RBC

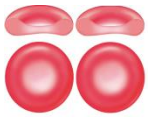
Incubation at 25^{0}c

Resealing buffer

Resealed Loaded RBC

Efficiency → 1-8%

Isotonic Osmotic Lysis



Physical rupturing

Chemical rupturing

Isotonically ruptured RBC

Drug

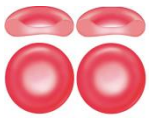
Isotonic Buffer

Loaded RBC

Incubation at
25^0 C

Resealed RBC

Preswell Dilutional Haemolysis



0.6% w/v NaCl

Swelled RBC

5 min incubation at 0°C

Drug + Loading Buffer

Loaded RBC

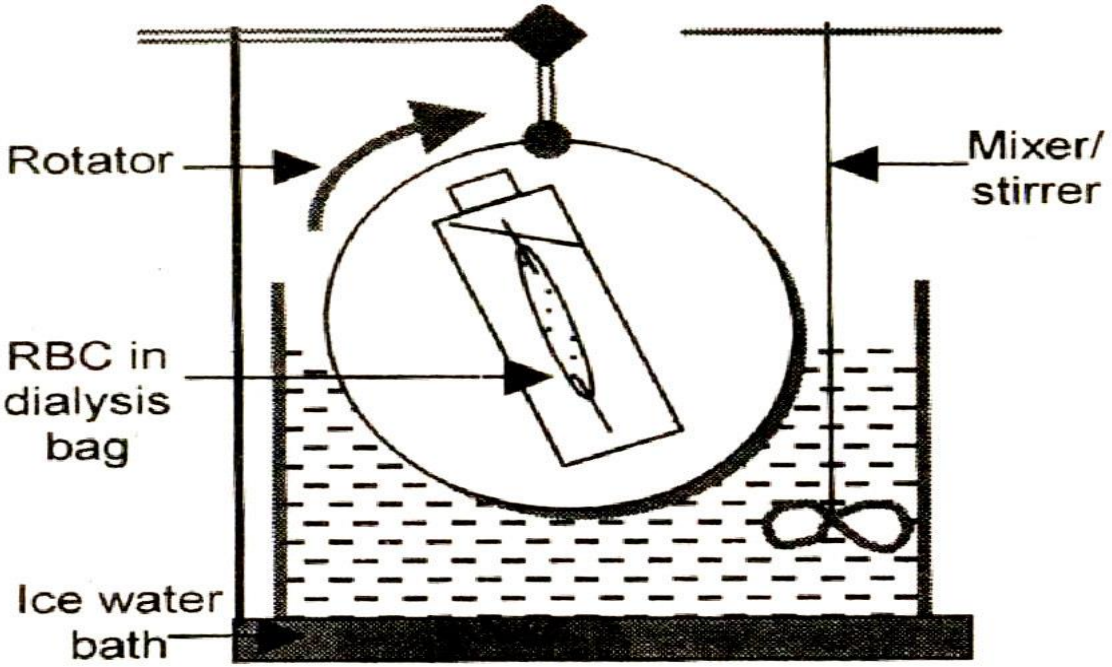
5 min incubation at 25°C

Resealing Buffer

Loaded RBC

Efficiency → 72%

Dialysis



80 %
Haematocrit
value

RBC
+
Phosphate
buffer

Placed in dialysis bag with air bubble

Dialysis bag placed in 200ml of lysis buffer with mechanical rotator 2hrs. 4c.

Loading buffer

Drug

Loaded RBC

Dialysis bag placed in Resealing buffer with mechanical rotator 30 min 37c.

Resealed RBC

Efficiency → 30-45%

Electro-insertion or Electro-encapsulation

RBC + Pulsation medium

2.2 Kv Current for 20 micro sec

At 25^0 C

Drug + Loading suspension

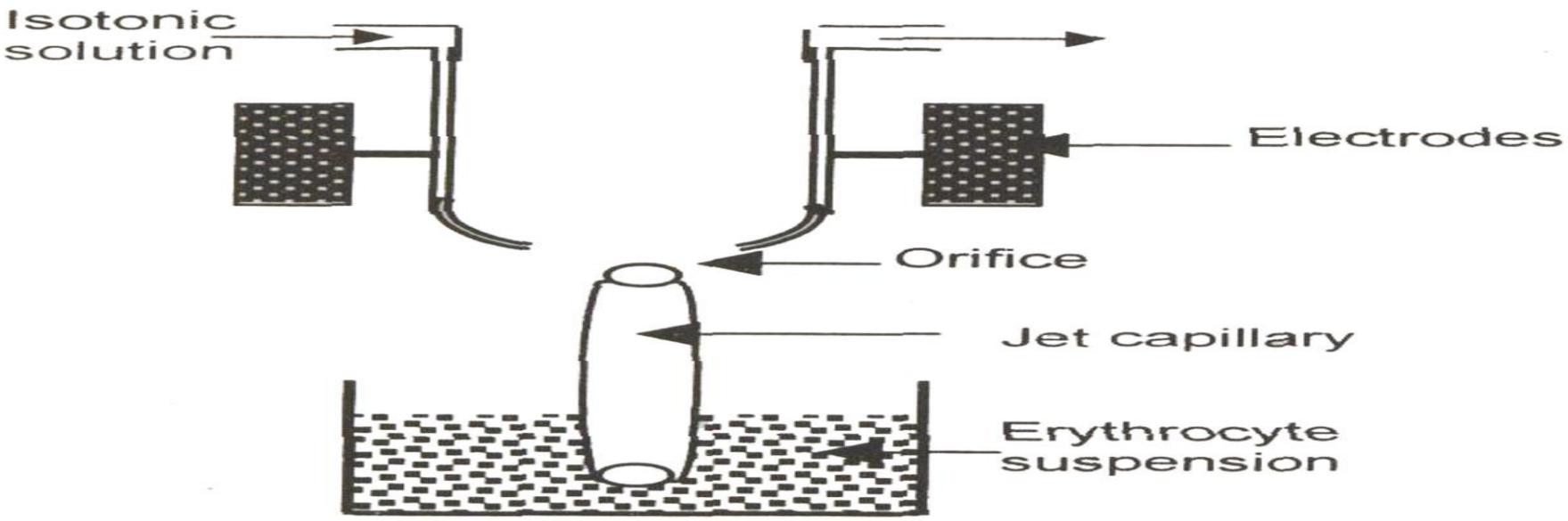
3.7 Kv Current for 20 micro sec

Isotonic NaCl

Loaded RBC

Resealing Buffer

Resealed RBC



Entrapment By Endocytosis:-

RBC

+

Drug Suspension

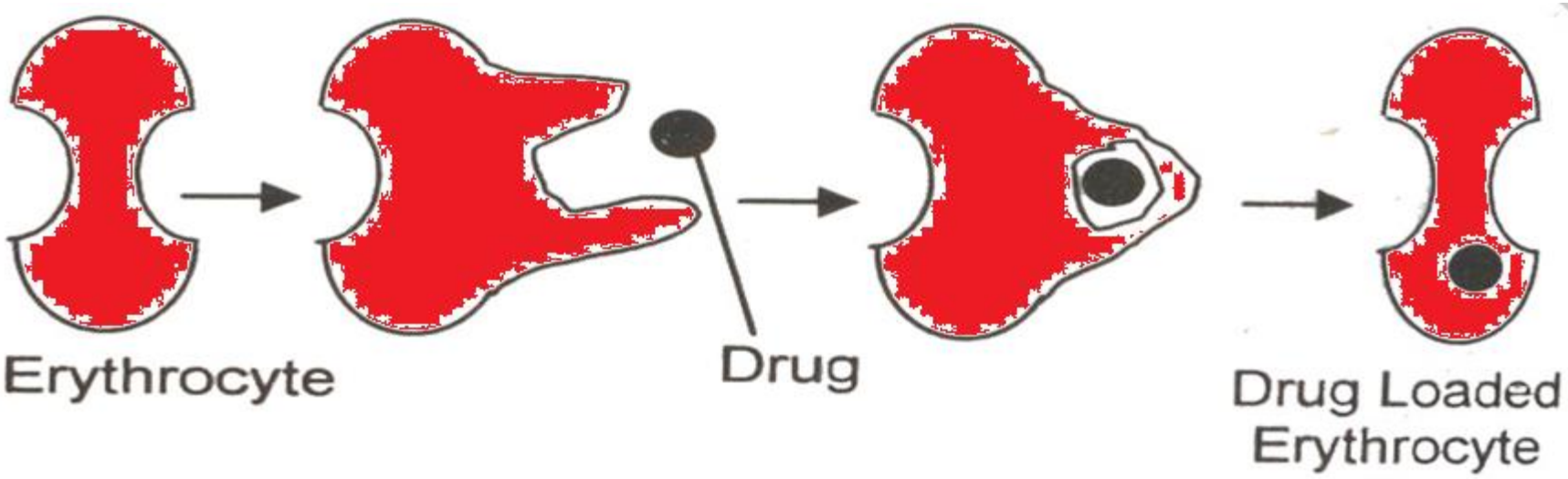
Buffer containing ATP, $MgCl_2$, and $CaCl_2$

At 25^0 C

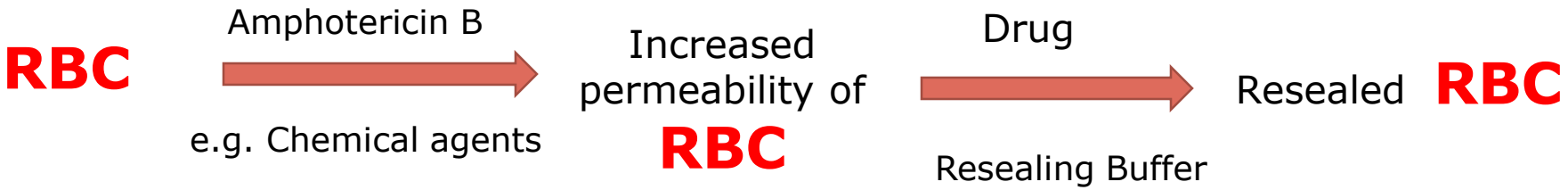
Loaded RBC

Resealing Buffer

Resealed RBC



Membrane Perturbation Method



In vitro Characterization

Parameter	Method/instrument used
I. Physical characterization	
Shape and surface morphology	Transmission electron microscopy, scanning electron microscopy, phase contrast microscopy, optical microscopy.
Vesicle size and size distribution	Transmission electron microscopy, optical microscopy.
Drug release	Diffusion cell, dialysis
Drug content	Deproteinization of cell membrane followed by assay of resealed drug, radiolabelling
Surface electrical potential	Zeta potential measurement
Surface pH	pH-sensitive probes
Deformability	Capillary method

In vitro Characterization

II. Cellular characterization	
% Hb content	Deproteinization of cell membrane followed by hemoglobin assay
Cell volume	Laser light scattering
% Cell recovery	Neubaur's chamber, hematological analyzer
Osmotic fragility	Stepwise incubation with isotonic to hypotonic saline solutions and determination of drug and hemoglobin assay
Osmotic shock	Dilution with distilled water and estimation of drug and hemoglobin
Turbulent shock	Passage of cell suspension through 30-gauge hypodermic needle at 10 mL/min flow rate and estimation of residual drug and hemoglobin, vigorous shaking followed by hemoglobin estimation
Erythrocyte sedimentation rate	ESR methods
III. Biological characterization	
Sterility	Sterility test
Pyrogenicity	Rabbit method, LAL test
Animal toxicity	Toxicity tests

Applications of resealed erythrocytes

- Erythrocytes as carrier for enzymes
- Erythrocytes as carrier for drugs
- Erythrocytes for drug targeting
  Drug targeting to reticuloendothelial system
- Drug targeting to liver
  - Treatment of liver tumors
  - Treatment of parasitic diseases
  - Removal of RES iron overload
  - Removal of toxic agents

Applications of Resealed Erythrocytes

- Delivery of antiviral agents
- Oxygen deficiency therapy
- Microinjection of macromolecules

Novel systems

Nanoerythrosomes

Erythrosomes