Nanoparticulate drug delivery systems

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Program: « nanoparticles in medicine »

Introduction to drug delivery systems (DDS)

Formulation processes

Nanoparticulate DDS properties for pharmaceutical and medical applications

Cyclodextrins

Applications to:
Vaccines

Cancer
Gene therapy
Diabetes

Introduction to Nanoparticles
As Drug Delivery Systems

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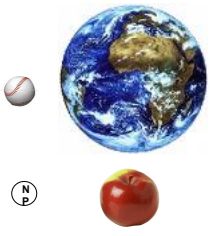
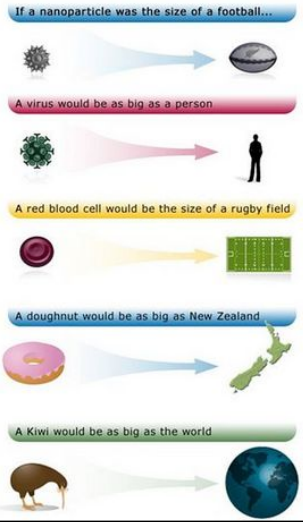
I. Nano

'Nano' derives from the Greek word "nanos", which means dwarf or extremely small. It can be used as a prefix for any unit. A nanometer is a billionth of a meter or 10^{-9} m

Nanometer-length scale

If a baseball is the size of Earth, a nanoparticle would be the size of an apple.





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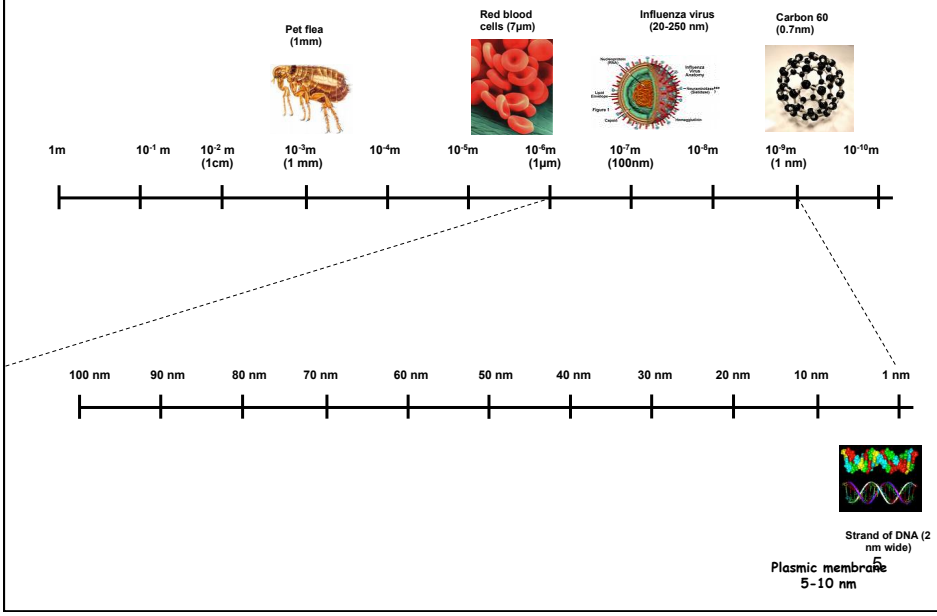

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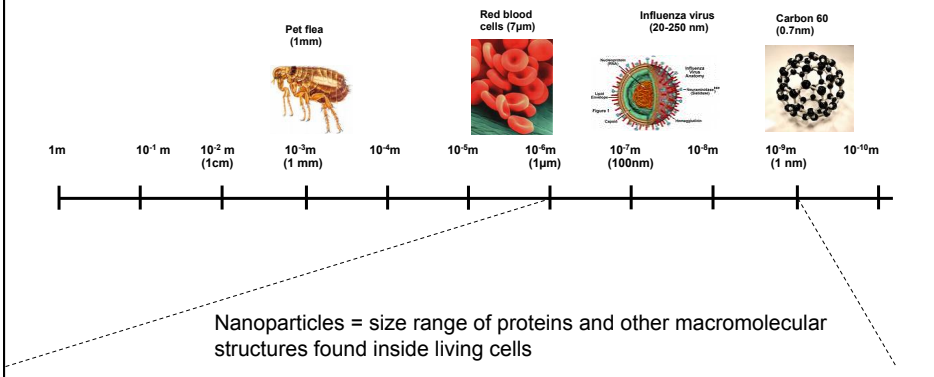

Nanoparticles = size range of proteins and other macromolecular structures found inside living cells

⇒ Nanoparticles are able to interact with existing cellular machinery to facilitate the drug delivery to the cells
endocytosis, targetting….

Drug delivery field also uses submicron particles (100nm to 1µm) called nanoparticles in the litterature !

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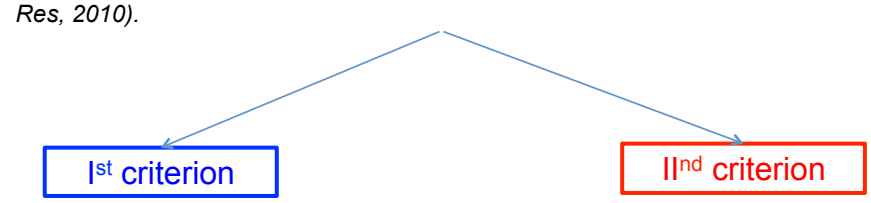
Definitions

Nanoparticle : an intentionally produced particle that has at least one dimension in the nanoscale range (1-100 nm) (National Institute of Health).

Nanopharmaceuticals = pharmaceuticals **engineered on the nanoscale**. Pharmaceuticals where the nanomaterial plays the **pivotal therapeutic role** or adds **additional functionality** to the previous compound *(Rivera et al, Pharmacol Res, 2010).*



Nanoengineering plays a major role in the manufacturing process

Nanomaterial either essential for the therapeutic activity or adds new functionality to the original molecule

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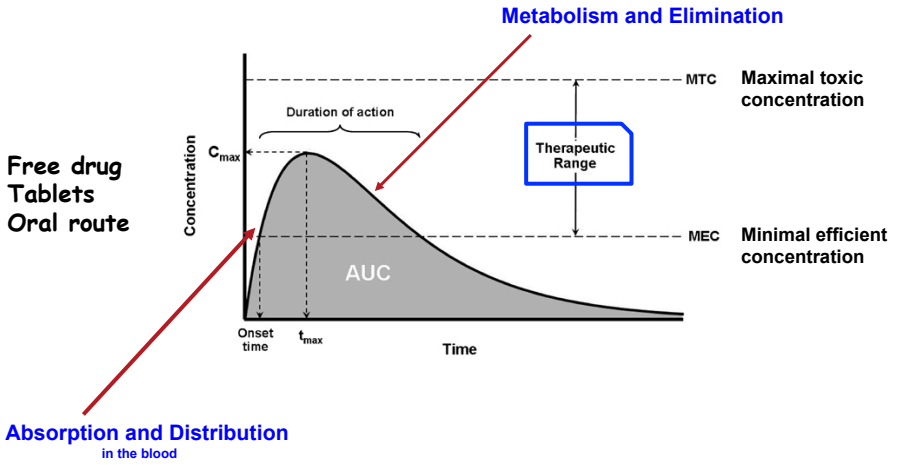
Weissig et al, Int J Nanomedicine, 2014

II. Free drug: pharmacokinetic



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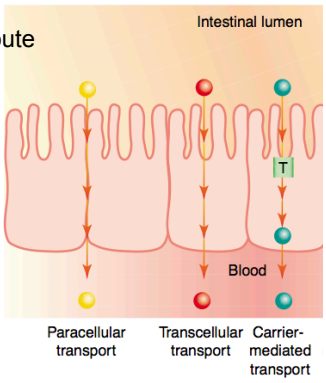
Overview of ADME

Most drugs :

enter the body (by mouth or injection…) - must cross barriers to entry (skin, gut wall, alveolar membrane…..)

Ex: oral route



ABSORPTION

Amphiphilic molecules are better absorbed.

Clarck design, Baltimore MD, USA
A. Li, DDT, 2001

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Overview ADME

Most drugs :

are **distributed by the blood to the site of action** - intra- or extra- cellular - cross barriers to distribution (capillaries, cell wall….) - distribution affects concentration at site of action and sites of excretion and biotransformation

DISTRIBUTION

are **biotransformed** to one or several different compounds by enzymes evolved to cope with natural materials - this may increase, decrease or change drug actions

METABOLISM

are **excreted** (by kidney…….) which removes them and/or their metabolites from the body

EXCRETION

→ **Steps based on drug properties**
A lot of barriers

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III. Barriers to free drug delivery

Physicochemical barriers (properties)

Molecular weight
Solubility
Partition coefficient
pKa
Dissolution rate
Salt formation
Prodrugs
Particle size, surface area and shape
Crystallinity, polymorphism
Stereochemical factors
Drug stability (in GIT)
…

Biological barriers

Biodegradation by digestive enzymes
Short *in vivo* half-life
Immunogenicity
Difficulty in crossing mucosal barriers
No access to some compartments

⇒ Barriers impair drug efficiency

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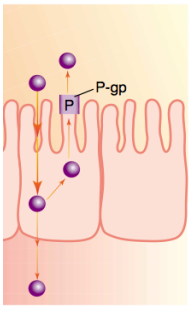
Examples

	Degradation	Membranes
Oral administration of proteins (insulin)	Enzymatic degradation pH	Mucosal barriers (hydrophilic drug)
Poorly soluble drugs		Oral route : no molecular state Parenteral (injection) route : embolism
Central nervous system		Tight junctions of BBB Pglycoproteins (efflux)



P-gp mediated efflux

Drug DiscoveryToday

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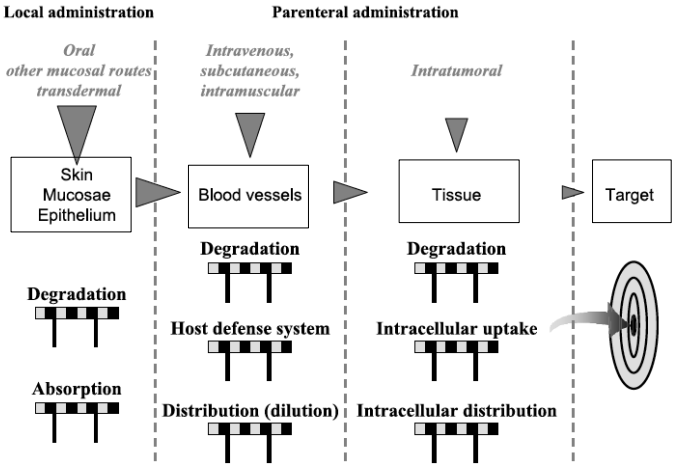
Barriers



From Couvreur P. and Vauthier, C., 2006, Pharm. Res.

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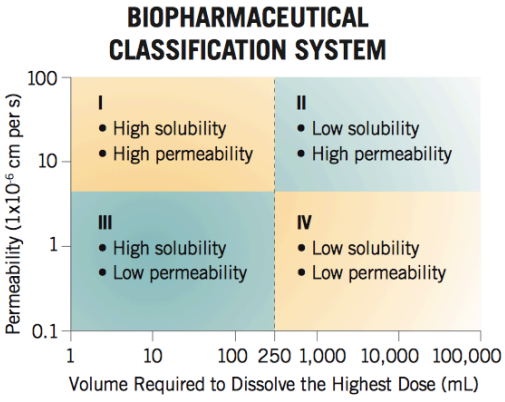
Biopharmaceutical Classification System (BCS)



Developped for solid dosage forms

Solubility in aqueous media and permeability of cellular layer such as Caco2

No problem with class I

But recent molecules mainly belong to class IV with low solubility and low permeability

Need for enhanced formulation technique (compared to oral forms)

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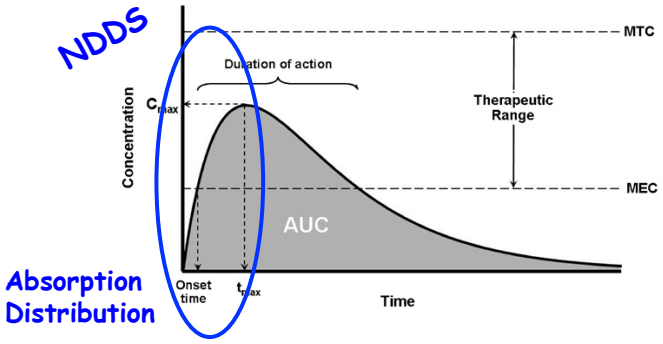
IV. Drug Delivery Systems (DDS)

PURPOSE

These systems are exploited for therapeutic purpose to carry the drug in the body in a controlled manner from the site of administration to the therapeutic target (P. Couvreur, 2006)



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Definition

Aim : To make the distribution of active drug independant of its own physicochemical properties.

pKa
size, molecular weight
solubility…

The fate of the active drug depends on the delivery system.

« Controlled Drug Delivery System (DDS) »

Any pharmaceutical formulation is « drug delivery system » (creams, eye drops, tablets….)

However, most would be considered to be « conventional » as no specific new technology needs to be used in their preparation or use.

More specialized systems to overcome delivery problems

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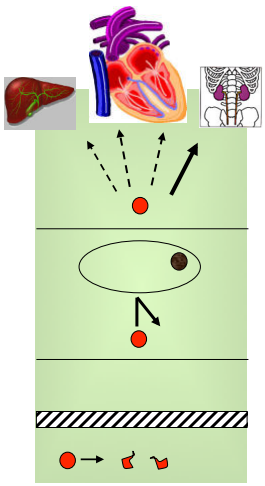
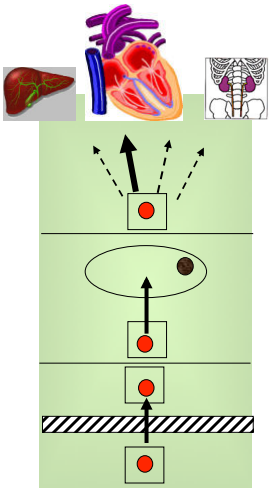
V. Advantages of drug delivery systems



- ✓ Targeting/Specificity
- ✓ Membrane crossing
- ✓ Drug protection
- ✓ Decrease toxicity



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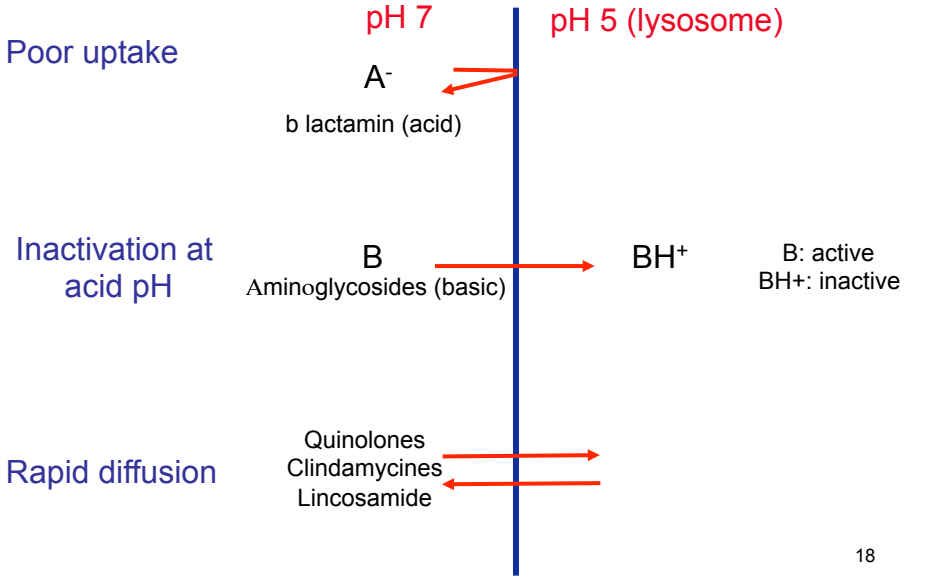
Example of application: Infectious diseases



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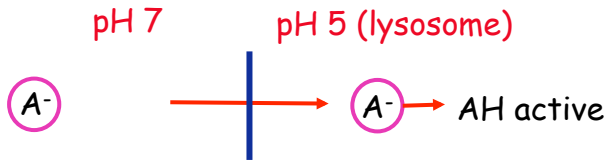
Adapted from Couvreur and Vauthier, Pharm.Res, 2006

Interest of nanodevices



Increases the intracellular uptake (through endocytosis)

Allows the drug to diffuse freely into the cell (i.e., by lysosomal fusion mechanisms)

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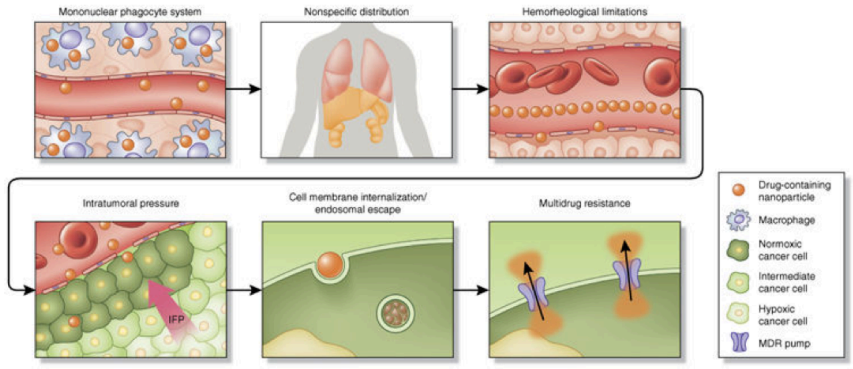
VII. Obstacles to overcome for drug delivery



Opzonization and subsequent uptake by macrophages: accumulation in spleen, liver
Nonspecific distribution of nanotherapeutics to healthy organs
Size and geometry contributes to rheological limitations in blood. Cell free layer
Intratumoral pressure
Cellular internalization and endosomal affect route of internalization and intracellular fate
Drug efflux pumps confer therapy resistance to the cell

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Blanco et al, Nature biotechnology, 2015

Therapeutic challenges of drug delivery systems

Protection against degradation
Improved membrane absorption
Controlled and sustained release
Controlling biodistribution
Improving intracellular penetration
Improving the bioavailability

⇒ Increased efficacy
Reduced toxicity

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Rational for developping controlled release of drugs

Increased patient compliance
- less frequent dosing
- more « acceptable » (eg, needle-less)

Safety
- can control PK to remain within therapeutic index « window »
- decrease side effects

Improved therapy
- can time release
- environmentally-responsive systems

Decreased cost
- lower doses: more efficient use of drug

Greater profits/Commercial
- patent extension for drug
- the marketing edge
- controlled release feature more profitable

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Which drugs?

Highly toxic compounds for healthy tissues

Poorly soluble in both aqueous and organic media: new drugs from biotechnology

Rapidly degraded: peptides, proteins, nucleic acids

Rapidly metabolised

Hardly cross biological barriers

Need to reach a target (tissue or cell): nucleic acids

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To design a DDS it is important to know

Which drug (physicochemical properties)?
How much drug is needed?
At what delivery rate?
Over what period of time (duration)?
With what bioavailability?
Acting at which sites or on which cells?

Innovation approach

- rapidly,
- with minimal expenditure of corporate resources, and
- with a system design (drug delivery product) that makes minimal compromises from the ideal system (IDDS).

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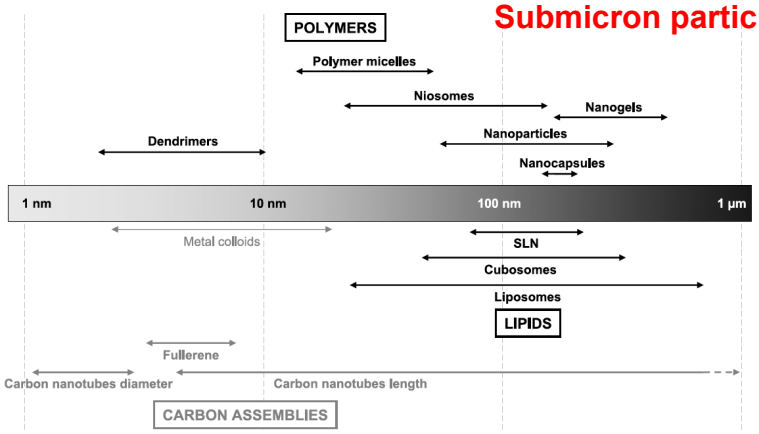
VIII. Classification



Submicron particles

Nanoparticles

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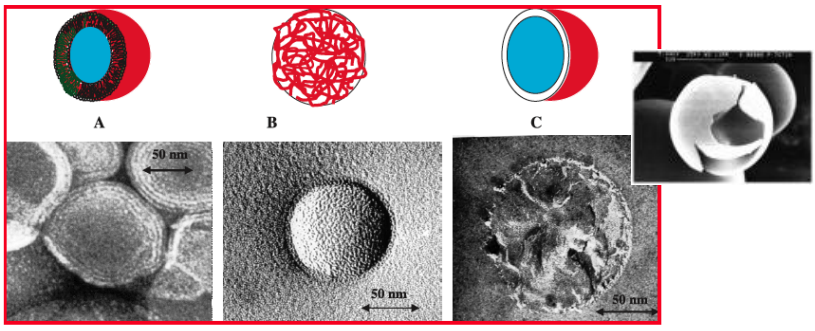
From Couvreur P. and Vauthier, C., 2006, Pharm. Res.

Classification: Structure

Capsules / Spheres
Reservoir / Matrix



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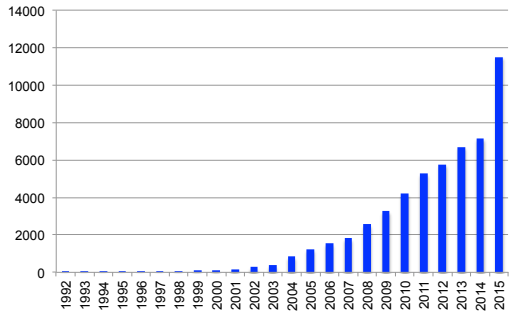
Andrieux et al., l' actualité chimique, 2003

SCIENTIFIC PUBLICATIONS on nanoparticles

Number of publications



Nanoparticles in the title
PubMed (september, 2016)

However, success rate is very low (number of clinical products)
The path for FDA approval for nanomedicines is long and risky.
43 approved nanopharmaceuticals on the market
Only four products have been approved after 2010…

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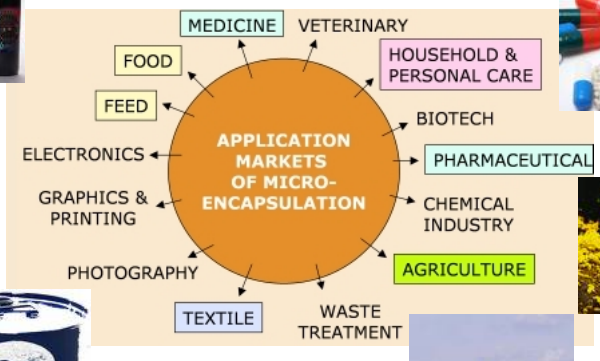
Weissig et al, Int J Nanomedicine, 2014

Application markets



http://w /presentation/amitmgupta31-147397-micro-encapsulation-amit-gupta-acp-lecture-wardha-education-ppt-powerpoint/

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FORMULATION PROCESSES

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Composition in brief

Polymers

- natural product: gelatin, albumin, polysaccharides, collagen, cellulose
- synthetic product: acrylic, cyanoacrylic, PLA, PLAGA

Lipids

- phospholipids: liposomes
- solid lipids: triacylglycerol, waxes, and paraffins **: SLN**

Metal

2

I

POLYMERIC PARTICLES

3

Polymeric particles

Poly(urethanes) for elasticity.
Poly(siloxanes) or silicones for insulating ability.
Poly(methyl methacrylate) for physical strength and transparency.
Poly(vinyl alcohol) for hydrophilicity and strength.
Poly(ethylene) for toughness and lack of swelling.
Poly(vinyl pyrrolidone) for suspension capabilities.

Physical properties

Poly(2-hydroxy ethyl methacrylate).
Poly(acrylic acid).
Polyacrylamide.
Poly(ethylene-co-vinyl acetate).
Poly(ethylene glycol).
Poly(methacrylic acid).

Chemically inert
Free of leachable impurities
Minimal undesired aging
Readily processing

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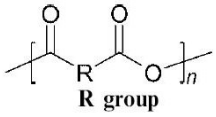
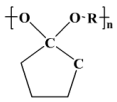
Polymeric particles

Absorbable suture

- Polylactides (PLA)
- Polyglycolides (PGA)
- Poly(lactide-co-glycolides) (PLGA)
- Polyanhydrides
- Polyorthoesters



Biodegradable polymers



Metabolic pathways

Biologically acceptable molecules

No need to remove
No adverse reactions

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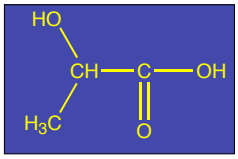
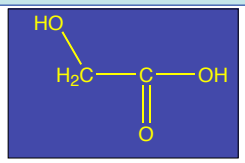
Lisa Branon-Peppas, 2007

Polymeric particles

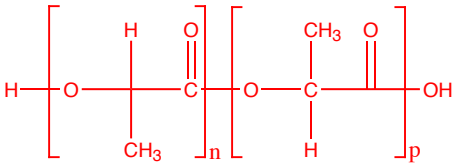


Lactic acid

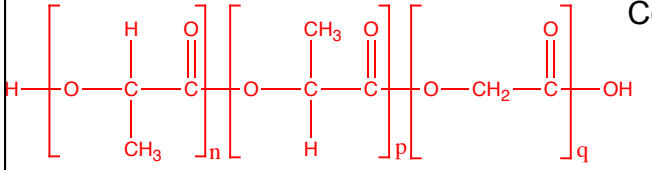


Glycolic acid

Intensive use in DDS



Lactic acid homopolymer (PLA)



Copolymer (PLGA)

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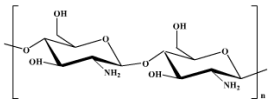
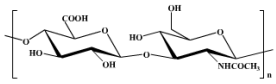
Polymeric particles

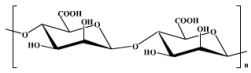
Natural polymers



Chitosan



Hyaluronic acid



Alginic acid

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PREPARATION OF POLYMERIC NANOPARTICLES

* Anionic polymerization
* Emulsion polymerization
* Interfacial polymerization
§ Solvent evaporation
§ Solvent deposition
§ Supercritical fluids

* From monomers
§ From preformed polymers

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SOLVENT EVAPORATION

Preformed polymer and the drug dissolved in a volatile, water-immiscible organic solvent (dichloromethan…)

The organic phase is then added to the aqueous phase under stirring (containing surfactants i.e. PVA…)

Homogenization, sometimes sonication

The organic solvent is removed by heating and/or under reduced pressure

The polymer precipitates

Formation of micro-or nanospheres instantaneously, containing the drug

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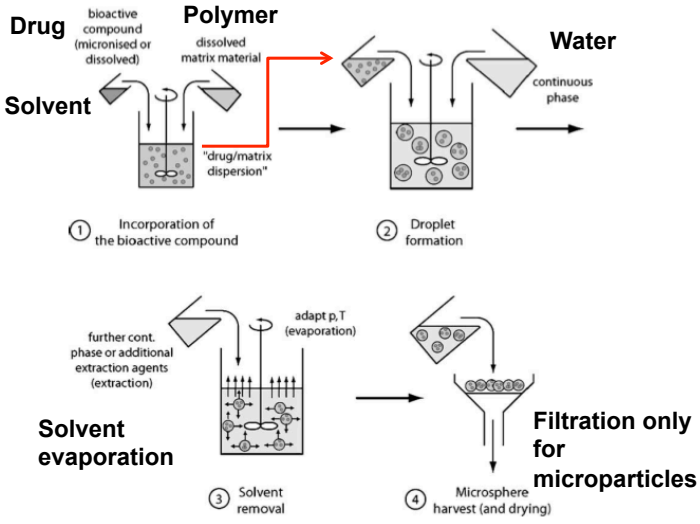
SOLVENT EVAPORATION



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Freitas et al, JCR, 2005

SOLVENT EVAPORATION

Well-established

Frequently used

Ex : poly(lactic acid)nanoparticles and poly(lactic-coglycolic acid) nanoparticles

Microparticles as well

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SOLVENT DEPOSITION = « précipitation » (Fessi, 1988)

The polymer (PLA) and surfactant(s) are dissolved in a **volatile** organic solvent such as acetone, **miscible** with water

Active drug suspended in the organic solvent

The reaction mixture is poured into the water phase, which contains surfactant under moderate stirring conditions

Nanocapsules are formed instantaneously

The organic solvent is then removed under reduced pressure

Partial removal of water also occurs

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INTERFACIAL POLYCONDENSATION/POLYADDITION

Chemical reaction at liquid/liquid interface

Polycondensation: growth of polymer by chemical reaction between functional groups of monomers

This reaction could eliminate (polycondensation) or not (polyaddition) a small molecule
Capsule formation occurs because monomers or oligomers react at an interface to grow a capsule wall membrane

Basic feature : formation of an emulsion

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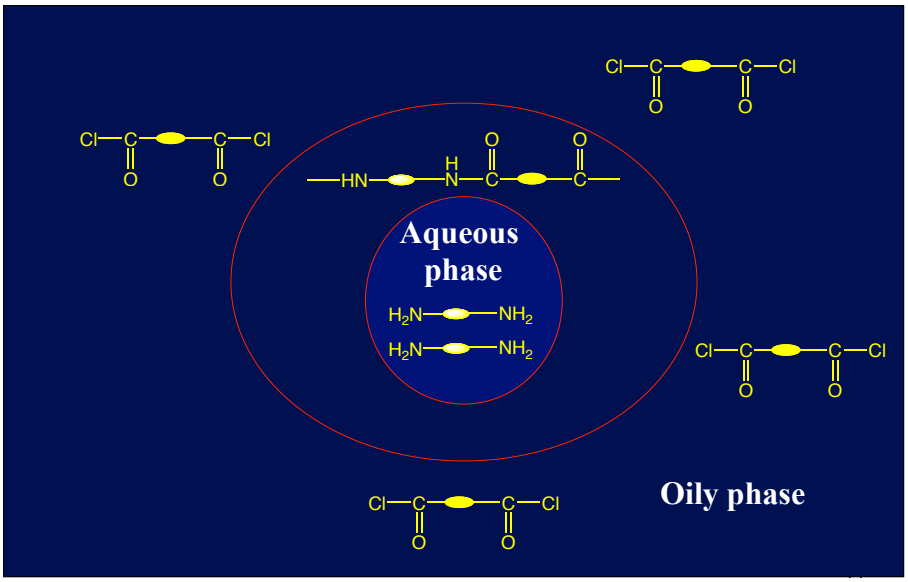
INTERFACIAL CONDENSATION



COMBINATIONS OF MONOMERS

Method for nano and microparticles

Two reactants, each one dissolved in a mutually immiscible liquid, diffuse to the interface between the two liquids where they react to form the capsule wall

Various polymers as a function of the monomers

Diamine + dichloroformiate : polyurethane
Dialcool + diacid chloride : polyester
Diamine + diisocyanate : polyurea
Diamine + diacid chloride : polyamide

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SUPERCRITICAL FLUID

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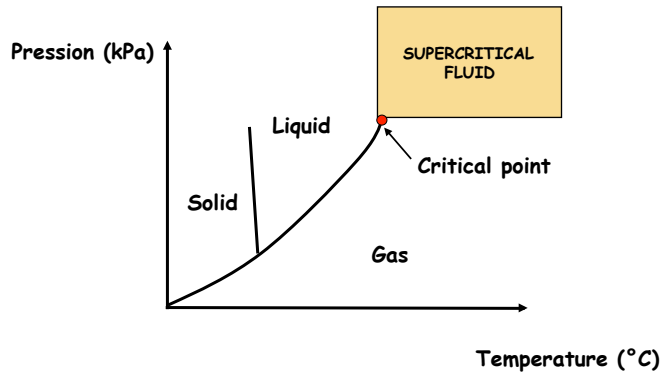
SUPERCRITICAL FLUIDS

Phase diagram

Liquid and gas together



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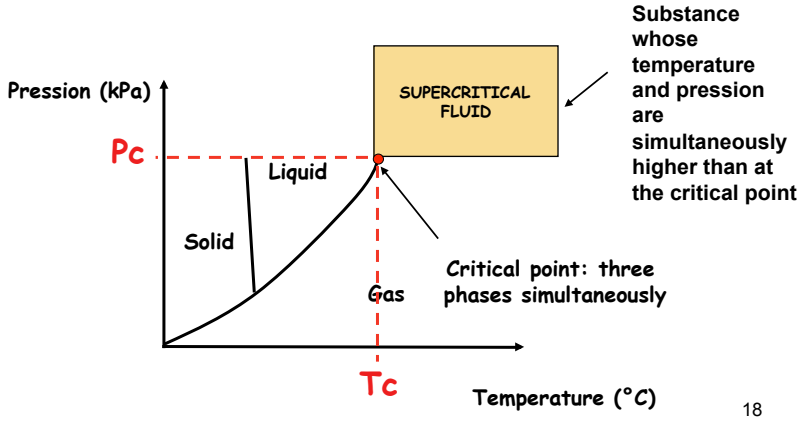
DEFINITION

Critical temperature : the temperature above which the substance can no longer exists as a liquid no matter how much pressure is applied

Critical pressure : the pressure above which the substance can no longer exists as a gas no matter how much temperature is applied



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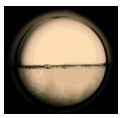
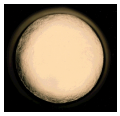
EXAMPLE OF SUPERCRITICAL CO_2



Phase separation between liquid and gas
Visible meniscus

Temperature and pression increasing,
progressive disapearance of the meniscus.
Closed densities



More than critical temperature and pression.
Complete disapearance of the meniscus
Phase boundary disappeared
One **homogeneous phase** : critical fluid

Properties?

http://advtechconsultants.com/SupercriticalFluids.htm

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PROPERTIES OF SUPERCRITICAL FLUIDS

Liquid have solubilizing nature
Gases have diffusivity and compressibility / Expandable

	Density (kg/m^3)	Viscosity (cP)	Diffusivity (mm^2/s)
Gas	1	0,01	1-10
Supercritical fluid	**100-800**	**0,05-0,1**	**0,01-0,1**
Liquid	1000	0,5-1,0	0,001

SCFs :

Liquid-like density and solubilizing capacity
Gas-like viscosity, compressibility and diffusivity

Substituant of organic solvents.

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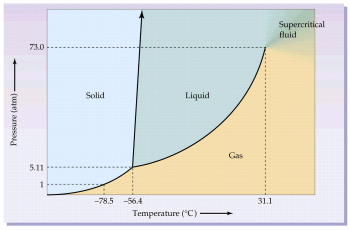
WHY CO_2 ?



- Critical temperature of 31.1°C
- Critical pression of 7.4 Mpa
- Low toxicity
- Non flammable
- Low reactivity
- High quantity
- Inexpensive
- GRAS status (generally regarded as safe)
- Approuved by FDA for use in food and pharmaceutical operations

Supercritical CO_2 is a non-polar **solvent** with dissolution properties that are comparable to **hexane**

But not a universal solvent…

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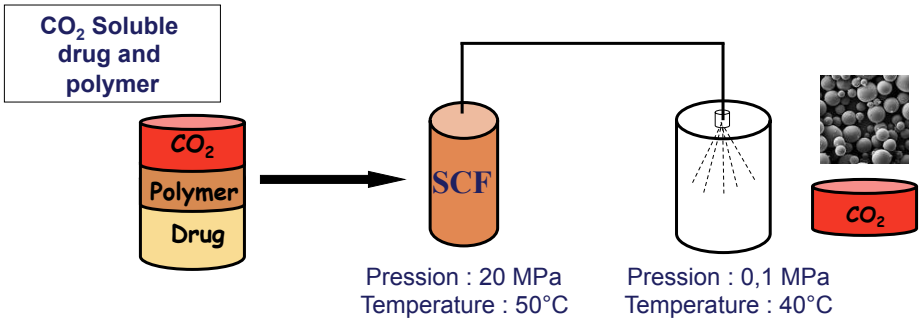
APPLICATIONS TO MICROENCAPSULATION

Principle : Solubility modification with the pression : solidification

Polymer or polymer and drug dissolution in the fluid
Decrease of pression allowing the fluid to go through a nozzle of pulverisation.



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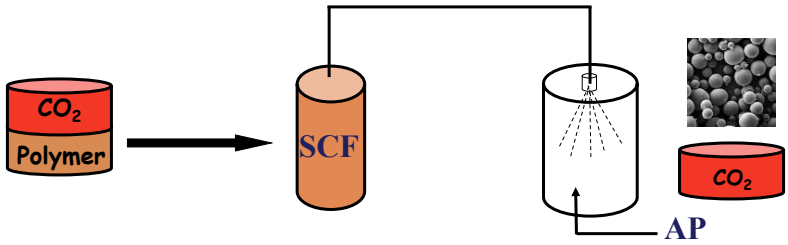
APPLICATIONS TO MICROENCAPSULATION

CO_2 Soluble polymer

Decrease of pression allowing the fluid to go through a nozzle of pulverisation



Pression : 20 MPa
Temperature : 50°C

Pression : 0,1 MPa
Temperature : 40°C

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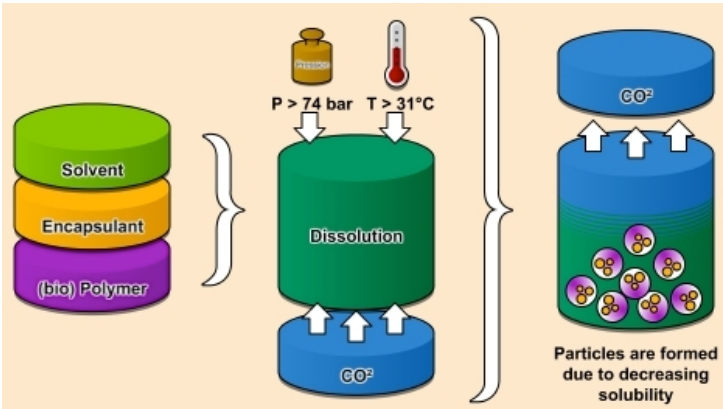
SUPERCRITICAL FLUIDS ANTI-SOLVENT



Microsphere formation and encapsulation by the Gas Anti-Solvent (GAS) or Supercritical fluid Anti-Solvent (SAS) method

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Gate2TECH, www.gate2tech.com

APPLICATIONS IN MICROENCAPSULATION

Rules

Carrier solvent
Drug solvent
Carrier and drug solvent

Advantages

Low temperature (thermolabile materials)
No need of solvent
The supercritical fluid returns to a gaseous state without condense and thus leaving no traces of liquid in the material, by slow depressurization.

II

LIPID NANOPARTICLES

SOLID LIPID NANOPARTICLES

Lipophilic colloidal delivery system

Efficient and non-toxic drug carrier specially for lipophilic drug molecules

Composed of physiological/well tolerated excipients **(GRAS)**

Possess **solid matrix** (similar to polymeric nanoparticles)
- Protective properties
- Controlled release properties

Colloidal dimensions and controlled release behaviour enable drug protection and administration by parenteral and non-parenteral routes.

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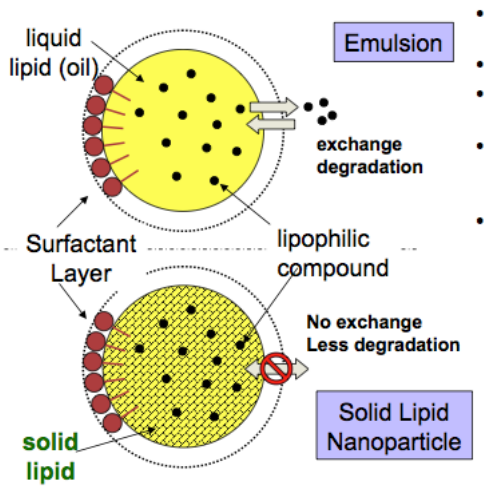
DIFFERENCE BETWEEN EMULSION AND SLN



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SOLID LIPID NANOPARTICLES

Polymeric nanoparticles

- possible toxicity of the polymer
- difficulties for scale-up

Solid lipid nanoparticles (SLN)

- Alternative for polymeric nanoparticles
- Lipid composition : safe (triacylglycerol, waxes, paraffin…). Good tolerability
- physical stability
- Large scale production possible (Müller et al, 2001)
- inexpensive

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PREPARATION OF SOLID LIPID NANOPARTICLES

Melt-emulsification by high pressure homogenisation

- heat solid lipid
- pour the viscous lipid in hot water
- high pression applied allowing the mixture crossing some little pores

Cold high pressure homogenisation (Muller et al.)

- same process

Precipitation from microemulsions (Gasco et al)

- precipitation from microemulsions

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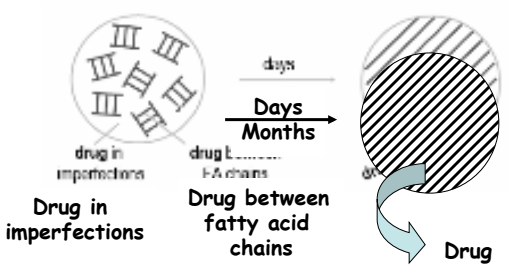
Solid Lipid Nanoparticles

But : - gel formation with time
- particle aggregation
- polymorphic transition during storage. Tend to form perfect crystals.



- insufficient loading capacity
- high water content of dispersions (70-99.9%)

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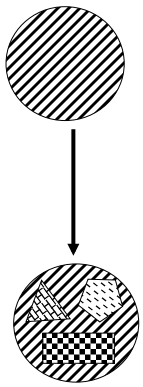
Almeida, 2007

Nanostructured Lipid Carriers (NLC)

Produced from blend of solid and liquid lipids
Particles are in solid state at body temperature

Inhibit crystallization process by mixing « spatially » very different molecules : imperfections in lattice

Controlled nanostructuring of lipid matrix
- to accommodate drug(s)
- to control release
- to trigger release



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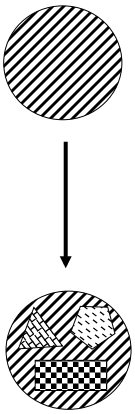
Nanostructured Lipid Carriers (NLC)

Multiple oil nanodroplets in solid fat nanoparticles

Not « just mixing » solid lipids but controlled

Higher drug load
1% retinol in SLN
6% retinol in NLC



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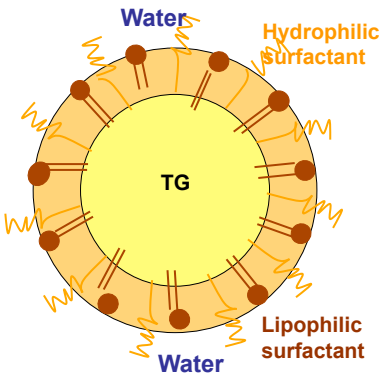
Shidhaye, Curr Drug Deliv, 2008

Lipid nanocapsules



25-100 nm
Stability
Solvent-free process
Lipophilic core

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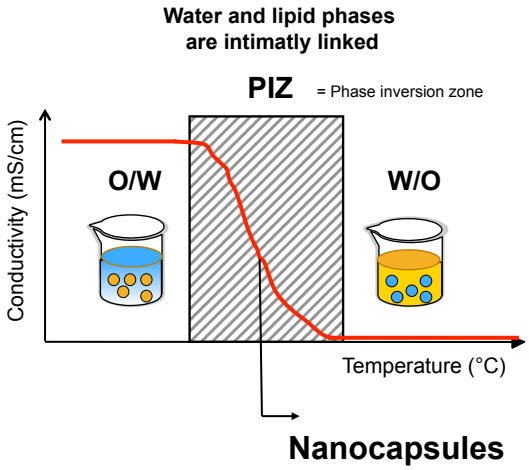
Heurtault et al, Pharm Res, 2002

Lipid nanocapsules (LNC)



Composition:

- **Medium chain triglycerides** *(TG d'acide caprylique et caprique)*
- **Lipophilic surfactant** *(egg L-α-phosphatidylcholine)*
- **Hydrophilic surfactant** *(polyethylen glycol 660 et 600 hydroxystearate, Solutol® HS 15)*
- **Water**
- **NaCl**

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Heurtault et al, Pharm Res, 2002

LIPID NANOCAPSULES. « A new platform for nanomedicine »

Various strategies for drug delivery to the sites of action using LNC.

Strategies	Examples	Encapsulated drugs	Encapsulation rates	Study designs	Results	Reference
P-gp inhibition	LNC coated with PEG-type nonionic surfactants such as Solutol®	Etoposide	89.9 ± 2.3%	*In vitro* on C6, F98, 9L glioma cell lines	Increase cytotoxicity on glioma cells due to high intracellular drug accumulation	Lamprecht and Benoit (2006)
		Paclitaxel	93.0 ± 3.1%	*In vitro* on 9L and F98 glioma cell lines *In vivo* on s.c. F98 tumor model, single i.t. treatment at Day 5	Significant reduction in cell survival Significant reduction in tumor mass and tumor volume evolution	Garcion et al. (2006)
Passive targeting	Post-insertion of longer PEG chains: DSPE-PEG 1500; DSPE-PEG 2000; DSPE-PEG-5000	Drug-free		Biodistribution after an i.v. injection into healthy rats	Half-life time over 5h *vs* under 21 min for conventional LNC	Hoarau et al. (2004), Ballot et al. (2006), Beduneau et al. (2006)
	Post-insertion of DSPE-PEG 2000	Docetaxel	>98%	C26 colon adenocarcinoma s.c. tumor, i.v. injection of treatments in mice	Significant and substantial accumulation in the tumor *vs* conventional LNC and control docetaxel formulation (Taxotere®)	Khalid et al. (2006)
Active targeting	Attachment of OX26 Mab or Fab' fragments at the LNC surface directed against TfR	Drug-free		*In vitro* cell binding on Y3.AG.1.2.3. cells and rat BCECs	Effective binding of immuno-nanocapsules on the cells *via* TfR	Beduneau et al. (2007a,b)
				Biodistribution after an i.v. injection into healthy rats	Significant accumulation in the brain 24h after administration *vs* non-targeted LNC	
Local administration (CED)	CED technique for delivery of LNC into the brain	^{188}Re-SSS; Fc-diOH	>98%	9L rat brain tumor intracranial xenograft model, CED treatment	Significant improvement in median survival time	Allard et al. (2008a)
Oral administration	LNC formulation to inhibit P-gp on the gastrointestinal tract	Paclitaxel	99.9 ± 1%	Oral administration by gastric intubation into healthy rats	Augmentation of mean plasmatic concentration of paclitaxel	Peltier et al. (2006)

P-gp: P-glycoprotein; LNC: lipid nanocapsules; PEG: polyethyleneglycol; s.c.: subcutaneous; i.t.: intratumoral; i.v.: intravenous; Mab: monoclonal antibodies; TfR: transferrin receptor; BCECs: brain cerebral endothelial cells; CED: convection-enhanced delivery; ^{188}Re-SSS: $^{188}Re(S_3CPh)_2(S_2CPh)$ complex; Fc-diOH: ferrocifenol.

Huynh et al, Int J Pharm, 2009

III

CARBON NANOPARTICLES

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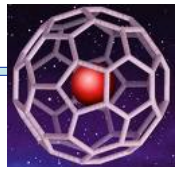
FULLERENES



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

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CARBON NANOTUBES (1991)

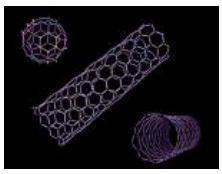
Only a few nm's in diameter, a single-walled carbon nanotube can grow as long as several micrometers

SWNTs may nest inside each other to form « russian dolls », known as **multi-walled carbon nanotubes** (MWNTs)

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

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



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IV

LIPOSOMES

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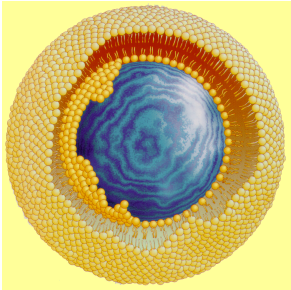
Introduction

Liposomes described by Bangham in 1961 (1964).

Used as models of cellular membranes.

Interest as DDS during the past 40 years



Synthetic structures

Microscopic phospholipid bubbles

Aqueous core

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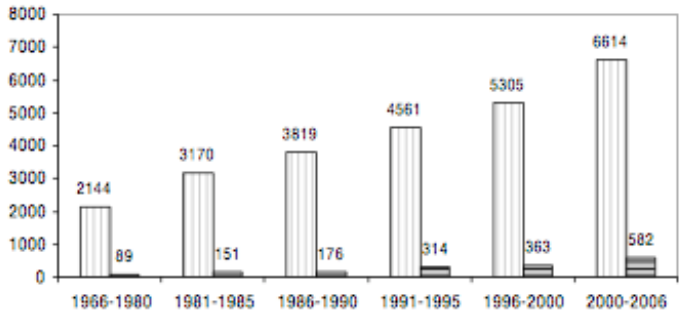
Scientific research on liposomes



papers

Older DDS
High experience

reviews

Figure 1 Increase in scientific research on liposomes: papers (vertical line) and reviews (horizontal line) published (total numbers on vertical axis). Data obtained from Ovid-Medline search keyword "liposomes".

2002-2016 : more than 32 000 publications, pubmed

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COMPOSITION

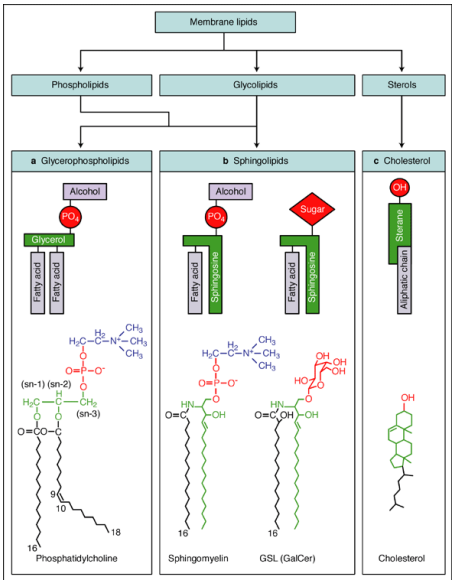
Composition of liposomes

Diacyl phosphoglycerides
Phosphatidylcholine

Sphingolipids

Sterols
Cholesterol

Fatty acids



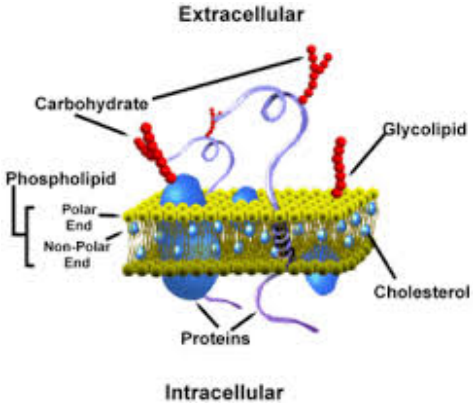
Structure-based classification of membrane lipids

Liposomes and membrane cells

Same composition as membrane cells

Models:
structure
function: diffusion of solutes

Low toxicity



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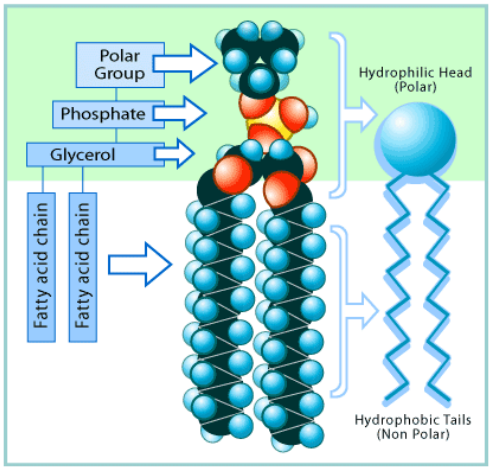
Phospholipids



Main components

Fatty acids
Carbon chain length,
Insaturation and their number

Polar head charge
Negative: PG, PS, PI
Neutral: PC, PE
Positive: cationic lipids

Addition of charged phospholids for stability
Amphiphilic nature of phospholipids

Sensibility to phospholipases

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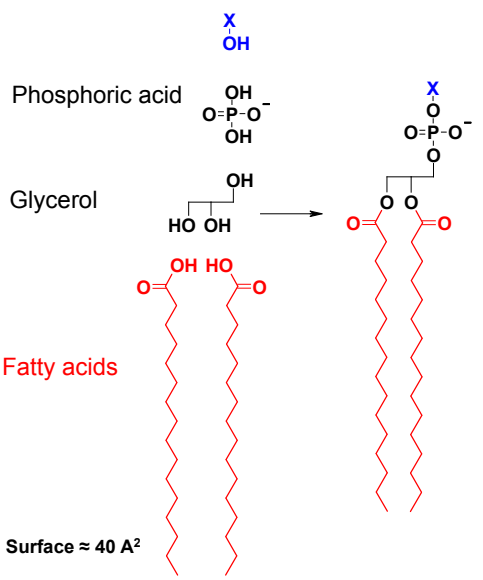
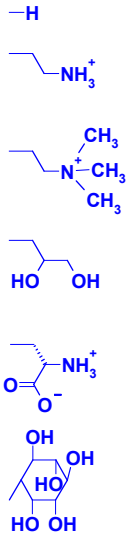
Phosphoglycerides (50%)





Phosphatidic acid =PA

Phosphatidylethanolamine = PE

Phosphatidylcholine = lecithine = PC

Phosphatidylglycerol = PG

Phosphatidylserine = PS

Phosphatidylinositol = PI

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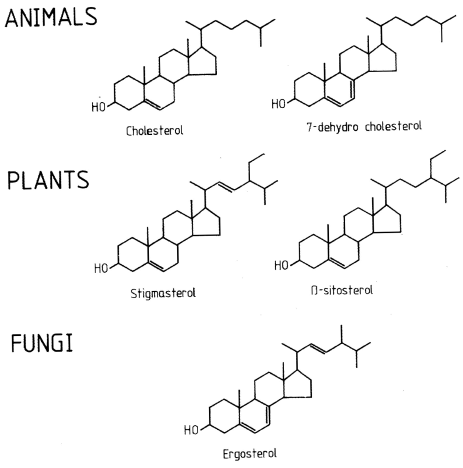
Sterols



Main sterols found in natural membrane

Cholesterol

Proportion 1:1 to 1:2 (cholesterol/PC).

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ORGANISATION

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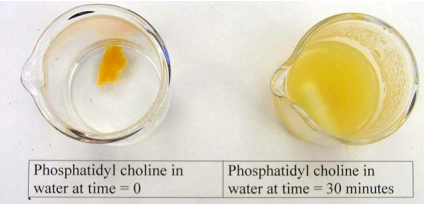
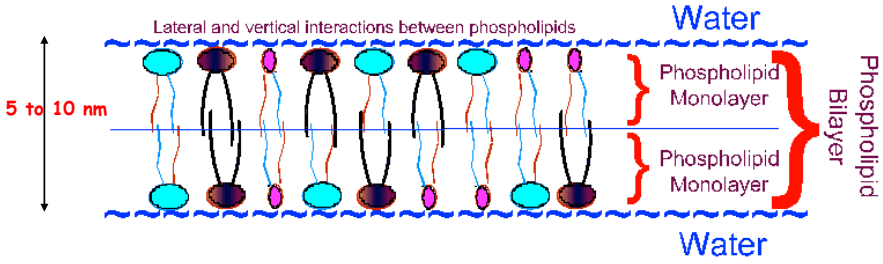
Phospholipids organization

Amphiphilic molecules
Spontaneous bilayer in water





Interactions:

Van der Waals: hydrophobic region
Hydrogen and electrostatic: hydrophilic regions

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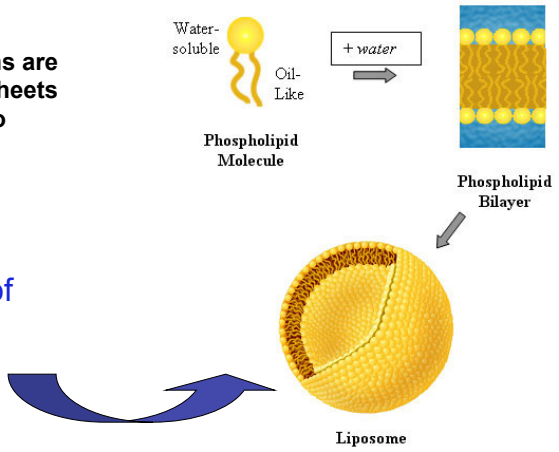
3-D organization

Favorable interactions are complete when the sheets fold on themselves to form closed sealed vesicles



Minimisation of **un**favorable interactions

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http://www.encapsula.com/company.html

Phospholipids and membrane properties

The shape of the membrane depends on the lipid structure

Double-chained lipids with large head-group areas, fluid chains : flexible bilayers, vesicles: PC, PG, PS, PI…

Lipid	Critical packing parameter $v/a_0 l_c$	Critical packing shape	Structures formed
Single-chained lipids (surfactants) with large head-group areas: *SDS in low salt*	< 1/3	Cone	Spherical micelles
Single-chained lipids with small head-group areas: *SDS and CTAB in high salt, nonionic lipids*	1/3-1/2	Truncated cone	Cylindrical micelles
Double-chained lipids with large head-group areas, fluid chains: *Phosphatidyl choline (lecithin), phosphatidyl serine, phosphatidyl glycerol, phosphatidyl inositol, phosphatidic acid, sphingomyelin, DGDG[a], dihexadecyl phosphate, dialkyl dimethyl ammonium salts*	1/2-1	Truncated cone	Flexible bilayers, vesicles
Double-chained lipids with small head-group areas, anionic lipids in high salt, saturated frozen chains: *phosphatidyl ethanolamine, phosphatidyl serine + Ca^{2+}*	~1	Cylinder	Planar bilayers
Double-chained lipids with small head-group areas, nonionic lipids, poly *(cis)* unsaturated chains, high *T*: *unsat. phosphatidyl ethanolamine, cardiolipin + Ca^{2+} phosphatidic acid + Ca^{2+} cholesterol, MGDG[b]*	> 1	Inverted truncated cone or wedge	Inverted micelles

[a] DGDG, digalactosyl diglyceride, diglucosyl diglyceride.
[b] MGDG, monogalactosyl diglyceride, monoglucosyl diglyceride.

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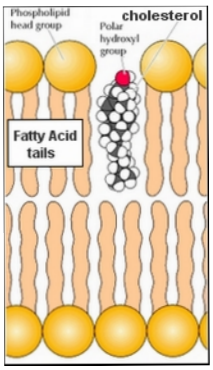
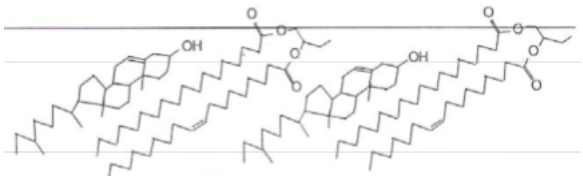
Cholesterol



Cholesterol increases the membrane stability if the temperature is above the phase transition temperature and increase fluidity and permeability if the temperature is lower than the phase transition temperature.

Around 50% cholesterol/phospholipids

No bilayer if only sterols.



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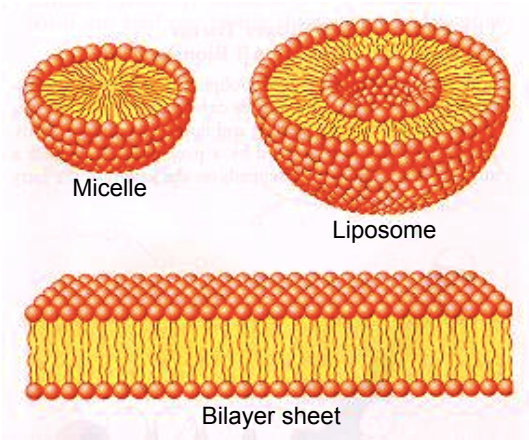
Liposomes vs micelles

Single chain lipids



Double chain lipids

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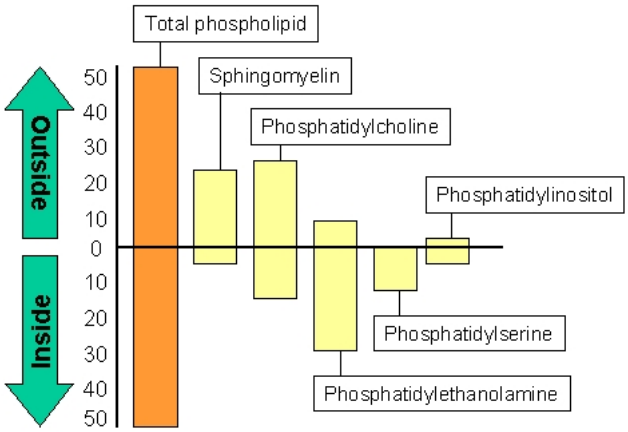
Distribution of the phospholipids in the membrane



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DYNAMIC STRUCTURES

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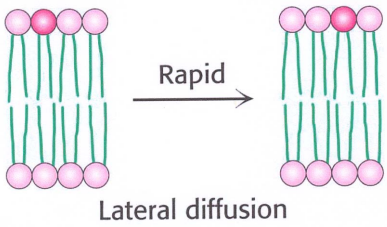
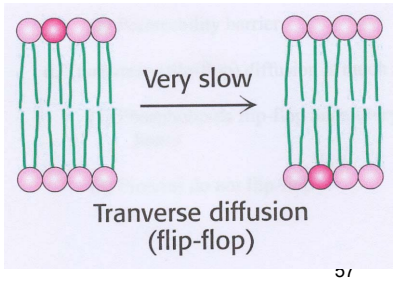
Dynamic systems

- **Lateral diffusion: fast (µs).**
- **Flip-flop: slow (h or days).**
- **It depends on**
  - **temperature**
  - **composition**

The permeability is related to these movements.





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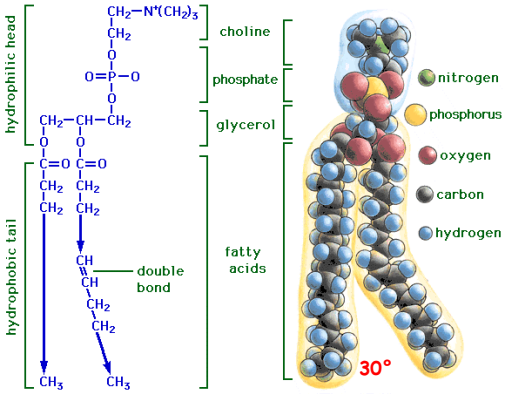
Phase transition temperature



Double bond

Formation of angle

Consequence on PTT

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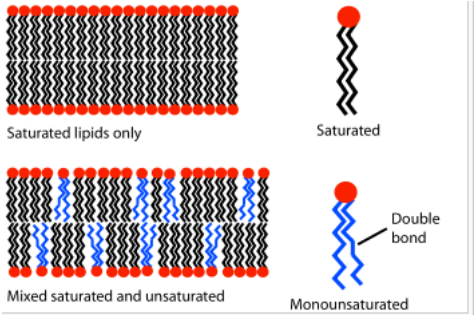
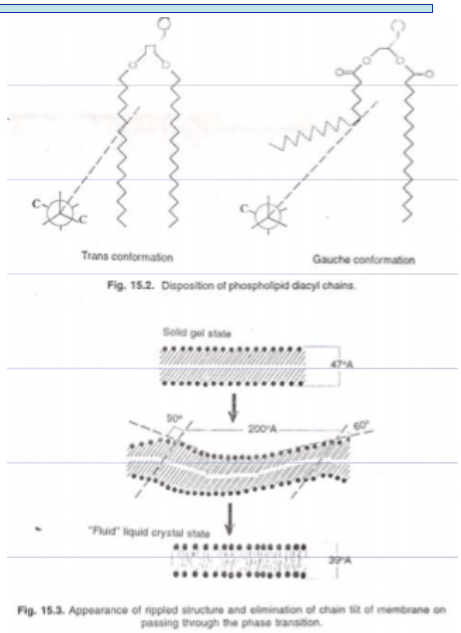
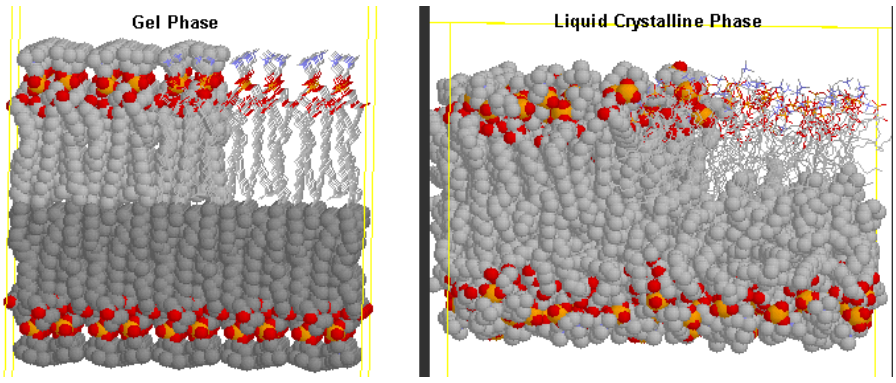
Phase transition temperature





Phase transition temperature



Solid → **Fluid**

Temperature

Phase transition temperature: T required to induce a change from the gel phase to the liquid crystalline phase.
Depends on the PL
Fluidity necessary for formulation

60

Dr. Jakubowski, 2013

Phase transition temperatures for diacyl phospholipids

1) The length of the acyl chain in the lipid
2) The degree of insaturation of the hydrocarbon chains in lipid
3) The type of the polar head group

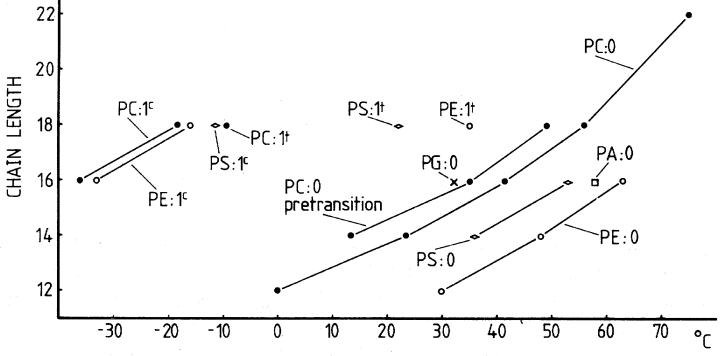


Figure 6. Phase transition temperatures for diacyl phospholipids with different headgroups as a function of chain length. Data from different sources is presented for synthetic phospholipids containing fatty acids of the same chain length and unsaturation in both 1- and 2- positions. Unless otherwise stated, all values quoted are for the main transition. The mono-unsaturated acids all have their unsaturation (either *cis* or *trans*) in the 9-position, which is the position in the chain giving the lowest phase transition temperature (i.e. the position in which the *cis* double bond maximally inhibits close packing of fatty acid chains in the gel phase).

CLASSIFICATION

62

Classification as a function of size

- Small unilamellar vesicles SUV, 30-100 nm
- Large unilamellar vesicles LUV 100-5000 nm
- Giant unilamellar vesicles GUV 5 - 100 microns
- Small multilamellar vesicles SMV, 30-100 nm
- LMV, etc.

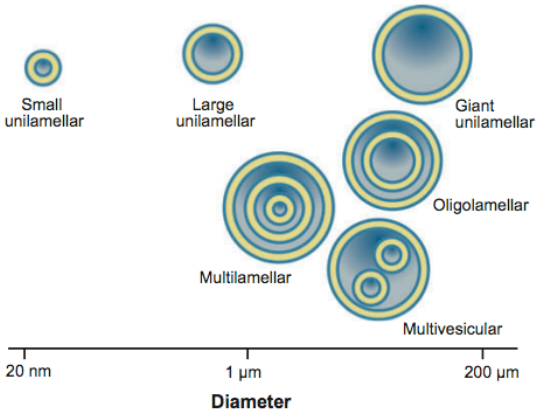
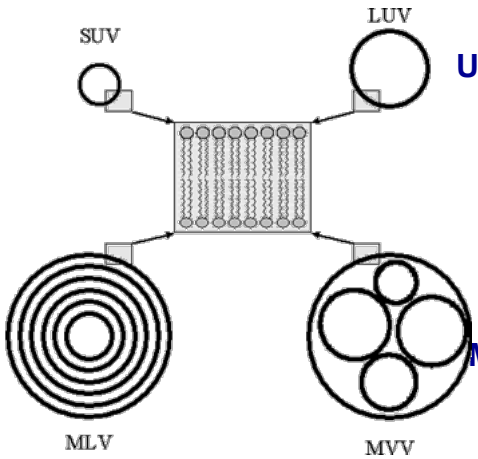


Figure 2

Schematic representation of the commonly applied classification scheme for liposomes. Small unilamellar vesicles (~0.02 µm to ~0.2 µm), large unilamellar vesicles (~0.2 µm to ~1 µm), and giant unilamellar vesicles (>1 µm) are the three most important groups for analytical applications. Multilamellar vesicles are frequently used in pharmaceutical and cosmetic applications (56). Multivesicular vesicles are giant vesicles encapsulating smaller liposomes and have been used in nanoreactor assemblies (141) and as drug delivery tools (vesosomes) (142). The drawings are not to scale.

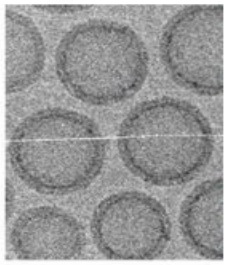
Classification as a function of structure



Unilamellar

Multilamellar

Cryo-TEM



64

Nanomedicine Laboratory, Dr Muller

Classification based on composition and mode of drug delivery

- **Conventional liposomes**
  - Neutral or negatively charged phospholipids
  - Subject to endocytosis. Useful for macrophages targeting. Rapid and saturable uptake by macrophages

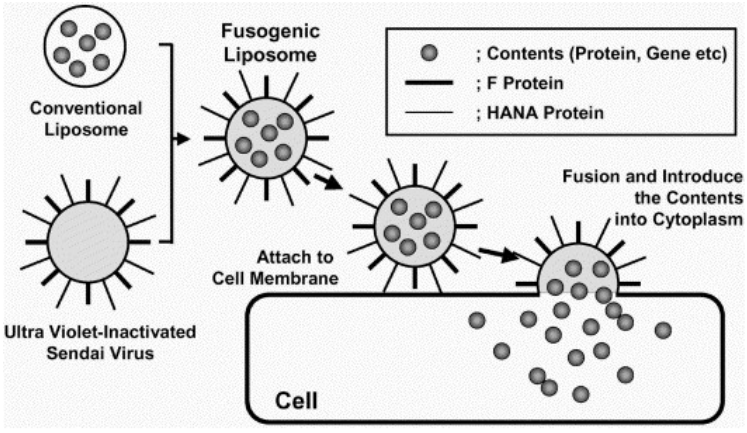
pH sensitive/ fusogenic/cationic/immuno/ long-circulating

- **pH sensitive liposomes**
  - Phospholipids such as PE or DOPE with either Cholesteryl hemisuccinate (CHEMS) or oleic acid (OA)
  - Subject to endocytosis. At low pH, fuse with cell or endosome membranes and release their contents in cytoplasm.
  - Suitable for intracellular delivery of weak bases and macromolecules.
  - Biodistribution and PK similar to conventional liposomes.
  - Structurally unstable;

65

Classification based on composition and mode of drug delivery

- **Fusogenic liposomes**
  - **Conventional liposomes with the Sendai virus (HANA proteins)**



HANA: hemagglutinating and neuraminidase proteins: binding to the sialic acid R
F protein: fusion protein interacting with the lipid layer: cell fusion

66

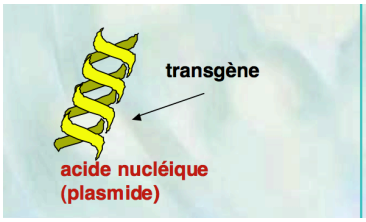
Kunisawa et al, ADDR, 2001

Classification based on composition and application

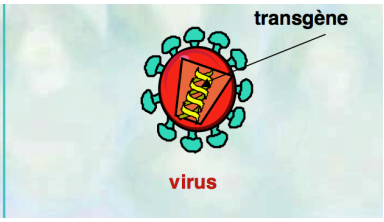
- **Cationic liposomes**
  - **Cationic lipids: DDAB, DOGS, DOSPA, DOTAP, DOTMA…**
  - **Possibly fuse with cell**
  - **or endosome membranes; suitable for delivery of negatively charges macromolecules (DNA, RNA..)**

Gene therapy

Non viral



Viral



Liposomes, nanoparticles
Polycations, cationic lipids
No immune response
Less expensive

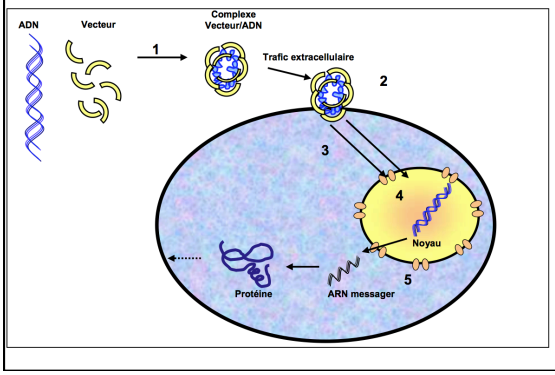
67

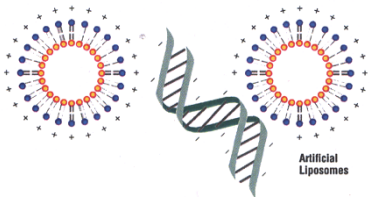
Classification based on composition and application

Liposomes = cationic lipids (+ neutral lipids)

Interaction with nucleic acids negatively charged

DNA compaction = liposomes-DNA complexe





68

Classification based on composition and application

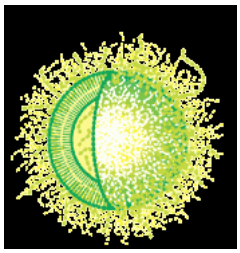
- **Immunoliposomes**
  - **Liposomes with attached antibody or recognition sequence.**
  - **Subject to receptor-mediated endocytosis cell-specific binding (targeting).;**

Can release contents extracellularly near the target tissue and Drugs may diffuse through plasma membrane to produce their effects.

69

Classification based on composition and application

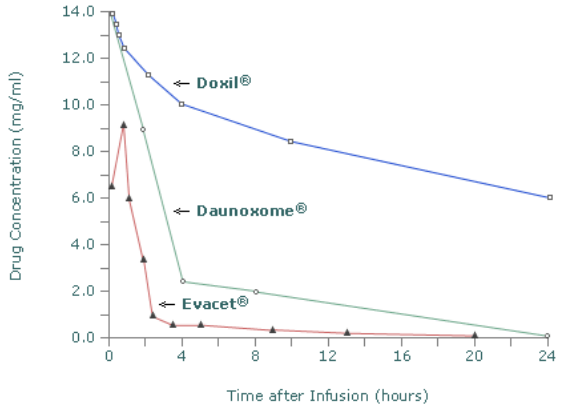
- **Stealth liposomes**
  - **Presence of PEG in the basic composition**
  - **Hydrophilic surface coating; low opsonization and low rate of uptake by RES; long circulation half-life;**



PEG coating
Doxorubicin encapsulation
Doxil®
Kaposi sarcoma and refractory ovarian cance
Approved by FDA in 2005

70

Classification based on composition and application



Daunoxome : Liposomes of daunorubicine
Evacet: liposomes of doxorubicine
Doxil : stealth liposomes of doxorubicine

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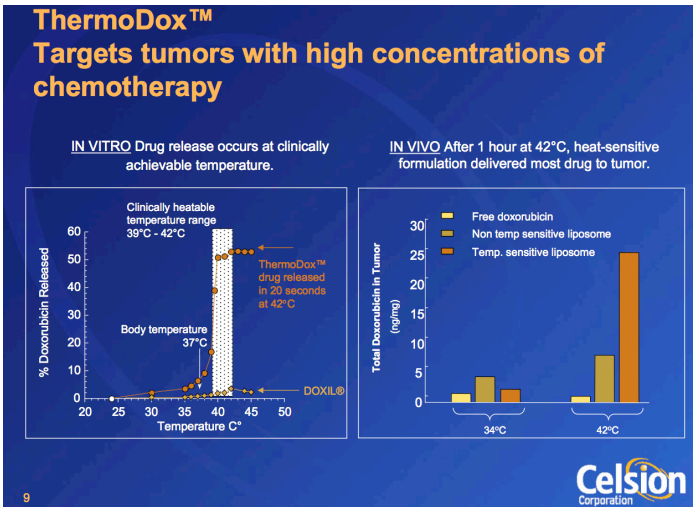
http://www.alza.com/alza/stealth_more
ALZA's patented STEALTH® technology

Classification based on composition and application

- **Thermoliposomes**



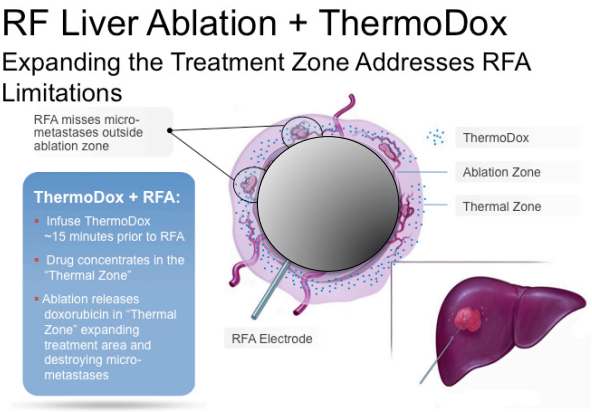
72

The LTSL formulation was composed of DPPC:MPPC:DSPE-PEG-2000 in the molar ratio of 90:10:4
Needham [CANCER RESEARCH 60, 1197-1201, March 1, 2000]

Classification based on composition and application

- Thermoliposomes

RF : radiofrequency ablation



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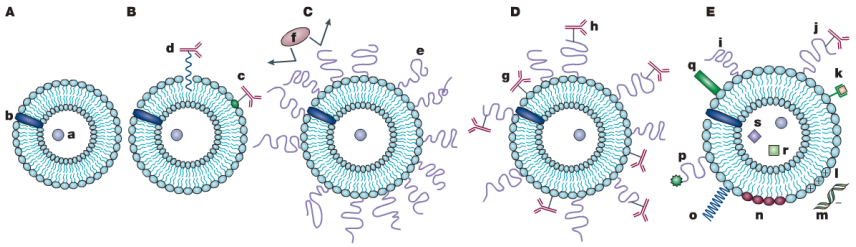
Classification based on composition and application

B. Immunoliposomes

D. Targeted and stealth liposomes



A. Conventional liposomes

C. Stealth liposomes

E. Last generation

p. CPP
k. diagnostic label
n. Stimuli sensitive lipids
…

74

Torchilin, Nature Reviews, 2005

Classification based on method of preparation

Reverse phase evaporation method: REV

Multilamellar vesicles made by REV: MLV-REV

Extrusion technique: VET

Deshydratation-Rehydratation: DRV

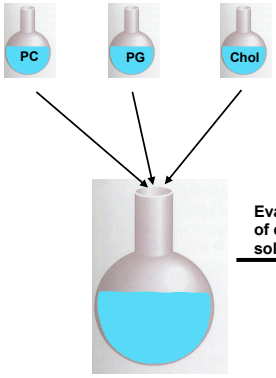
Frozen and thawed: FATMLV

75

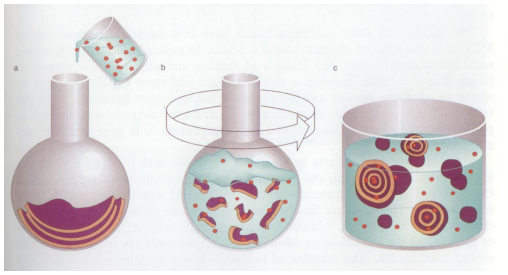
METHODS OF PREPARATION

76

Lipid film hydratation: MLV



Evaporation of organic solvent



Mixture of phospholipids in organic solvent

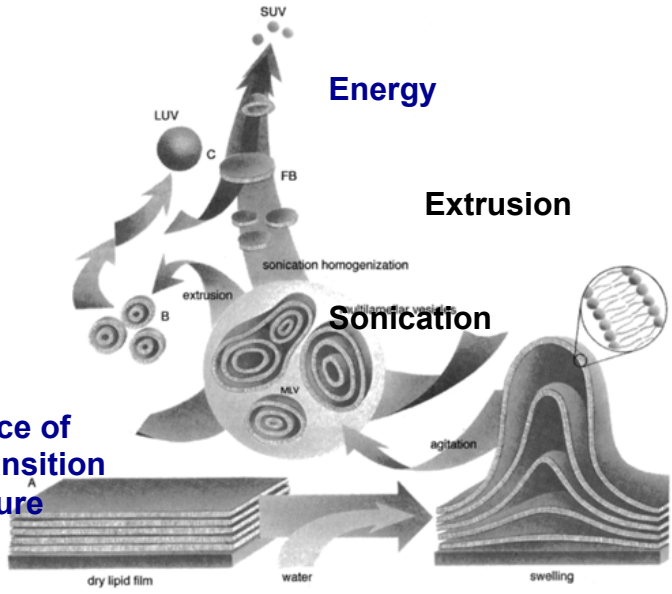
Addition of an aqueous phase to a dry thin lipid film

Swelling and peeling of the lipid film under vigourous agitation

Milky suspension of MLVs

77

Lipid film hydratation



Energy

Extrusion

Sonication

Importance of phase transition temperature

78

www.avantilipids.com

Lipid film hydratation

ADVANTAGES

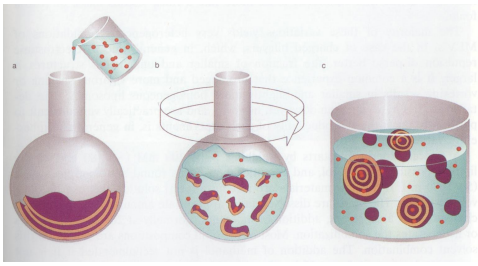
Simple
Fast

DRAWBACKS

Low encapsulation rates

Heterogeneous sizes (MLV)

Not industrial production



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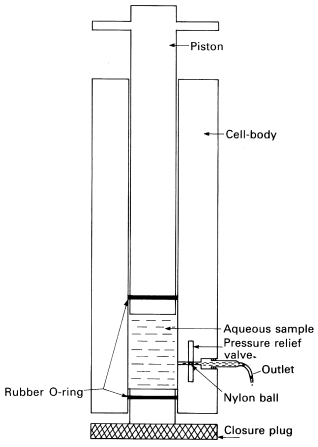
French press: extrusion

MLV → **SUV**



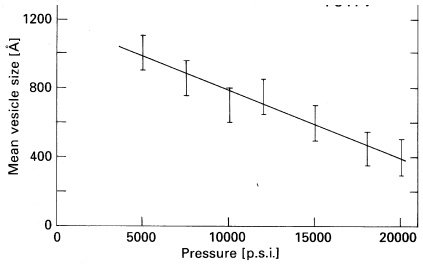
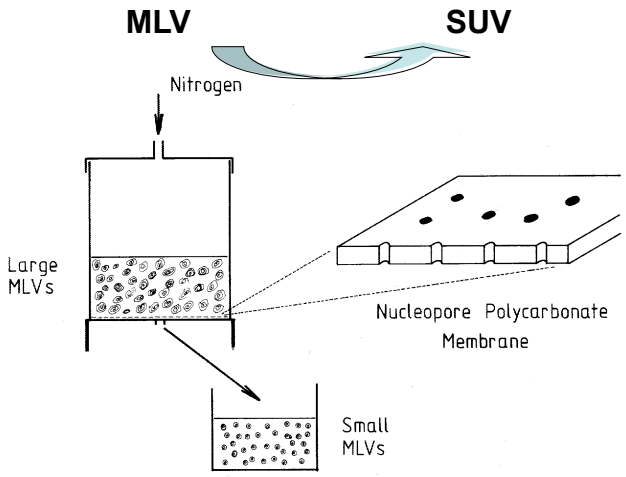


Figure 6. French press technique. (**a**) Diagram of a French pressure cell. Small vesicles are obtained when phospholipid dipsersions are extruded through the small orifice (lower right) at pressures of 20 000 p.s.i. or greater. (**b**) Graph showing relationship between pressure and liposome diameter.

Temperature increases
Several passages
MLV rupture: SUV

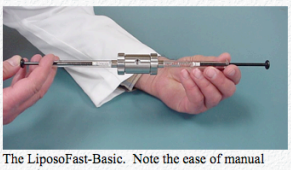
80

Extrusion membrane



Polycarbonate membrane with pores
Several passages
Decrease number of lamellas: MLV=> SUV

LiposoFast



The LiposoFast-Basic. Note the ease of manual operation.

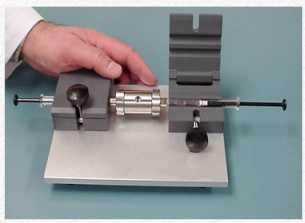
Click here to see a short video demonstrating this product.

LiposoFast-Basic

1. Principle of Operation
The LiposoFast-Basic produces unilamellar liposomes by the manual extrusion of a multilamellar liposome suspension through a polycarbonate membrane of defined pore size, using gas-tight, glass syringes. The sample is passed through the membrane by pushing the sample back and forth between two syringes.

2. Temperature Control
The entire LiposoFast-Basic can be immersed in a water bath for use with high transition temperature lipids or heat sensitive compounds.

3. Cleaning/Sterilization
All components of the instrument are easily cleaned and can be autoclaved.



The LiposoFast-Stabilizer with a LiposoFast-Basic installed.

LiposoFast-Stabilizer

1. Principle of Operation
The LiposoFast-Stabilizer was designed to simplify the repetitive use of the LiposoFast-Basic as well as the extrusion of highly concentrated samples. It accommodates both 0.5mL and 1.0mL syringes.

2. Temperature Control
The LiposoFast-Stabilizer can be immersed in a water bath for use with high transition temperature lipids or heat sensitive compounds.

3. Cleaning/Sterilization
All components of the LiposoFast-Stabilizer are easily cleaned and can be autoclaved.

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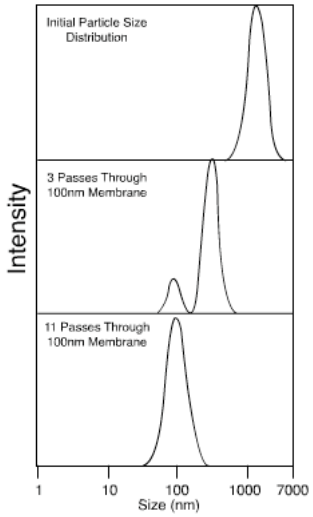
LiposoFast-Basic (www.avestin.com)

LiposoFast



Number of passages
Decrease the diameter
Increase the homogeneity

11 passages recommanded

83

French press/membrane extrusion

ADVANTAGES

Rapid
Simple
Reproducible
Non agressive
High encapsulation rates
Industrial production

Sizes related to the membrane pores

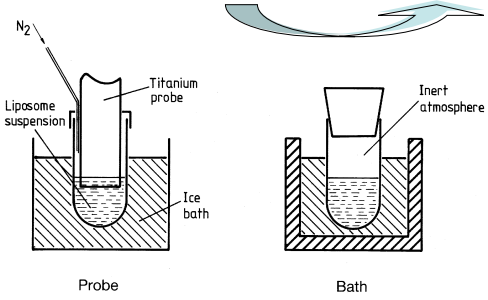
DRAWBACKS

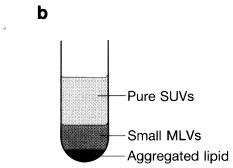
Price
MLV prior to SUV
Low encapsulation volumes

84

Sonication

MLV → **SUV**





ADVANTAGES

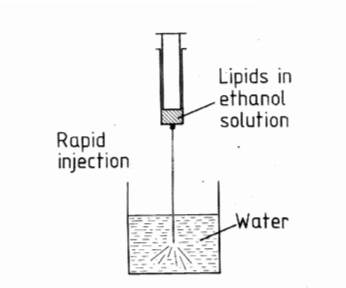
Homogeneous diameters

DRAWBACKS

Low encapsulation rate
Low encapsulation volume
Temperature
Degradation
Aerosol
Titane particles
MLV at first

85

Ethanol injection: SUV



Final ethanol-in-water concentration not > 7.5%.
Rate of injection.
Extremely simple
Low risk of degradation of sensitive lipids
Variation of the concentration of lipid in ethanol
of the rate of injection of ethanol

86

Reverse phase evaporation

REV: reverse evaporation phase vesicles

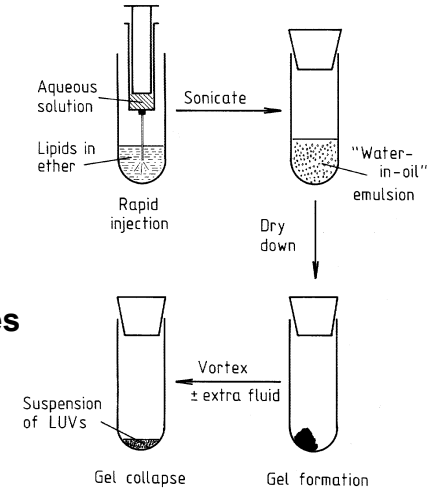


Figure 15. Stages in the preparation of liposomes by reverse phase evaporation. After formation of a water-in-oil emulsion by sonication of the aqueous solute in an organic solution of lipids, the organic solvent is evaporated off to yield a gel. The gel then collapses either naturally, as drying is continued, or as a result of mechanical shaking, to give a free-flowing aqueous suspension of liposomes.

7

Reverse phase evaporation

PL solubilisation in organic solvent (ether)

Addition of aqueous phase (1/3) containing drug

Phospholipids at the interface

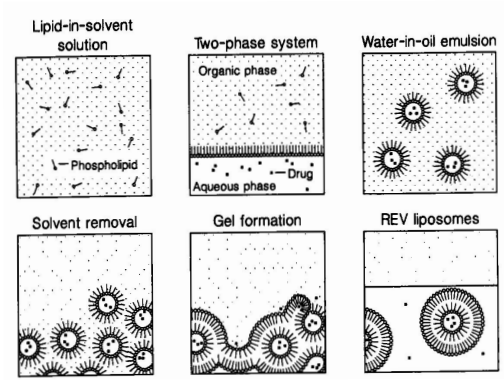
Sonication leading to water-in-oil emulsion

Solvent evaporation: globule concentration

Gel formation

Complete evaporation

Gel destruction/vortex



Unilamellar vesicles. 500 nm

88

Common preparation techniques

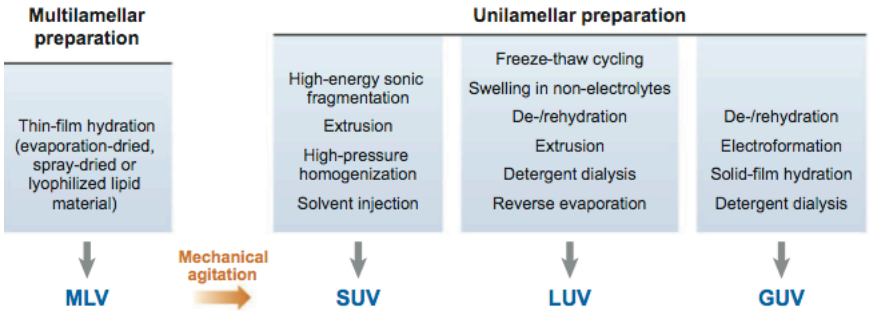


Figure 4

Common preparation techniques for different types of liposomes, categorized by lamellarity and size range. Multilamellar vesicles (MLVs) can be transformed into unilamellar vesicles by various means of mechanical treatment (5). Abbreviations: GUV, giant unilamellar vesicle; LUV, large unilamellar vesicle; SUV, small unilamellar vesicle.

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EQUIPMENT FOR NANOPARTICLES FORMULATION

Homogenizer

Nanosizer
Zetasizer

Ultra sonicator

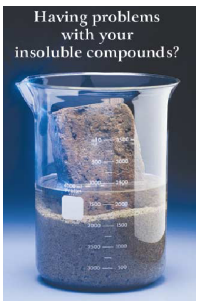
Evaporator

90

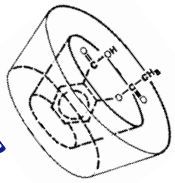
UNIVERSITÉ DE STRASBOURG

FRC
International Center for Frontier Research in Chemistry

cnrs

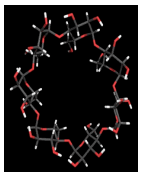


CYCLODEXTRINS



Béatrice Heurtault

European School On Nanosciences and Nanotechnologies
2016



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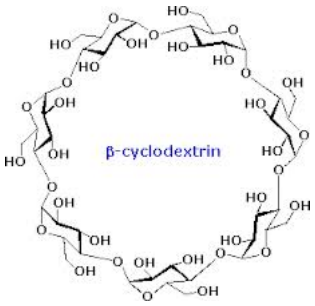
Tel: 33 (0)3 90 24 41 71 ; Fax: 33 (0)3 90 24 43 06 ; e-mail: bheurtault@unistra.fr

1

2D-Structure

Glycopyranose-based cyclic oligosaccharides.
« Chair » conformation
α-1,4-glycosidic bonds

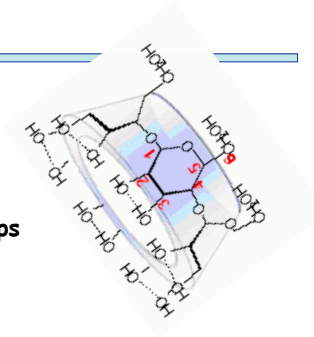
Central hydrophobic cavity: secondary OH
External hydrophilic surface: primary OH

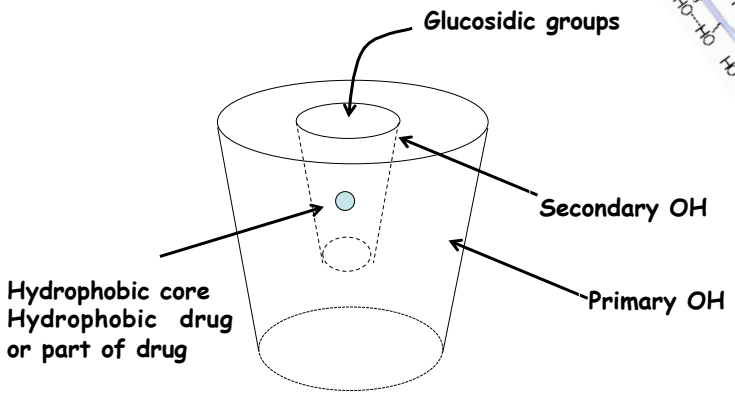


2

3D-Structure

Hydrophilic: outside
Hydrophobic: inside





Truncated cone

3

How to get cyclodextrins?

Enzymatic Hydrolysis of scartch: cyclic array of glucose monomers

Variable number of glucopyranose units 6, 7 or 8
respectively called α, β and γ

* If < 6: impossible to get a ring (high tension no closure, thermodynamically instable)

* If > 8: flexible cycle, kinetically obtained but difficult to purify

4

Physicochemical properties

	Nb of units	MW	Diam (Å)	Water sol. 25°C
α	6	972	5,7	14.5 g%
β	7	1135	8	1.8 g%
γ	8	1297	9,5	23.2 g%

(x g/100 ml)

Size and water solubility differ
β **is the most used**
Availability, size of the cavity
But solubility in water is rather weak (1.8 g%)

5

Usual modified cyclodextrins

Commercially available in large amounts:

Cyclodextrins	R
HP-β-**CD**	$CH_2CHOHCH_3$
SBE-β-**CD**	$(CH_2)_4SO_3^-\ Na^+$
RM-β-**CD**	CH_3

- Modified properties:
  - solubility
  - properties of the core

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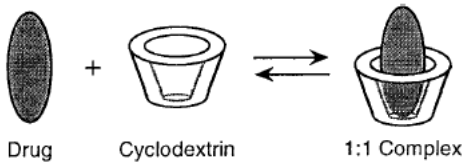
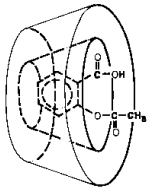
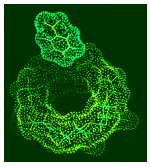
Drug solubilization





Aspirin-loaded CD

Central cavity
Polarity of ethanolic solution
Suitable sized molecule
No covalent bounds



Loftsson et al, JPS, 1996

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Inclusion mechanism

Insoluble **drug** ⇌ **Drug** in solution

\+

CD

Kd ⇕ *Ka*

Soluble inclusion complex

⇕

Insoluble inclusion complex

The limit in solubility permits the separation

*Ka association constant
*Kd dissociation constant

*Constants are defined for a particular couple **API** / **CD**

Active Pharmaceutical Ingredient

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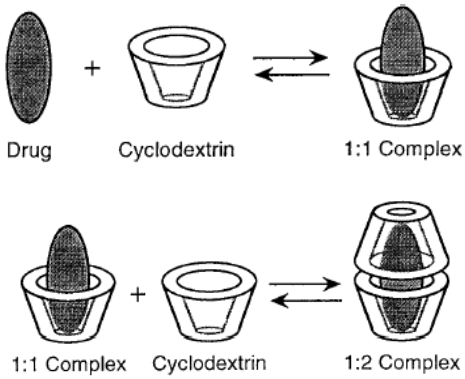
Inclusion parameters

Molecule size
Cavity diameter
Ratio CD/drug:1/1 ; 2/1 ; 1/2 …
Partial drug inclusion



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Roles of cyclodextrins

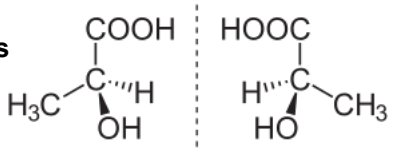
Enhance drug solubility in aqueous solutions

Table 5—Solubility of Drugs in Different Cyclodextrin Solutions at Room Temperature

Drug	Cyclodextrin[a]	Concn[b] (% w/v)	Solubility (mM)	Enhancement[c] Factor
Hydrocortisone (MW 362)	None		0.993	
	Glucosyl-α-CD	10	7.45	7.50
	Maltosyl-α-CD	10	11.3	11.4
	HP-β-CD MS 0.6	10	33.7	33.9
	HE-β-CD	10	48.3	48.6
	RM-β-CD MS 0.6	10	72.2	72.7
	RM-β-CD MS 1.8	10	50.8	51.2
	HTMAP-β-CD MS 0.5	10	30.3	30.1

Enhance drug stability in solutions

Separation of enantiomers



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Pharmaceutical applications of cyclodextrins

Usual CDs

Biocompatibility, « GRAS » (*generally recognized as safe*)

Propency to encapsulate lipophilic molecules

Modification of the apparent water solubility

Increase of the stability in solution of the molecules included, especially the pharmaceutical active ingredient (PAI)

As the water solubility is increased, doses to be administered can be lowered and toxicity can be limited.

Semisynthetic CDs

Hydrophilic solubilise hydrophobic **PAI**
Hydrophobic delay the dissolution of hydrophilic **PAI** *ex : isosorbide dinitrate*

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CD vs organic solvent

Usually

- Less toxic than solvents
- Less irritant after parenteral injection (IV, IM)
- No precipitation of the PAI after injection
- **CD** can be used in solid forms

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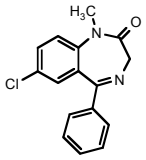
Diazepam formulation

Benzodiazepin
Anxiolytic
Sedative/hypnotic
Anticonvulsivant
Muscle relaxant



Diazepam weakly water soluble

Valium®		CD formulation	
Diazepam	5.0 mg	Diazepam	5.0 mg
Benzylic alcohol	15.7 mg	HPβCD	60.0 mg
Ethanol	85.3 mg	Na chloride	6.0 mg
Propylene glycol	414.0 mg	Water	ad 1.00 ml
Benzoic acid	47.5 mg		
Water	ad 1.00 ml		

⇨ *Around 45% water.*

⇨ *Around 93% water.*

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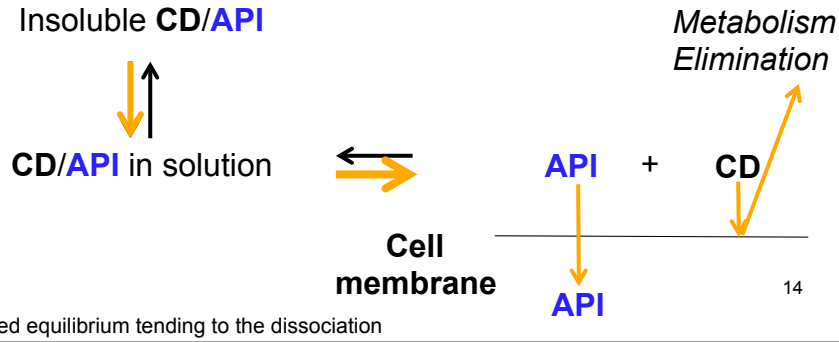
Drug release

Dissolution of the inclusion complex in the biological fluids
Release of the drug (equilibrium)
CD do not get through membranes (external hydrophily and MW +++)
Elimination of the **CD** (metabolism)
Absorption of the **API**

The equilibrium is deplaced and the major part of the drug is progressively released



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Displaced equilibrium tending to the dissociation

Administration routes

Oral route

Metabolism slower than starch

β amylases unefficient (no end, cycle !).
Only α amylases act at the colon level

Glycopyranose units are obtained

Speed: γ>β>α (α hardly not metabolised)

Toxicity almost nul: oral absorption # 0 !
But methylated: 10% are absorbed orally.

CD can (only) mask **bitter taste**

Oxyphenonium bromide (anticholinergic)

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Administration routes

Parenteral route

Decrease pain when injecting lipophilic drugs (IV or IM).

but

Risk of hemolysis with β > α > γ
Amphiphilic CDs modified on the IIary OH less hemolysis

Crystallisation in kidney: nephrotoxicity

Limits parenteral route!

γ CD are preferred as well as semisynthetic derivatives:
HP-γ CD and SBE-γ CD

Ocular route

Increase drug solubility, absorption, stability
Decrease local cornea irritation

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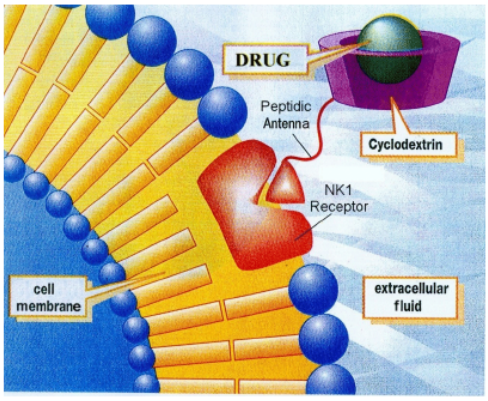
Targeting CDs



Covalently modified

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On the market

CD	PA	Commercial name
αCD	PEG_1 iv	PROSTAVASTIN (Eur.), CAVERJECT (USA)
βCD	Piroxicam tablets	BREXIN (Eur.)
HP-βCD	Intraconazole oral solution and iv	SPORANOX (Eur. et USA)
SBE-βCD	Ziprasidone maleate solution IM	ZELDOX (Eur.), GEODON (USA)
RM-βCD	Estradiol nasal spray	AERODIOL (Eur.)
γCD	OP-1206 tablets	OPALMON (Japan)
HP-βCD	Diclofenac sodium ocular solution	VOLTARENE (EUR.)

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