Nanoparticulate drug delivery systems

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European School On Nanosciences and Nanotechnologies
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Program: « nanoparticles in medicine »

Introduction to drug delivery systems (DDS)
Formulation processes
Nanoparticulate DDS properties

Applications
- Vaccines
- Cancer
- Gene therapy
- Diabetes

Nanoparticles
- Liposomes
- Cyclodextrins
- Polymer nanoparticles
- …

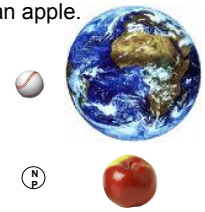
Introduction to Nanoparticles As Drug Delivery Systems

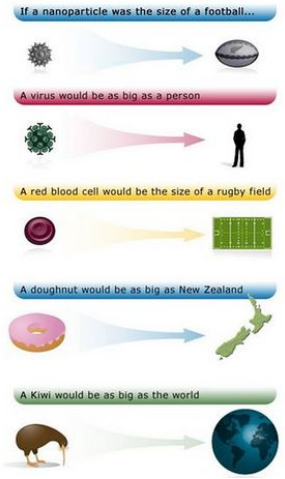
I. Nanoscale

'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

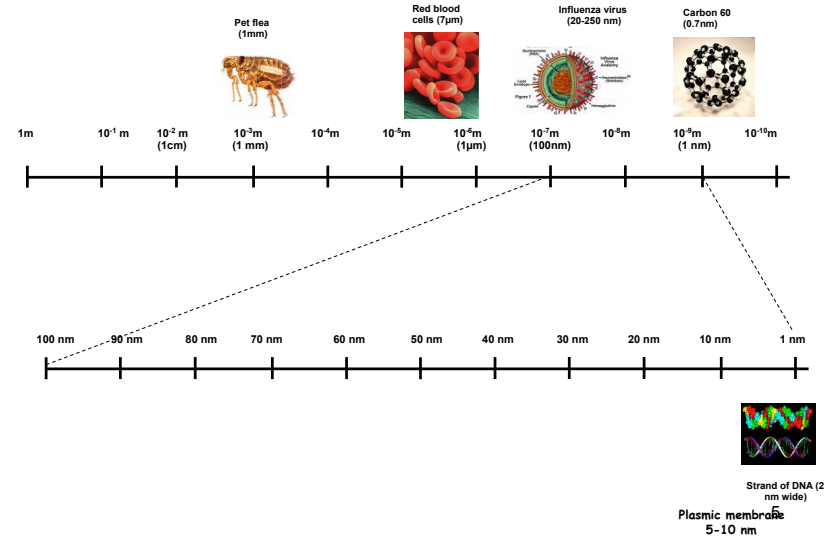
Nanometer-length scale

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

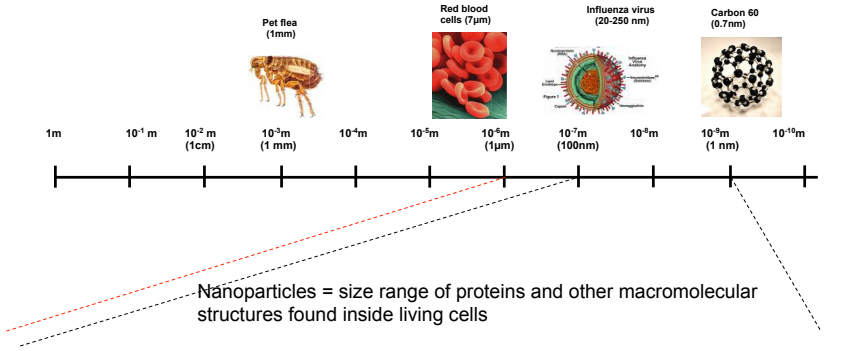




« Biology scale »



Scale



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 (100 nm to 1µm) called nanoparticles in the litterature !

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)*.

Ist criterion

Nanoengineering plays a major role in the manufacturing process

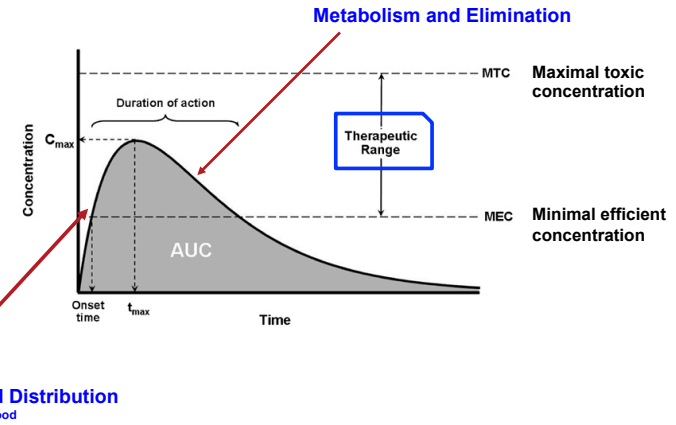
IInd criterion

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

Weissig et al, Int J Nanomedicine, 2014

II. Free drug: pharmacokinetic

Free drug
Tablets
Oral route

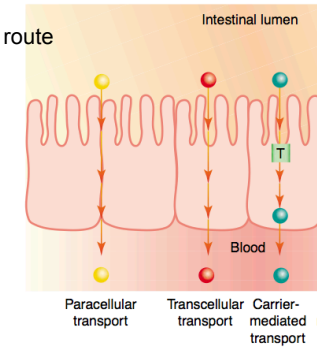


Overview of ADME

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

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

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)
…

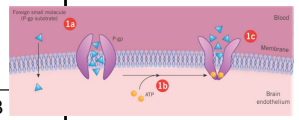
Biological barriers

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

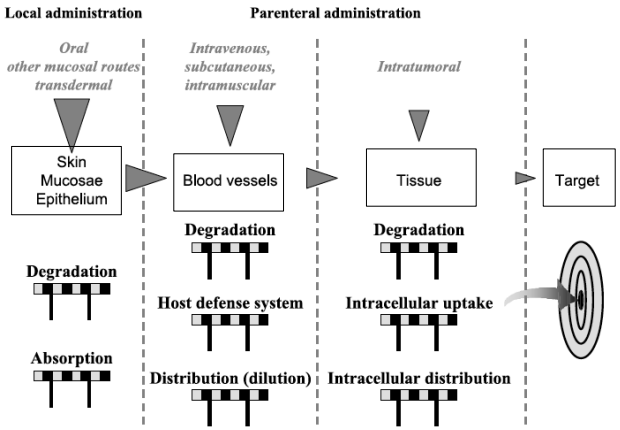
⇒ Barriers impair drug efficiency

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)

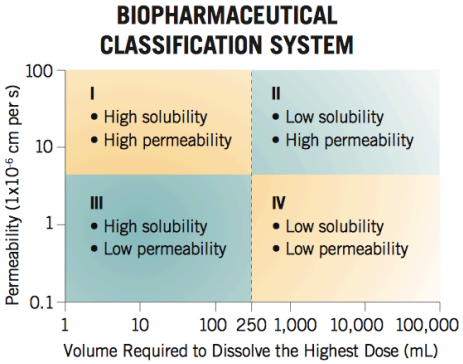


Barriers



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

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

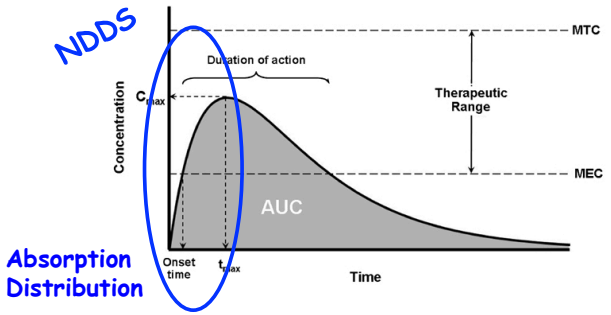
But recent molecules mainly belong to class IV with low solubility and low permeability

Need for enhanced formulation technique (compared to oral forms)

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)



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.

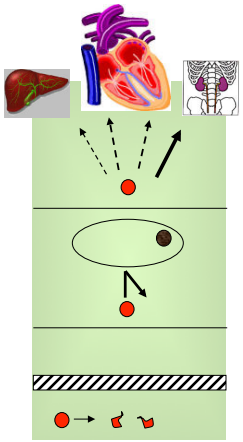
« Controlled Drug Delivery System (DDS) »

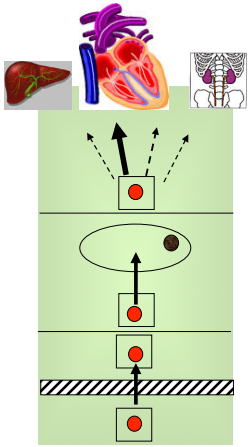
Any pharmaceutical formulation is « drug delivery system » (creams, eye drops, tablets….)

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

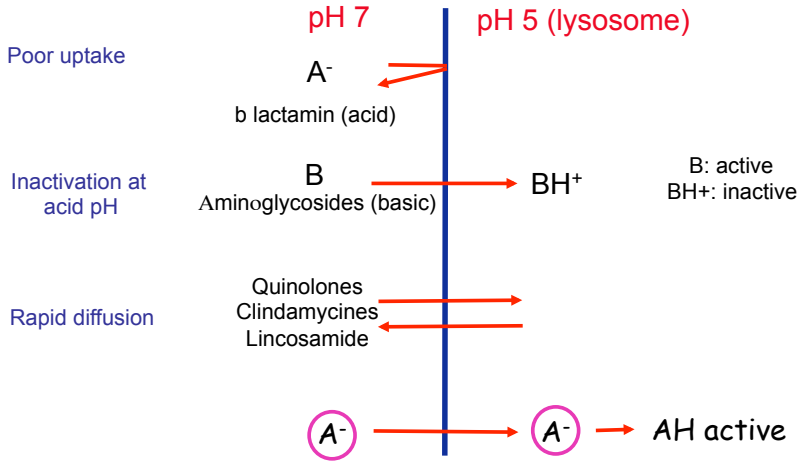
V. Advantages of drug delivery systems





Piotr Grodzinski, NCI, 2006

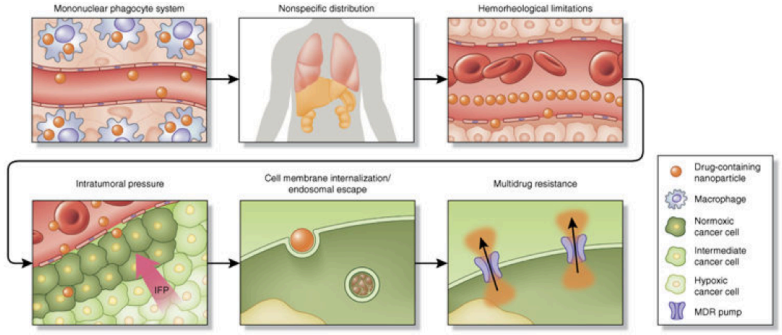
Example of application: Infectious diseases



Increases the intracellular uptake (through endocytosis)
Allows the drug to diffuse freely into the cell (i.e., by lysosomal fusion mechanisms)

Adapted from Couvreur and Vauthier, Pharm.Res, 2006

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

Blanco et al, Nature biotechnology, 2015

VIII. Pharmacokinetic/biodistribution profiles of np

Parameters to take into account when designing new carriers

Various steps:

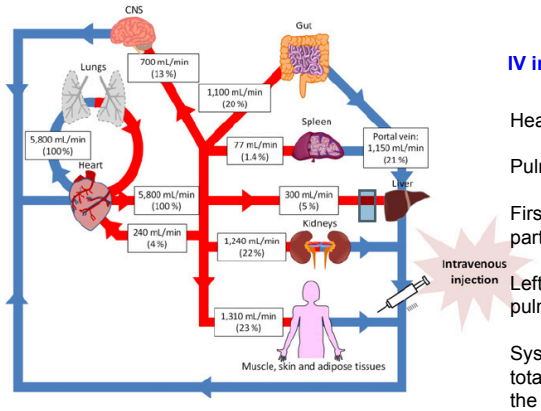
Entering the systemic circulation
Interactions with blood
Filtration by the kidney
Capture by the liver
Sieving through the spleen

Remaining in the blood circulation

Bertrand and Leroux, JCR, 2012
David R, BASF

VIII. Pharmacokinetic/biodistribution profiles of np

Entering the systemic circulation



IV injection: most reproducible way

Heart

Pulmonary circulation

First sieving by lung capillarires for particles >3µm

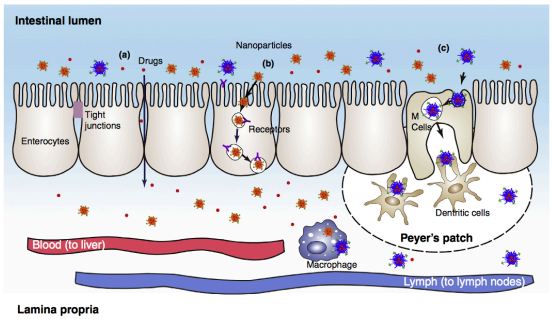
Left ventricle of the heart via the pulmonary veins

Systemic circulation where the total cardiac output is chared by the different organs

Bertrand and Leroux, JCR, 2012

VIII. Pharmacokinetic/biodistribution profiles of np

Entering the systemic circulation



Oral administration

-Diffusion through the mucus layer

- Contact with enterocytes and/or M-cells

- Uptake

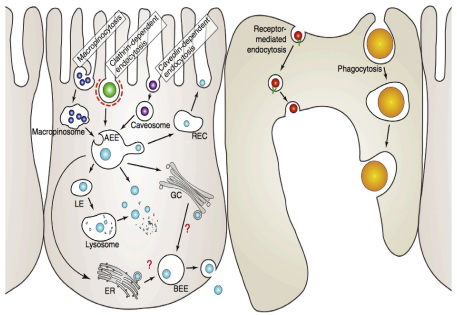
Larger particles remains within the submucosa or Galt of the intestine and colon
Smaller entered the bloodstream.
Surface charge influence the absorption. Better for non-ionic particles

Ye and al, Drug Discovery Today, 2016
Bergin and Witzmann, Int J. Biomed Nanoscience and Nanotech, 2013

VIII. Pharmacokinetic/biodistribution profiles of np

Entering the systemic circulation

Oral administration



Uptake :
cellular entry
paracellular transport

Most common mechanism: endocytosis

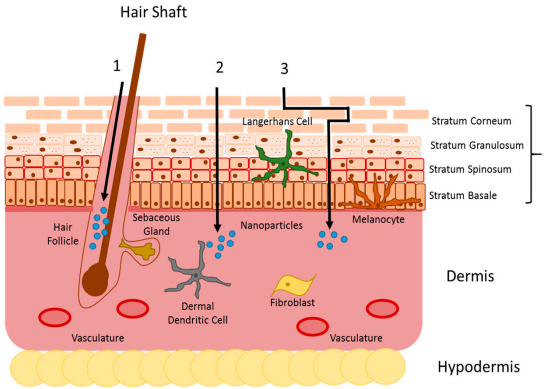
Greater absorption of smaller polystyrene particles (50nm) compared to larger (100nm).
>300 nm were not absorbed

Ye and al, Drug Discovery Today, 2016

VIII. Pharmacokinetic/biodistribution profiles of np

Entering the systemic circulation

Skin deposition



Appendageal route
Intracellular route
Intercellular route

Palmer et al, Molecules, 2016

Size, shape and charge play a role in dermal penetration

VIII. Pharmacokinetic/biodistribution profiles of np

Interactions with blood

Blood cells: red blood cells, leukocytes, platelets

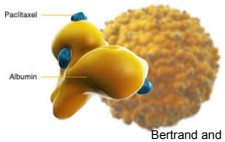
Plasma proteins

Interactions with plasma proteins
Guided by the physicochemical properties
Influence their circulation time and deposition in tissues
Albumin, lipoproteins, proteins of the complement and others

Albumin

Rapid
Non-specific interactions
Ionic and hydrophobic

Albumin-based paclitaxel np: Abraxane
- improved tolerance
- enhanced responses

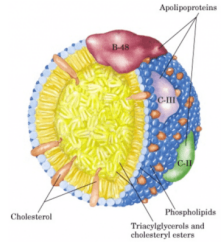


VIII. Pharmacokinetic/biodistribution profiles of np

Interactions with blood

Lipoproteins (HDL/VLDL) and apolipoproteins

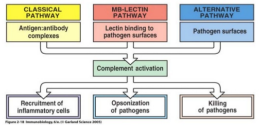
Responsable of lipid transport in bloodstream
Interactions with hydrophobic np
Exchange of PL between liposomes and lipoproteins
Triggering of the release of they payload



Proteins of the complement

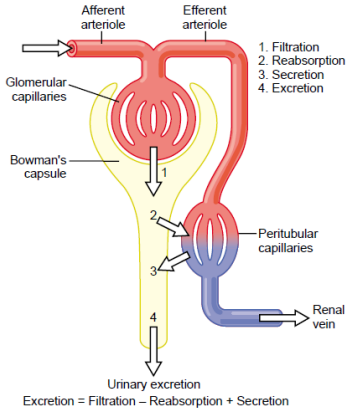
Abundant interactions with np (around 3g/L of proteins)
Activation of the complement cascade… immune response
Triggering of np phagocytosis
Depends on np properties

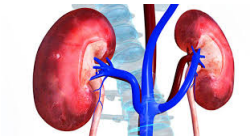
The complement cascade



VIII. Pharmacokinetic/biodistribution profiles of np

Filtration by the kidney





Kidneys are responsible for blood filtration

Proteins < 5-6 nm are filtered out by kidney: renal clearance

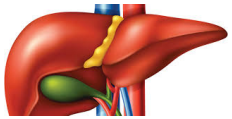
Most of np too large to be filtered

Soluble np filtered through the glomerulus can be reabsorbed (PEG-protein). Limited data

VIII. Pharmacokinetic/biodistribution profiles of np

Capture by the liver

Macrophages: Kupffer cells with phagocytic activity



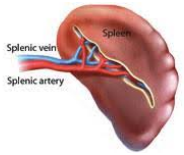
Defence system

Mainly phagocytosis

Various parameters influence internalization: size (upper size limit for phagocytosis around 20µm), shape, flexibility, deformability, surface properties

Sieving through the spleen

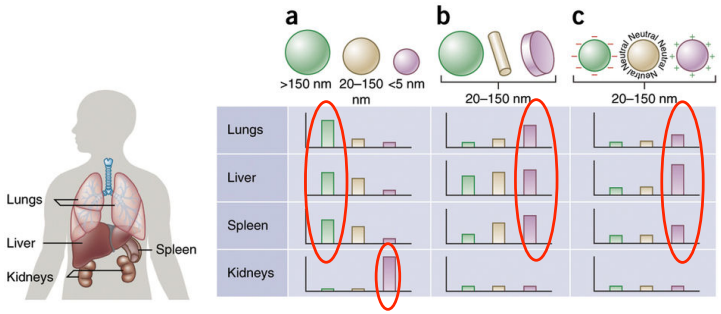
Highly irrigated organ



Sequestration for np with high rigidity, large size (>200 nm, irregular shapes…)

Concentration of np in liver and spleen

VIII. Pharmacokinetic/biodistribution profiles of np



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

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

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

…

To design a DDS it is important to know

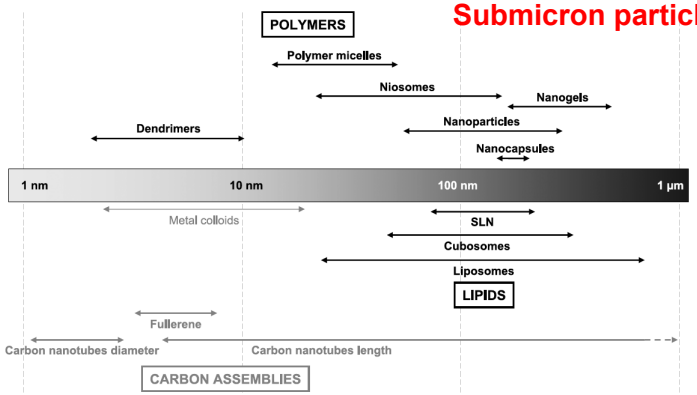
Which drug (physicochemical properties)?
How much drug is needed?
At what delivery rate?
Over what period of time (duration)?
With what bioavailability?
Acting at which sites or on which cells?

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).

IX. Classification

Submicron particles

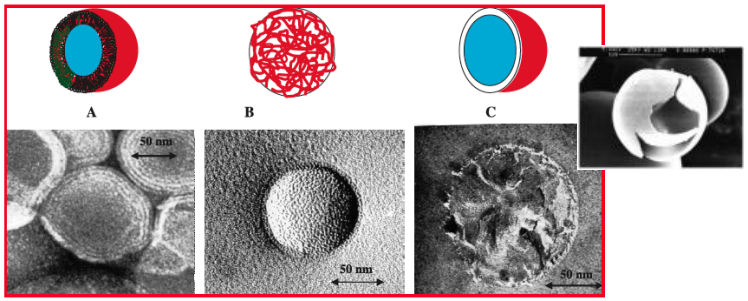


Nanoparticles

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

Classification: Structure

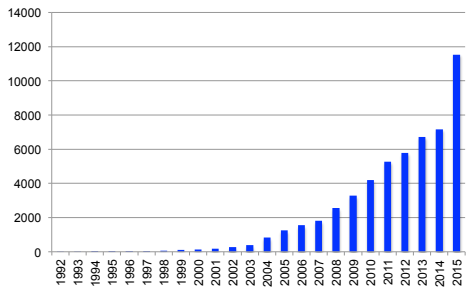
Capsules / Spheres
Reservoir / Matrix



Andrieux et al., l' actualité chimique, 2003

SCIENTIFIC PUBLICATIONS on nanoparticles

Number of publications

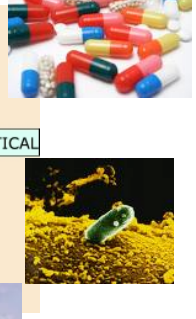


Nanoparticles in the title
PubMed
19825 for 2016 (sept., 2017)

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…

Weissig et al, Int J Nanomedicine, 2014

Application markets



http://www.authorstream.com/presentation/amitmgupta31-147397-micro-encapsulation-amit-gupta-acp-lecture-wardha-education-ppt-powerpoint/

FORMULATION PROCESSES

Béatrice Heurtault

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I

POLYMERIC PARTICLES

Composition in brief

Polymers

- natural products: gelatin, albumin, polysaccharides, collagen, cellulose

- synthetic products: acrylic, cyanoacrylic, PLA, PLAGA

Lipids

- phospholipids: liposomes

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

Metal

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

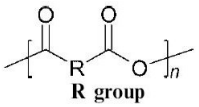
Poly(2-hydroxy ethyl methacrylate).
Poly(acrylic acid).
Polyacrylamide.
Poly(ethylene-co-vinyl acetate).
Poly(ethylene glycol).
Poly(methacrylic acid).

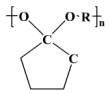
Chemically inert
Free of leachable impurities
Minimal undesired aging
Readily processing

Polymeric particles

Absorbable suture

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





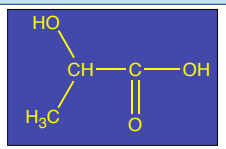
Biodegradable polymers

Metabolic pathways

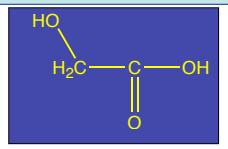
No need to remove
No adverse reactions

Biologically acceptable molecules

Polymeric particles

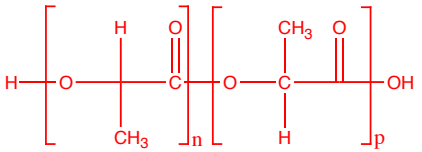


Lactic acid

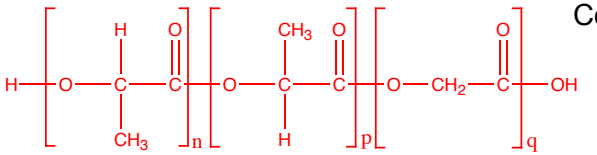


Glycolic acid

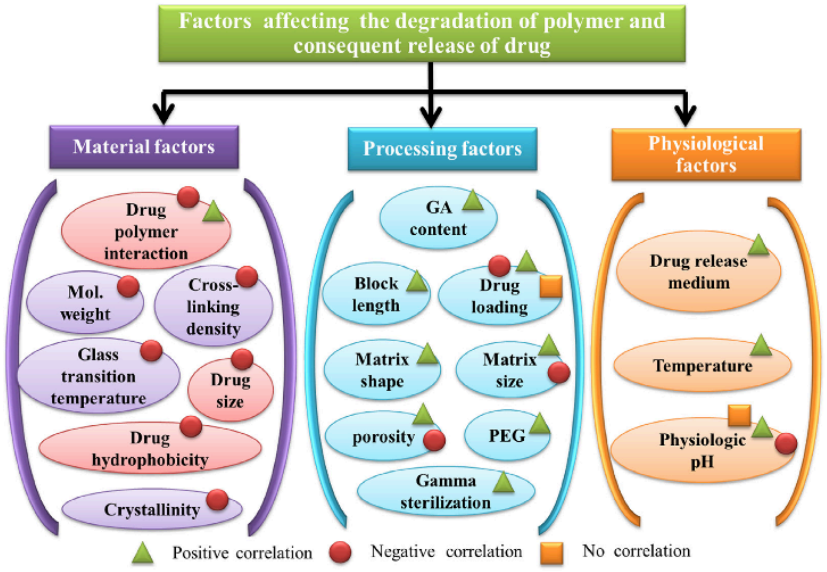
Intensive use in DDS



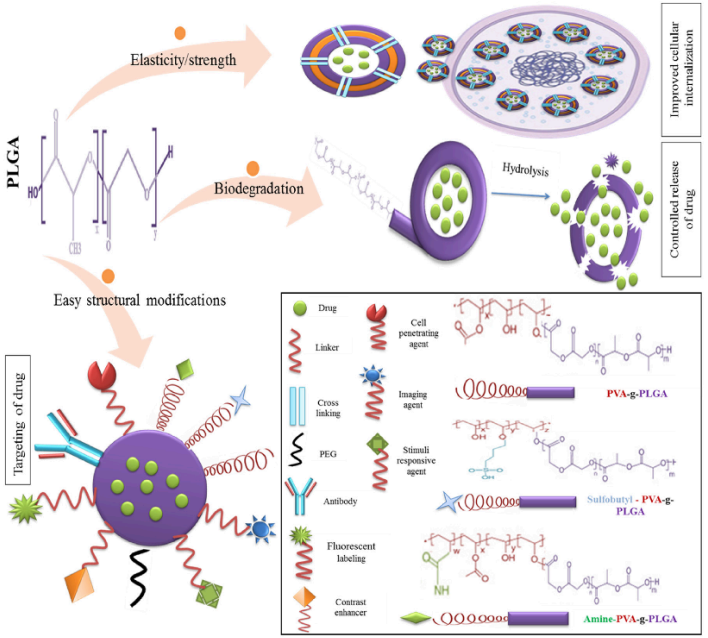
Lactic acid homopolymer (PLA)



Copolymer (PLGA)

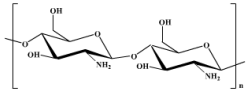


Rehman et al, Colloids and suface B, 2017

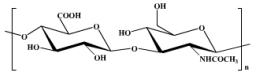


Polymeric particles

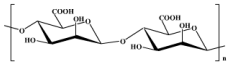
Natural polymers



Chitosan



Hyaluronic acid



Alginic acid

PREPARATION OF POLYMERIC NANOPARTICLES

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

* From monomers
§ From preformed polymers

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…)

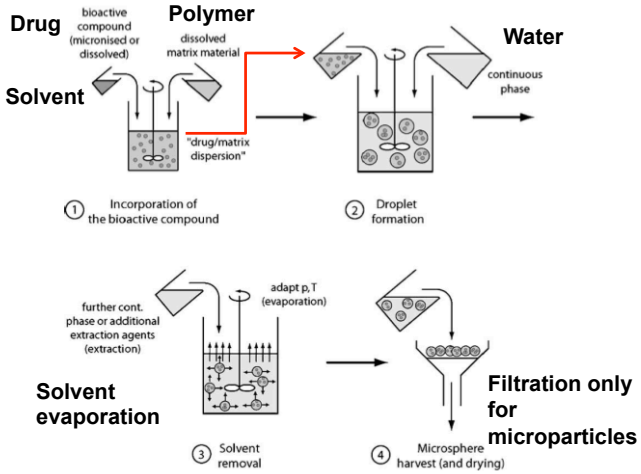
Homogenization, sometimes sonication

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

The polymer precipitates

Formation of micro-or nanospheres instantaneously, containing the drug

SOLVENT EVAPORATION



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

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

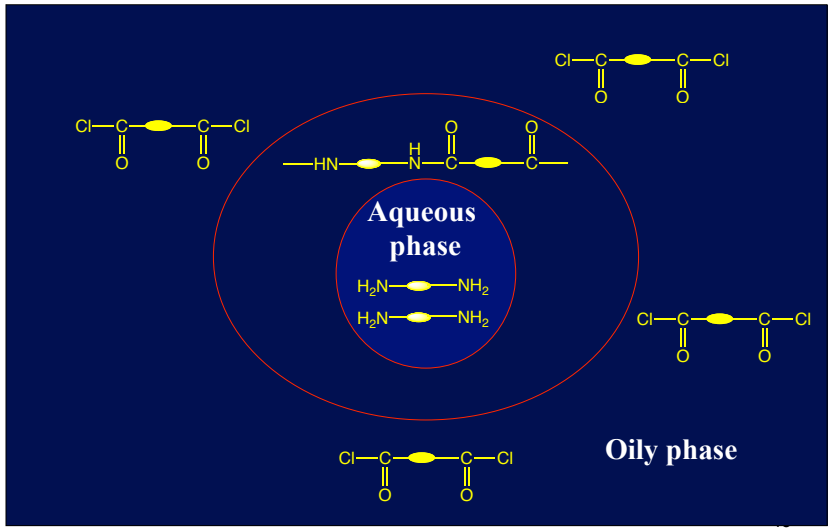
The reaction mixture is poured into the water phase, which contains surfactant under moderate stirring conditions

Nanocapsules are formed instantaneously

The organic solvent is then removed under reduced pressure

Partial removal of water also occurs

INTERFACIAL CONDENSATION



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

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

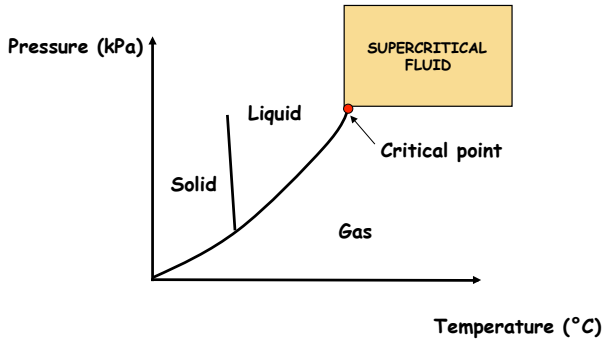
Diamine + dichloroformiate : polyurethane
Dialcohol + diacid chloride : polyester
Diamine + diisocyanate : polyurea
Diamine + diacid chloride : polyamide

SUPERCRITICAL FLUID

SUPERCRITICAL FLUIDS

Phase diagram

Liquid and gas together

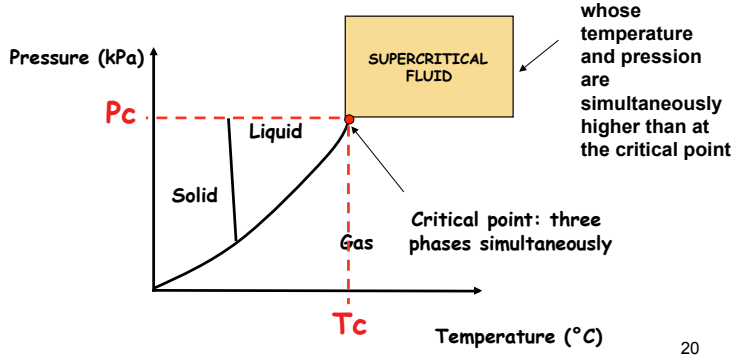


DEFINITION

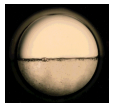
Critical temperature : the temperature above which the substance can no longer exists as a liquid no matter how much pressure is applied

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

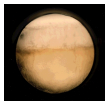
Substance whose temperature and pression are simultaneously higher than at the critical point



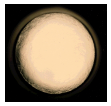
EXAMPLE OF SUPERCRITICAL CO_2



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

PROPERTIES OF SUPERCRITICAL FLUIDS

Liquid have solubilizing nature
Gases have diffusivity and compressibility / Expandable

	Density (kg/m³)	Viscosity (cP)	Diffusivity (mm²/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

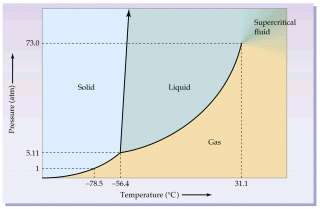
SCFs :

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

Substituant of organic solvents.

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



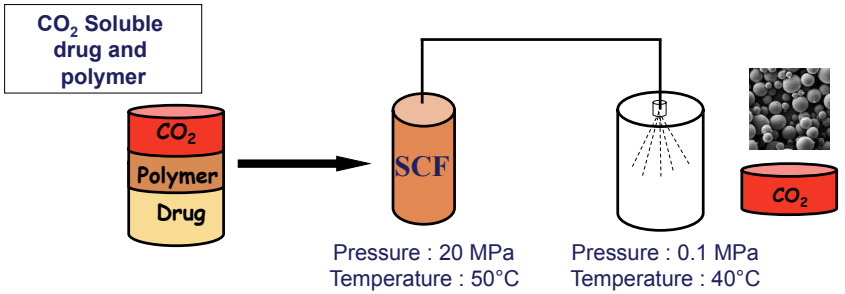
Supercritical CO_2 is a non-polar **solvent** with dissolution properties that are comparable to **hexane**

But not a universal solvent…

APPLICATIONS TO MICROENCAPSULATION

Principle : Solubility modification with the pression : solidification

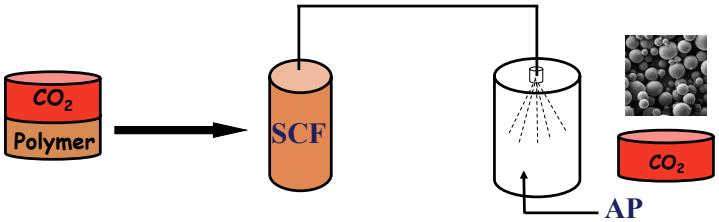
Polymer or polymer and drug dissolution in the fluid
Decrease of pressure allowing the fluid to go through a nozzle of pulverisation.



APPLICATIONS TO MICROENCAPSULATION

CO_2 Soluble polymer

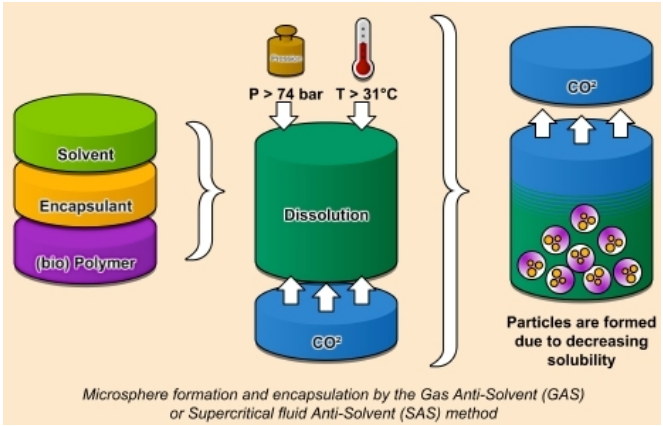
Decrease of pression allowing the fluid to go through a nozzle of pulverisation



Pressure : 20 MPa
Temperature : 50°C

Pressure : 0.1 MPa
Temperature : 40°C

SUPERCRITICAL FLUIDS ANTI-SOLVENT



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

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.

Nano and microparticles

II

LIPID NANOPARTICLES

SOLID LIPID NANOPARTICLES

Lipophilic colloidal delivery system

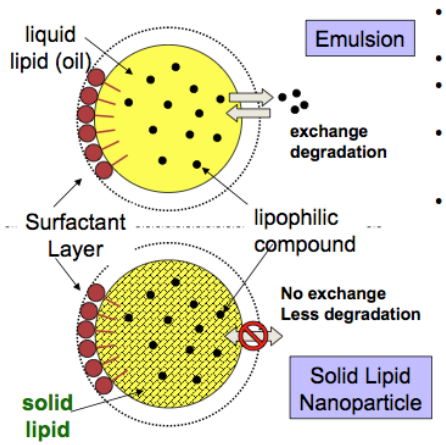
Efficient and non-toxic drug carrier specially for lipophilic drug molecules

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.

DIFFERENCE BETWEEN EMULSION AND SLN



SOLID LIPID NANOPARTICLES

Polymer 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

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

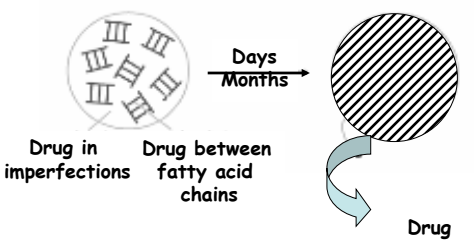
Cold high pressure homogenisation (Muller et al.)
- same process

Precipitation from microemulsions (Gasco et al)

- precipitation from microemulsions

Solid Lipid Nanoparticles

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



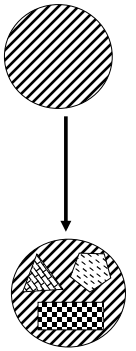
- insufficient loading capacity
- high water content of dispersions (70-99.9%)

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

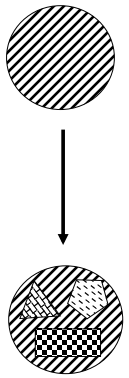


Nanostructured Lipid Carriers (NLC)

Multiple oil nanodroplets in solid fat nanoparticles

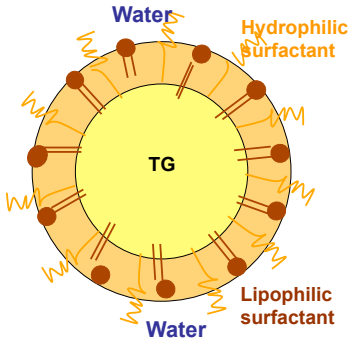
Not « just mixing » solid lipids but controlled

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



Shidhaye, Curr Drug Deliv, 2008

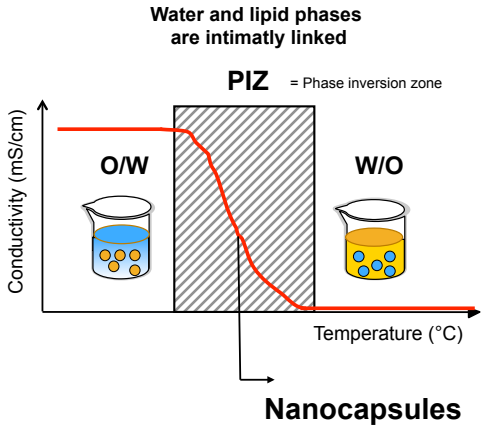
Lipid nanocapsules



25-100 nm
Stability
Solvent-free process
Lipophilic core

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**

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)

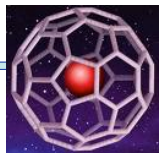
P-gp: P-glycoprotein; LNC: lipid nanocapsules; PEG: polyethyleneglycol; s.c.: subcutaneous; i.t.: intratumoral; i.v.: intravenous; Mab: monoclonal antibodies; TfR: transferrin receptor; BCECs: brain cerebral endothelial cells; CED: convection-enhanced delivery; ^{188}Re-SSS: $^{188}Re(S_2CPh)_2(S_2CPh)$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

Huynh et al, Int J Pharm, 2009

III

CARBON NANOPARTICLES

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

CARBON NANOTUBES (1991)

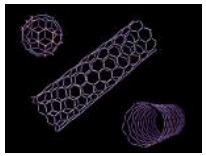
Only a few nm's in diameter, a single-walled carbon nanotube can grow as long as several micrometers

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



IV

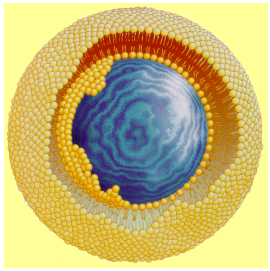
LIPOSOMES

Introduction

Liposomes described by Bangham in 1961 (1964).

Used as models of cellular membranes.

Interest as DDS during the past 40 years

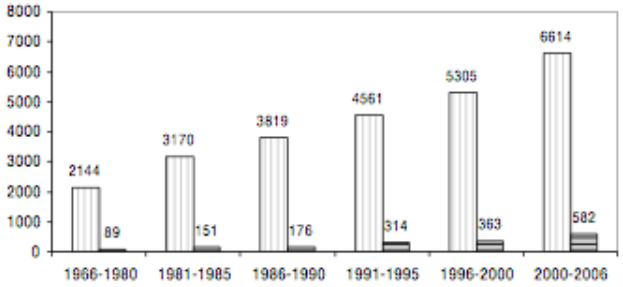


Synthetic structures

Microscopic phospholipid bubbles

Aqueous core

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

COMPOSITION

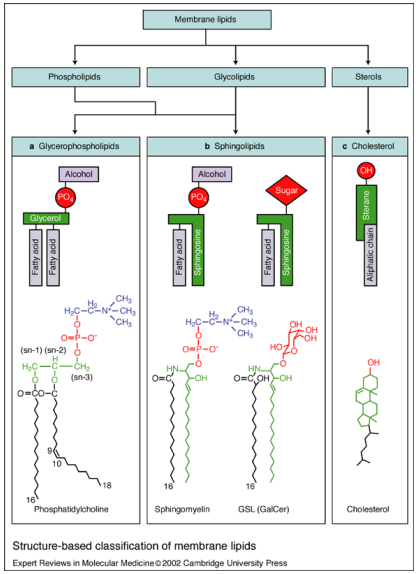
Composition of liposomes

Diacyl phosphoglycerides
- **Phosphatidylcholine**

Sphingolipids

Sterols
- **Cholesterol**

Fatty acids



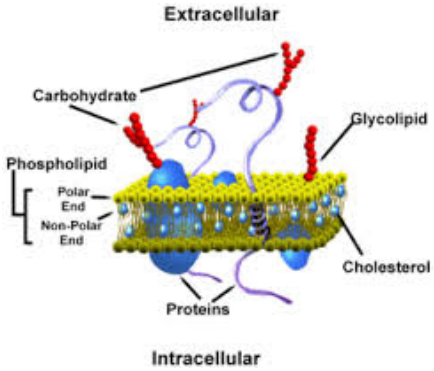
Structure-based classification of membrane lipids

Liposomes and membrane cells

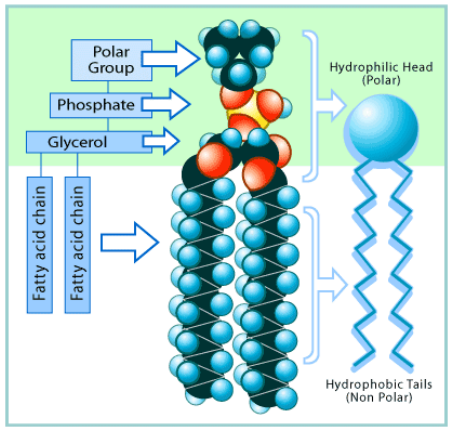
Same composition as membrane cells

Models:
- **structure**
- **function: diffusion of solutes**

Low toxicity



Phospholipids



Main components

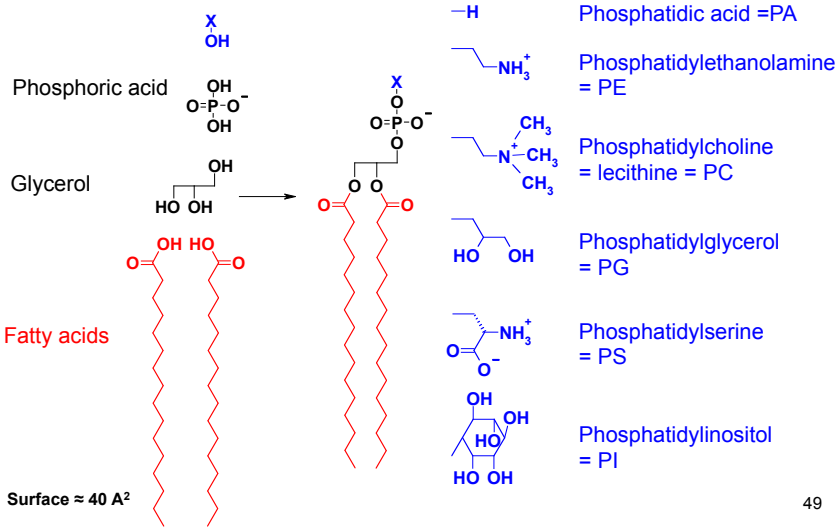
Fatty acids
Carbon chain length,
Insaturation and their number

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

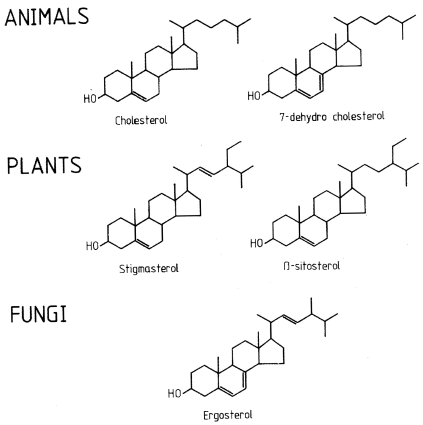
Addition of charged phospholids for stability
Amphiphilic nature of phospholipids

Sensibility to phospholipases

Phosphoglycerides (50%)



Sterols



Main sterols found in natural membrane

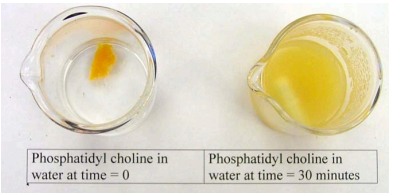
Cholesterol

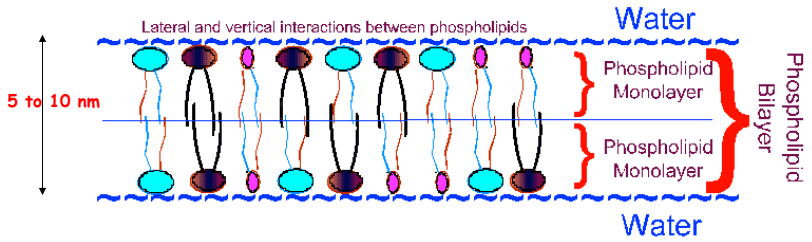
Proportion 1:1 to 1:2 (cholesterol/PC).

ORGANISATION

Phospholipids organization

Amphiphilic molecules
Spontaneous bilayer in water



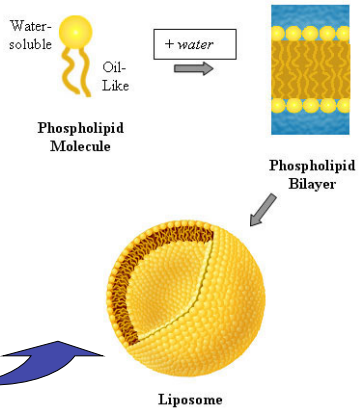


Interactions:

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

3-D organization

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



Minimisation of **un**favorable interactions

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

Phospholipids and membrane properties

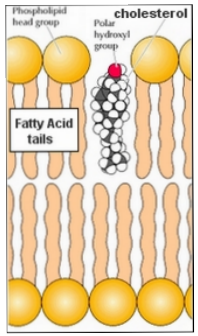
The shape of the membrane depends on the lipid structure

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_c 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.

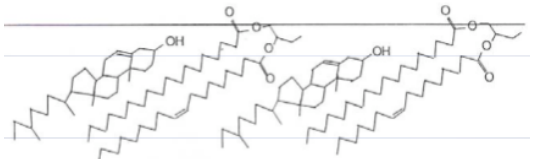
Cholesterol



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.

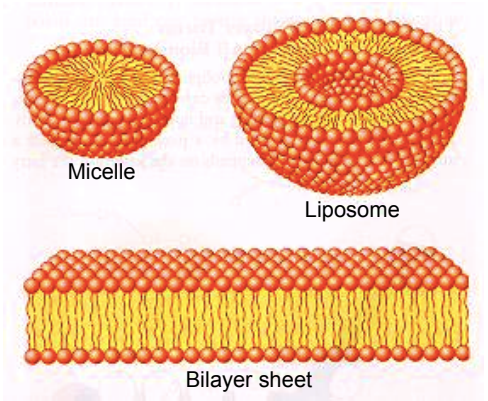
Around 50% cholesterol/phospholipids

No bilayer if only sterols.



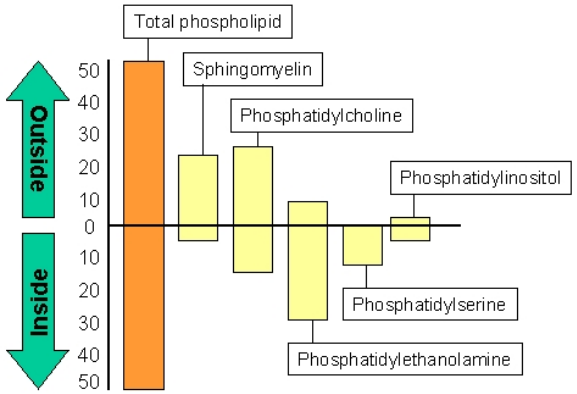
Liposomes vs micelles

Single chain lipids



Double chain lipids

Distribution of the phospholipids in the membrane

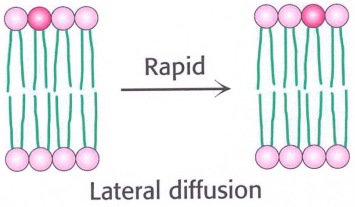


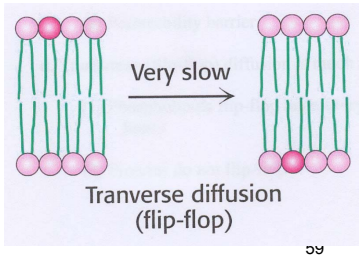
DYNAMIC STRUCTURES

Dynamic systems

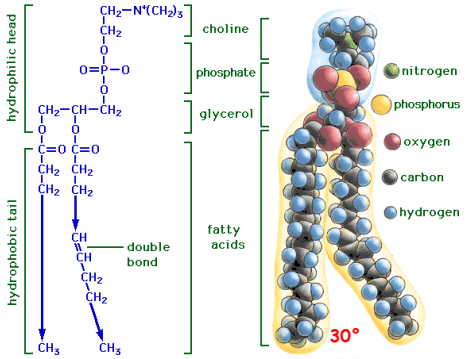
- **Lateral diffusion: fast (µs).**
- **Flip-flop: slow (h or days).**
- **It depends on**
  - **temperature**
  - **composition**

The permeability is related to these movements.





Phase transition temperature

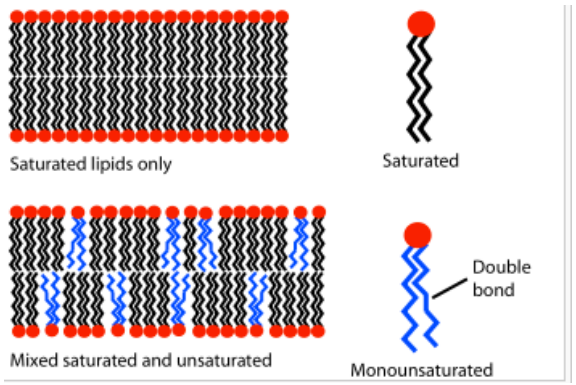


Double bond

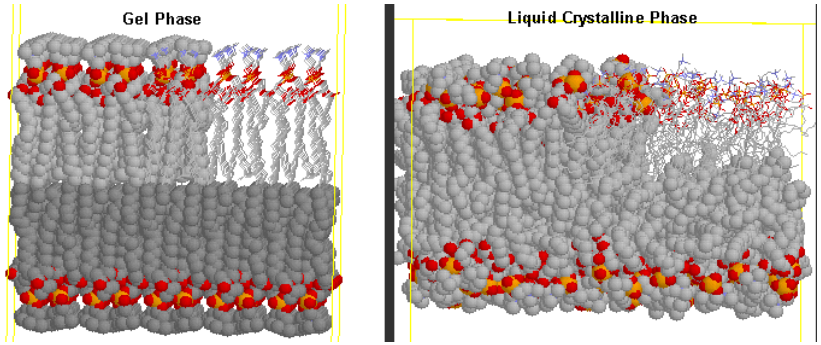
Formation of angle

Consequence on PTT

Phase transition temperature



Phase transition 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 : to be above the PTT

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

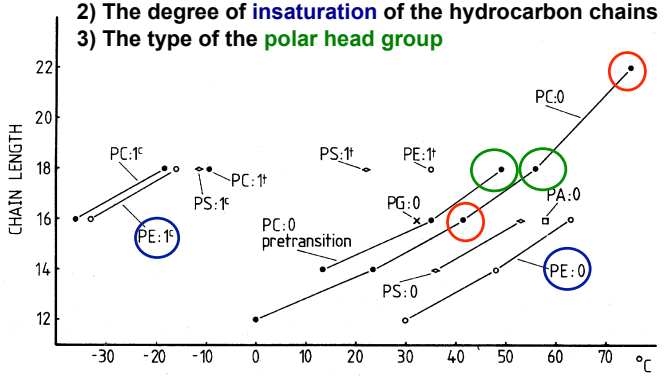


Figure 6. Phase transition temperatures for diacyl phospholipids with different headgroups as a function of chain length. Data from different sources is presented for synthetic phospholipids containing fatty acids of the same chain length and unsaturation in both 1- and 2- positions. Unless otherwise 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

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.**

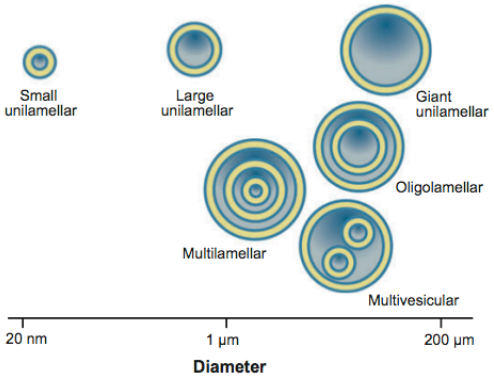
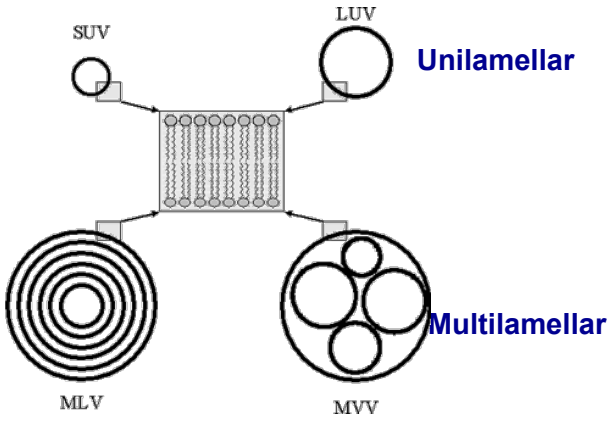


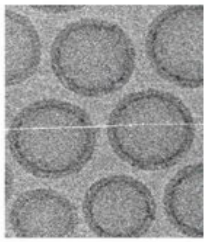
Figure 2

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



Cryo-TEM



Nanomedicine Laboratory, Dr Muller

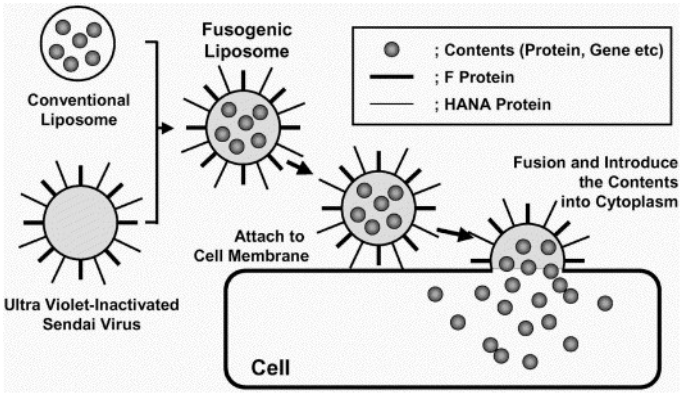
Classification based on composition and mode of drug delivery

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

- **pH sensitive 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;

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

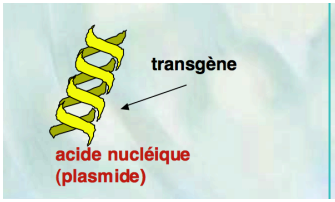
Kunisawa et al, ADDR, 2001

Classification based on composition and application

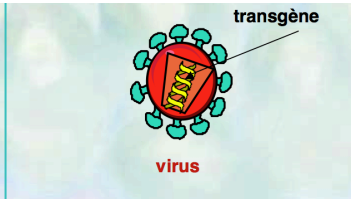
- **Cationic liposomes**
  - **Cationic lipids: DDAB, DOGS, DOSPA, DOTAP, DOTMA…**
  - **Possibly fuse with cell**
  - **or endosome membranes; suitable for delivery of negatively charges macromolecules (DNA, RNA..)**

Gene therapy

Non viral



Viral



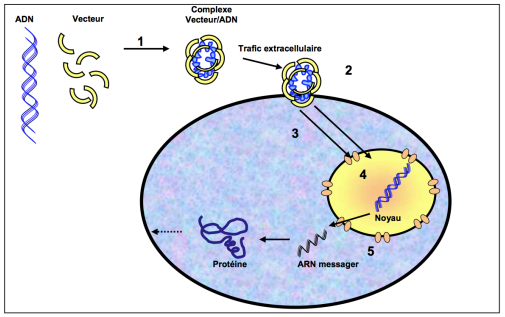
Liposomes, nanoparticles
Polycations, cationic lipids
No immune response
Less expensive

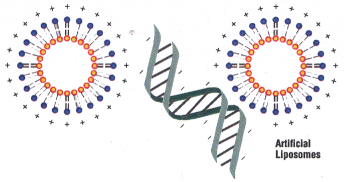
Classification based on composition and application

Liposomes = cationic lipids (+ neutral lipids)

Interaction with nucleic acids negatively charged

DNA compaction = liposomes-DNA complexe





Classification based on composition and application

- **Immunoliposomes**

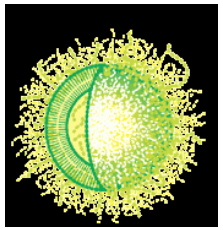
  - Liposomes with attached antibody or recognition sequence.

  - 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.

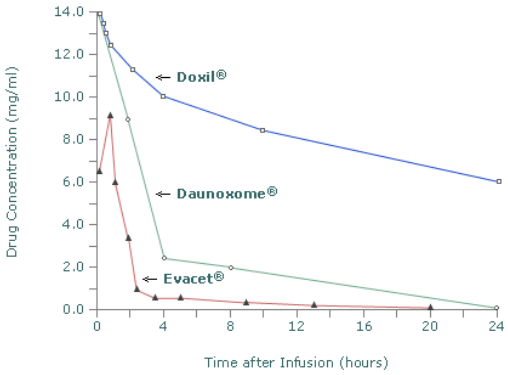
Classification based on composition and application

- **Stealth liposomes**
  - **Presence of PEG (polyethyleneglycol) in the basic composition**
  - **Hydrophilic surface coating; low opsonization and low rate of uptake by MPS (mononuclear phagocyte system; long circulation half-life;**



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

Classification based on composition and application



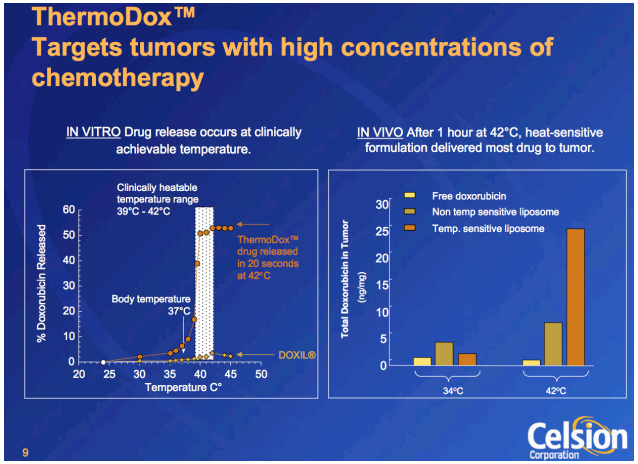
Daunoxome®: Liposomes of daunorubicine
Evacet®: liposomes of doxorubicine
Doxil®: stealth liposomes of doxorubicine

http://www.alza.com/alza/stealth_more.

ALZA's patented STEALTH® technology

Classification based on composition and application

- **Thermoliposomes**

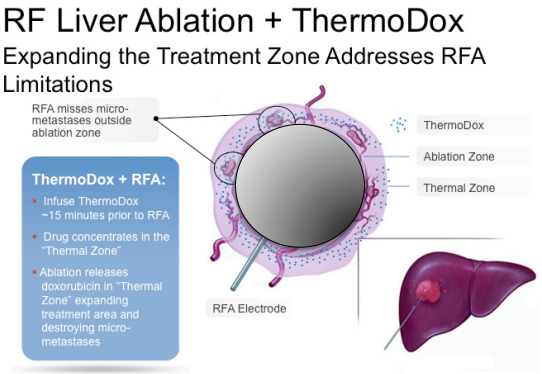


The LTSL formulation was composed of DPPC:MPPC:DSPE-PEG-2000 in the molar ratio of 90:10:4
Needham [CANCER RESEARCH 60, 1197-1201, March 1, 2000]

Classification based on composition and application

- Thermoliposomes

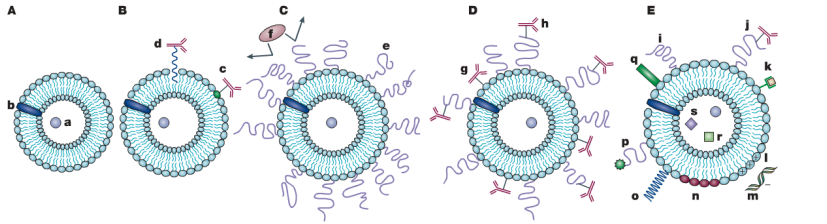
RF : radiofrequency ablation



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
…

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

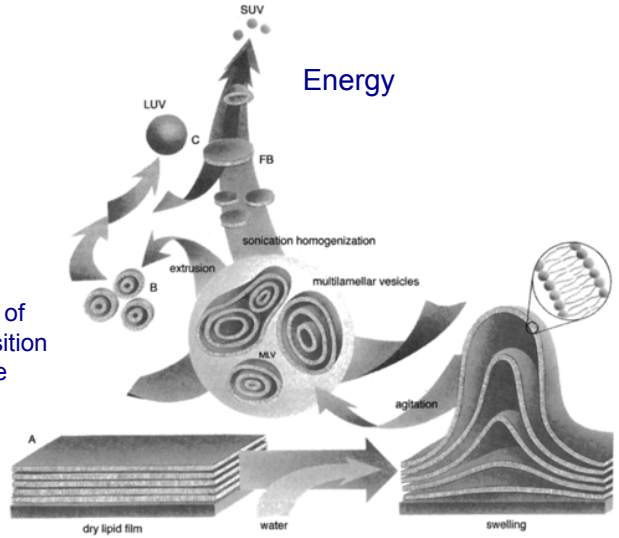
Deshydration-Rehydration: DRV

Frozen and thawed: FATMLV

METHODS OF PREPARATION

Lipid film hydration: MLV

Lipid film hydratation



Importance of phase transition temperature

Lipid film hydration

ADVANTAGES

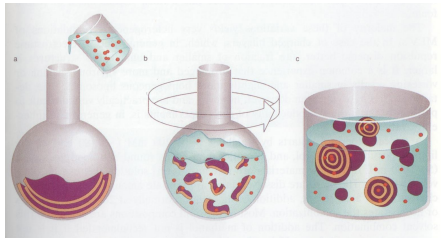
Simple
Fast

DRAWBACKS

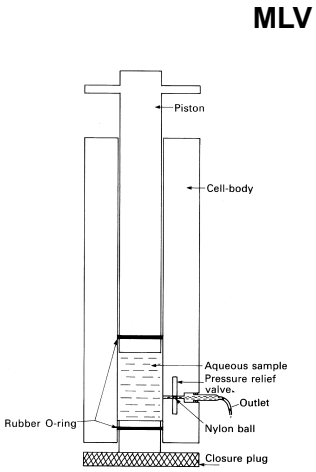
Low encapsulation rates

Heterogeneous sizes (MLV)

Not industrial production



French press: extrusion



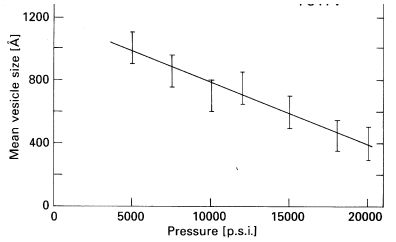
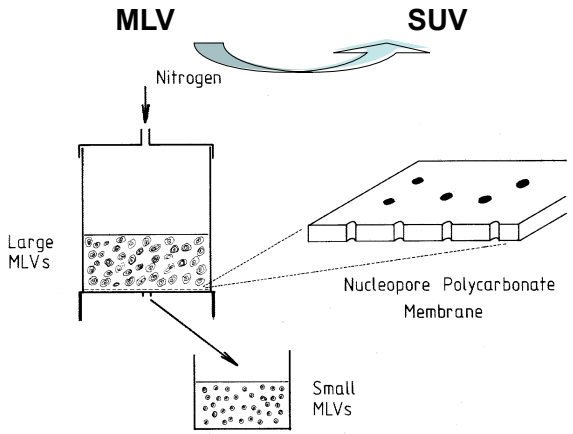


Figure 6. French press technique. (**a**) Diagram of a French pressure cell. Small vesicles are obtained when phospholipid dipsersions are extruded through the small orifice (lower right) at pressures of 20 000 p.s.i. or greater. (**b**) Graph showing relationship between pressure and liposome diameter.

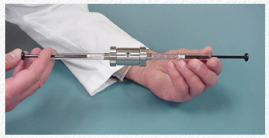
Temperature increases
Several passages
MLV rupture: SUV

Extrusion membrane



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

LiposoFast



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

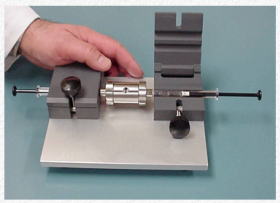
Click here to see a short video demonstrating this product.

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

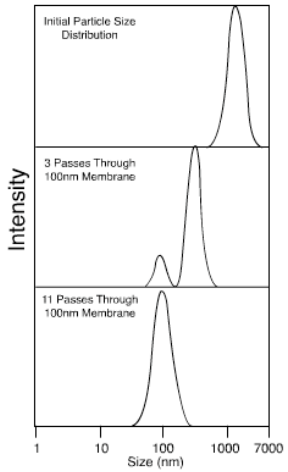
1. Principle of Operation
The LiposoFast-Stabilizer was designed to simplify the repetitive use of the LiposoFast-Basic as well as the extrusion of highly concentrated samples. It accommodates both 0.5mL and 1.0mL syringes.

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.

LiposoFast-Basic (www.avestin.com)

LiposoFast



Number of passages
Decrease the diameter
Increase the homogeneity

11 passages recommanded

French press/membrane extrusion

ADVANTAGES

Rapid
Simple
Reproducible
Non agressive
High encapsulation rates
Industrial production

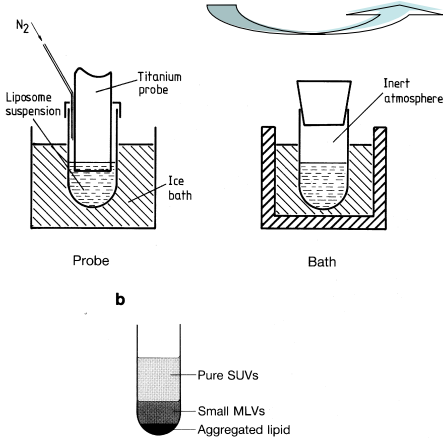
Sizes related to the membrane pores

DRAWBACKS

Price
MLV prior to SUV
Low encapsulation volumes

Sonication

MLV **SUV**



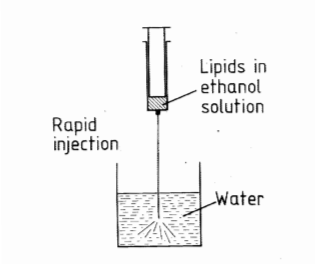
ADVANTAGES

Homogeneous diameters

DRAWBACKS

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

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

Reverse phase evaporation

REV: reverse evaporation phase vesicles

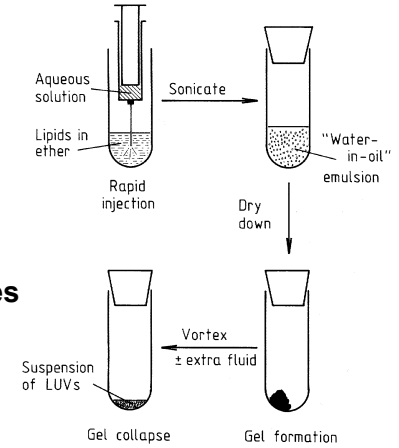


Figure 15. Stages in the preparation of liposomes by reverse phase evaporation. After formation of a water-in-oil emulsion by sonication of the aqueous solute in an organic solution of lipids, the organic solvent is evaporated off to yield a gel. The gel then collapses either naturally, as drying is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

Reverse phase evaporation

PL solubilisation in organic solvent (ether)

Addition of aqueous phase (1/3) containing drug

Phospholipids at the interface

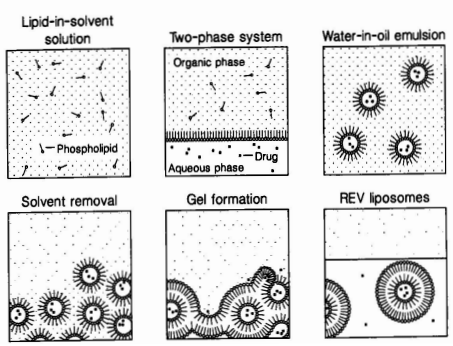
Sonication leading to water-in-oil emulsion

Solvent evaporation: globule concentration

Gel formation

Complete evaporation

Gel destruction/vortex



Unilamellar vesicles. 500 nm

Common preparation techniques

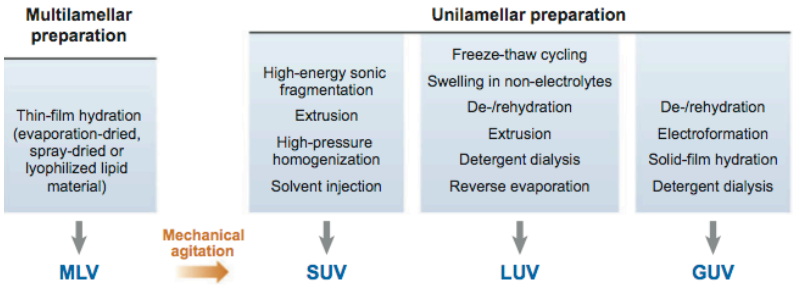


Figure 4

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.

EQUIPMENT FOR NANOPARTICLES FORMULATION

Ultra sonicator

Homogenizer

Nanosizer
Zetasizer

Evaporator

NANOPARTICULATE DDS PROPERTIES FOR PHARMACEUTICAL AND MEDICAL APPLICATIONS

Béatrice Heurtault

European School On Nanosciences and Nanotechnologies
2017

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1. SIZE

Relation size/surface/number

For 100 ng spherical particles of unit density

Particle diameter (nm)	Particle number	Surface area (mm^2)
2	2.4×10^{13}	300
20	2.4×10^{10}	30
1000	190 000	0.006

x 500 ; x 13×10^3 ; x 50 000

The volume of a sphere is proportional to the third power of the radius ($V = 4/3\pi r^3$), while the surface area is proportional to the second power ($SA = 4\pi r^2$). Hence, the surface area to volume ratio is inversely proportional to the radius. This has numerous effects on the nature of the particles and their functioning.

Relation size/surface

Consequences

Interaction through the surface with physiological medium, cells (cell membranes, red cells, tissues…) impacts:

- drug release increases with a decrease of diameter
- tissue retention increases with an increase of diameter
- biodistribution
- efficacy
- toxicity
- stability
- …

Relation size/surface

Nano/Microparticulate Drug Carriers

Microparticulate carriers

Controlled drug release
Protection against chemical, physical, enzymatic degradation

Nanoparticulate carriers

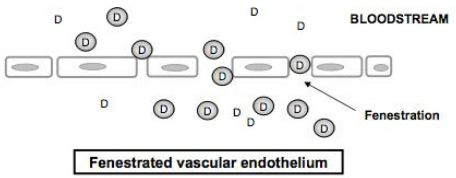
Controlled drug release
Protection
Biologically interacting functions
Phagocytosis, endocytosis, membrane permeability, cell targeting
Intravenous injection

Relation size/surface

Impact of size after IV administration

After IV administration

<20-30 nm: renal excretion
>30 nm: capture by the mononuclear phagocytic system cells: macrophages (liver, spleen, bone marrow)
30-150 nm: bone marrow, heart, kidney, stomach
150-300 nm: liver, spleen



Gaumet et al, EJPB, 2008

Organ or pathological situation	Fenestration size	Animal model
Kidney	20–30 nm	Guinea-pig, rabbit, rat
Liver	150 nm	Mice
Spleen	150 nm	Mice
Lung	1–400 nm	Dog
Bone marrow	85–150 nm	Guinea-pig, rabbit, rat
Skeletal, cardiac and smooth muscle	⩽6 nm	Mice
Skin, subcutaneous and mucuous membrane	⩽6 nm	Mice
Blood–brain barrier	No fenestrations	–
Tumor[a]	200–780 nm	Mice
Brain tumor[b]	100–380 nm	Rat
Inflamed organs	80 nm–1.4 μm	Hamster

I.V.: Biodistribution depends on size as well as « patients », pathologies…

Relation size/surface

Impact of size after in vivo SC administration

After SC administration

- nanoparticles
Vaccine delivery.
Contact with immune cells.
Possible diffusion

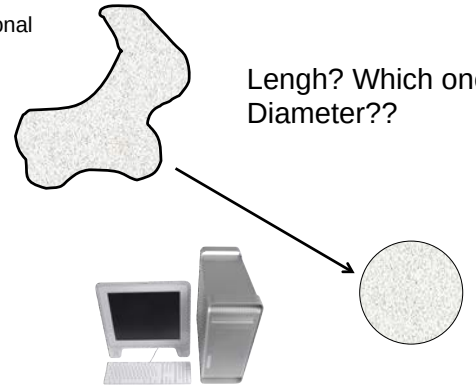
- microparticles
Local anesthetics: controlled release can achieve and maintain high local drug levels resulting in nerve blockade, while minimizing systemic distribution, which would cause systemic toxicity.

Systemic therapy: injections of human growth hormone microspheres.
Depot formulation
naltrexone microspheres: abstinence from alcohol
risperidone microspheres: schizophrenia

SC: Diffusion (distribution) and site of action depends on size

Size and shape measurements

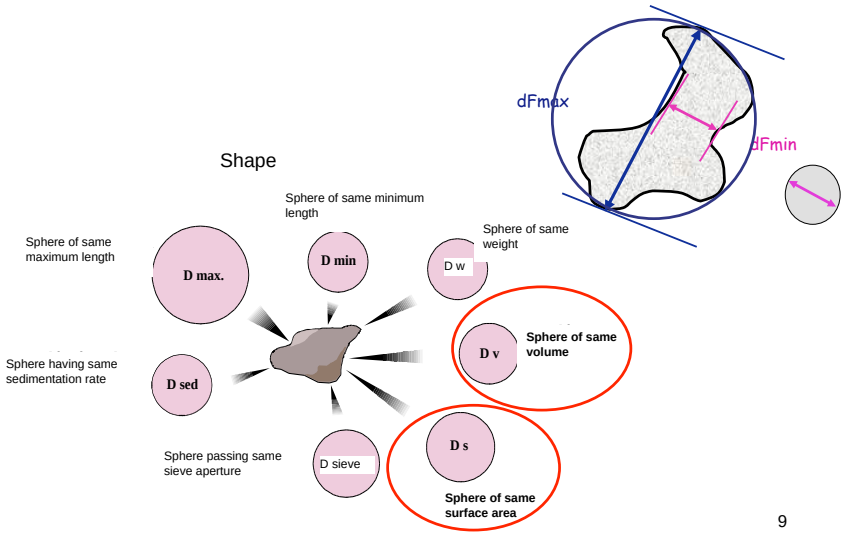
3 dimensional objects



Lengh? Which one?
Diameter??

Size reported as the diameter of the equivalent sphere»

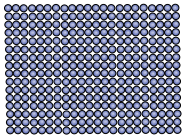
Size and shape measurements



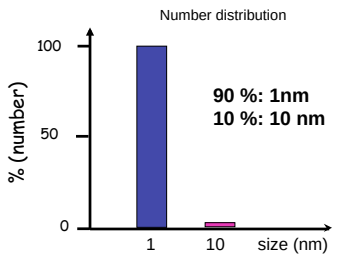
Size distribution

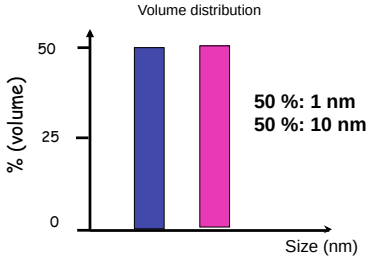
1000 particles = 1 particle

Rarely homogeneous sample!



Same sample!!!





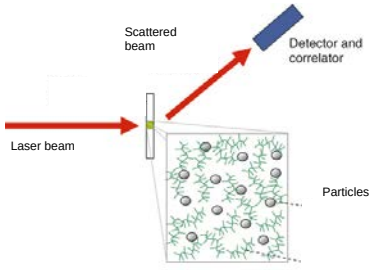
Size measurements

Photon correlation spectroscopy = quasi-elastic light scattering (QELS)

Brownian motion (movement in random direction)
Measurement as a function of time
Smaller particles move with higher velocity than larger particles

$D = K * T / 3\pi\eta d$

Stockes Einstein



Adapted from : http://www.esrf.eu/UsersAndScience/Publications/Highlights/2005/SCM/SCM2

K : constant
T : temperature
d : diameter
η : viscosity
D: diffusion coefficient

Diffraction of laser beam
Fluctuations in scattering intensity of the laser at a certain angle. Fluctuation depends on the speed which is related to the size
Calculation of the correlation function: diffusion coefficient
Conversion into particle size
Hydrodynamic diameter

Conclusion

Size

When working with nanoparticles

- **Average diameter** is essential AND

- « Type » of **diameter expression**: volume, number, intensity

Intensity measurement corresponds to the « true » value directly obtained from the correlation function. The other are deduced, through several assumptions (which can or not introduce a large bias, depending on the PDI of the sample)

- **Size distribution**: width, polydispersity index, variation coefficient

100 nm +/- 20 nm low PDI CV = 20/100=0.2
1000 nm +/- 20 nm low PDI CV=0.02

2. ZETA POTENTIAL

Zeta potential

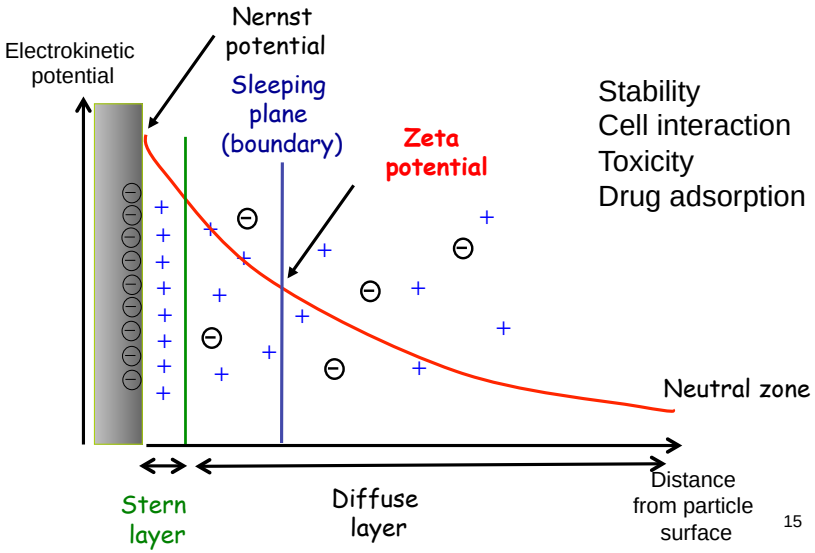
Particles in suspension usually carry an **electrical charge**.

Zeta potential **reflects** the charge of particles

The charge is more often negative than positive (chemical groups, adsorbtion of ions…).

The charges on the surface of each particle is counterbalanced by charges (ions) of opposite sign in the sourrounding solution: **counterions**.

Zeta potential



Stability
Cell interaction
Toxicity
Drug adsorption

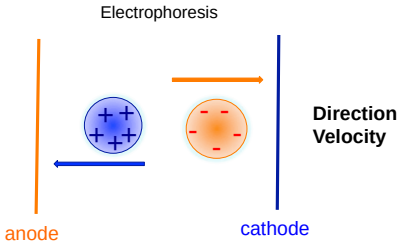
Measuring Zeta potential

Application of an electric field to the suspension

Attraction of the particles toward the electrode of opposite charge

Electrophoretic velocity as a function of the charge

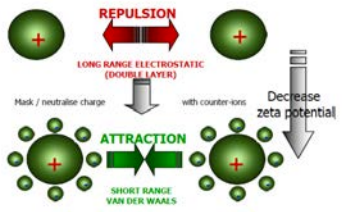
Measurement of the velocity for the determination of the zeta potential



Value depends on the:

Concentration of ions
Type of ions (mono or divalent one)

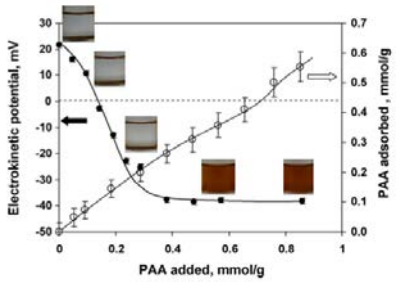
Zeta potential and colloidal stability



Malvern Instruments

Large negative or positive zeta potential

Low zeta potential



Magnetite nanoparticles (+)
Polyacrylate addition (-)

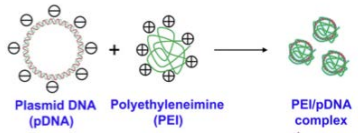
/30 mV/

Particle aggregation must be avoided for *in vivo* applications

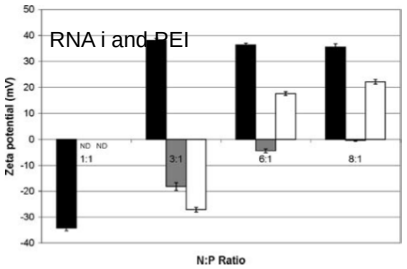
Hajdù et al, Colloids and surfaces B: Biointerfaces, 94(2012)242-249

Zeta potential and drug adsorption

Transfection: DNA or RNA delivery to cell nucleus

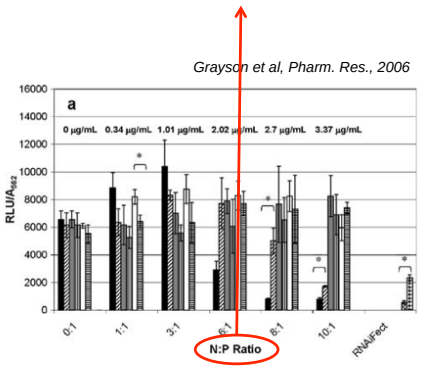


Park et al, Biomaterials, 2012



N/P (nitrogen/phosphate) ratio (PEI/DNA)
Transfection as a function of N/P ratio

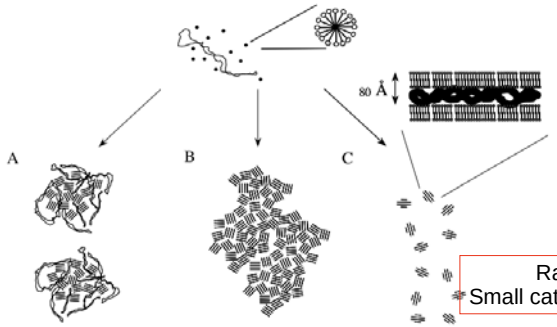
Grayson et al, Pharm. Res., 2006



Results as a function of N/P ratio (as a function of zeta potential)

Zeta potential and drug adsorption

Structure depends on the ratio between the cationic charge of the vector and the anionic charge of DNA



Ratio > 1
Small cationic particles

Ratio < 1
Limited association

Ratio = 1
Neutrality induces aggregation

Zeta potential and cellular uptake

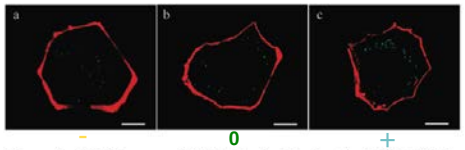
Surface charge affects cellular uptake and intracellular trafficking of **chitosan-based nanoparticles**
Yue et al, Biomacromolecules, 2011, 12, 2440-2446

Table 1. Zeta Potential, Average Diameter, and Polydispersity of As-Prepared NPs

nanoparticles	N-NPs	M-NPs	P-NPs
ζ potential (mV)	−45.84 ± 2.18	0.51 ± 1.31	39.25 ± 2.68
average diameter (nm)	215.70 ± 2.91	214.27 ± 1.36	216.12 ± 3.57
polydispersity	0.054 ± 0.0051	0.059 ± 0.0038	0.052 ± 0.0042

\- 0 +

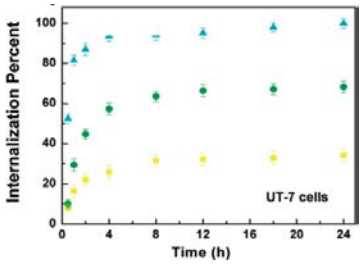
Intracellular trafficking



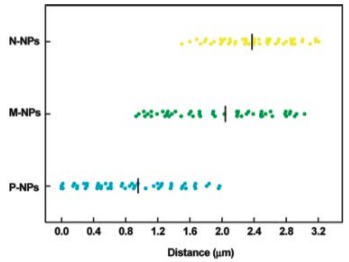
\- 0 +

Figure 5. CLSM images of HKC cells after incubated with (a) N-NPs, (b) M-NPs, and (c) P-NPs for 12 h. Cell membrane was labeled by rhodamine-phalloidin. NPs were tested by their autofluorescence and showed in pseudocolor here. Scale bar: 10 μm.

Internalization amount



Distance between nanoparticles and nucleus



Conclusion

Size

When working with nanoparticles, you have to measure:

Average diameter AND size distribution (width, polydispersity index, variation coefficient)

Expression of results: in intensity, volume, number?

Conclusion

Size

When working with nanoparticles, you have to measure:

Average diameter AND size distribution (width, polydispersity index, variation coefficient)

Expression of results: in intensity, volume, number?

Zeta potential

Value

Medium for measurement and if possible in physiological medium

Impact on: stability, drug adsorption, cell interaction/uptake, toxicity…

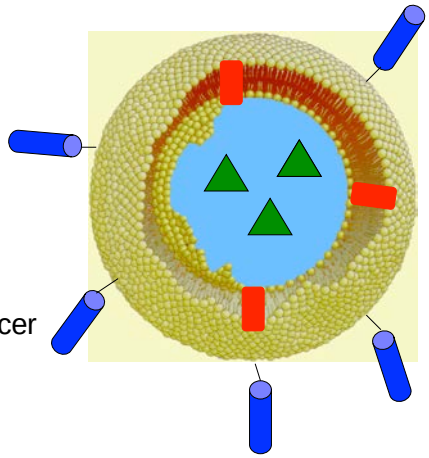
3.
ENCAPSULATION
DRUG RELEASE MECHANISMS

Drug association

bilayer insertion

encapsulation in the aqueous core

covalent ligation via spacer



Encapsulation efficiency

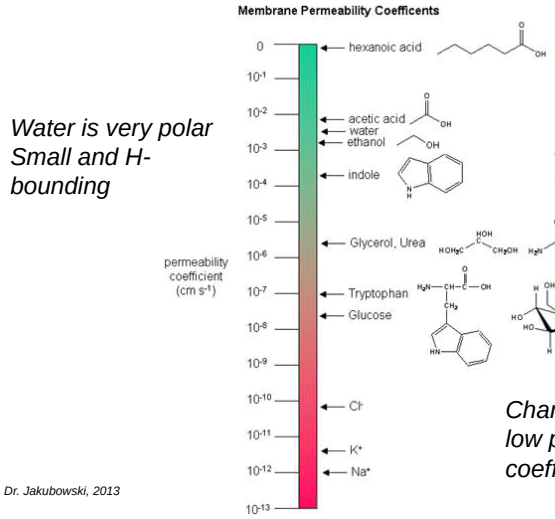
Internal volume and encapsulation efficiency of liposomes

Liposome Type		Internal Volume (μL/ μmole lipid)	(μL/ mg lipid)	Efficiency	Encapsulation Reference
MLV	(i) Hydration Method*	1-4	--	10-25	Hauser, 1982
	(ii) Solvent Spherule Method	0.3-2.7	0.5-4.0	0.3-1.6	Kim et al., 1985
SUV	(i) Sonication Method	0.02-0.05	--	0.1-1.0	Hauser, 1982
	(ii) French Pressure Method	0.2-1.5	--	--	Hauser, 1982
Multivesicular Liposome		10-79	15-127	11-89	Kim et al., 1983
Giant Liposomes		20	--	--	Oku et al., 1982
LUV	(i) REV Method	--	0.5-15.6	35-65	Szoka and Papahadjopoulos, 1978
	(ii) Modified REV Method	--	--	< 80	Handa et al., 1987
	(iii) Freeze Thaw Method	< 10	--	20-30	Pick, 1981
	(iv) Microfluidization Method	0.69-1.03	--	5-75	Mayhew et al., 1984
	(v) Extrusion through poly-carbonate filters under nitrogen	1.1-2.4	--	--	Hope et al., 1985

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.

Permeability of liposome bilayers

- Solubility
- Size
- Charge



Water is very polar Small and H-bounding

Little molecule, high permeability coefficient

Hydrophobic molecule, high permeability coefficient

Charged molecules, low permeability coefficient

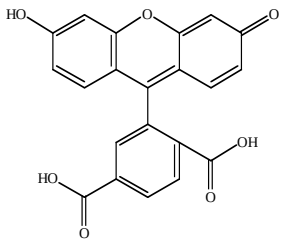
Dr. Jakubowski, 2013

Membrane integrity

Stability and controlled release

5,6 Carboxyfluorescein as reference

Small molecule



Fluorescence as a function of concentration

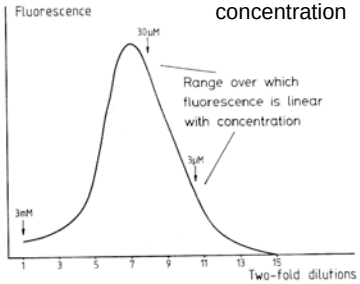
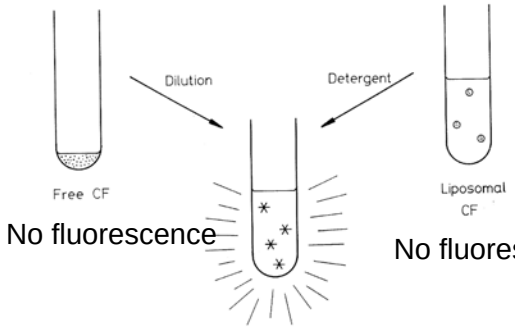


Figure 12. Change in carboxyfluorescein fluorescence with increasing dilution. Self-quenching of carboxyfluorescein is not completely relaxed until the solution has been diluted to 30 μmol concentration. For accurate quantitative work, it is important to carry out determinations at a concentration range below 30 μM. (Data provided by R.R.C.New and R.E.Stringer.)

Membrane integrity



No fluorescence

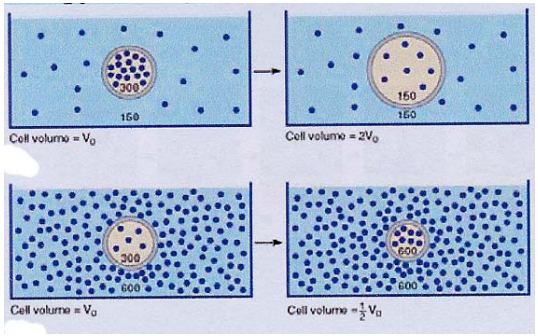
No fluorescence

Fluorescence

Isotony

Hypotonic solution : swelling and breaking-up

Hypertonic solution: decrease of the size



During formulation
During characterization

Encapsulation. Association

In nanoparticles the drug is either

- dissolved
- entrapped
- adsorbed
- attached
- encapsulated in the macromolecular material

A lot of release mechanisms

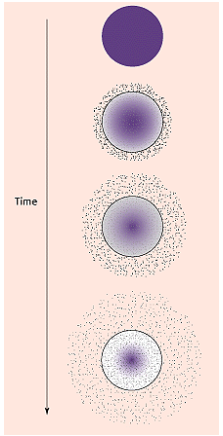
Most important release mechanisms

Diffusion

Degradation

Swelling followed by diffusion

Controlled-released mechanisms. Diffusion



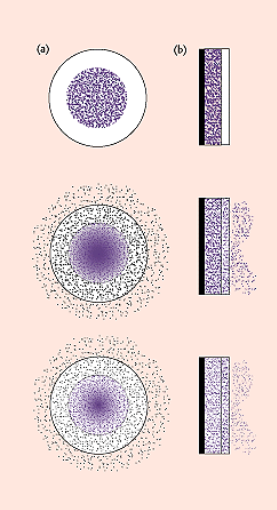
Initially an homogeneous system: matrix

Drug passes from the polymer matrix into the external environment

Decreased release rate: active agent has a progressively longer distance to travel

Diffusion from matrix drug delivery system

Controlled-released mechanisms. Diffusion



A reservoir surrounded by a film or membrane of a rate-controlling material.

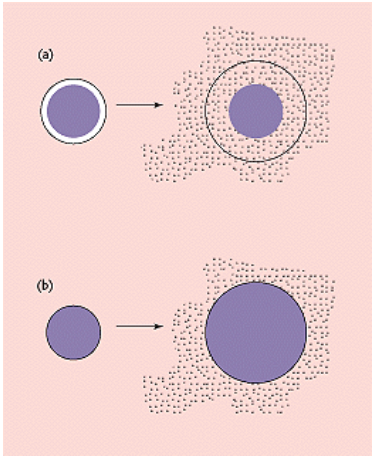
In the reservoir: solid drug, dilute solution or highly concentrated drug solution

Polymer layer: limits the release.

Drug delivery remains fairly constant.

Diffusion from typical reservoir devices (a) implantable or oral systems, and (b) transdermal systems

Controlled-released mechanisms. Swelling



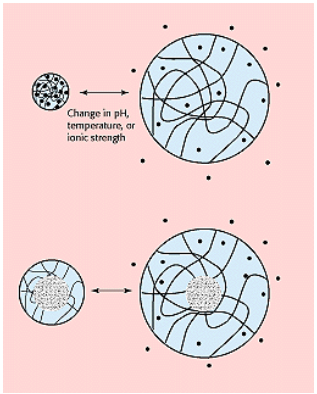
Drug delivery from (a) reservoir and (b) matrix swelling-controlled release systems

Absorbtion of water
Swelling
Diffusion of the drug through the swollen

Hydrogels

Controlled-released mechanisms. Swelling

Environmentally sensitive release systems



Swelling triggered by a change in the environment

Sometimes: reversible phenomenon

Environmentally sensitive systems

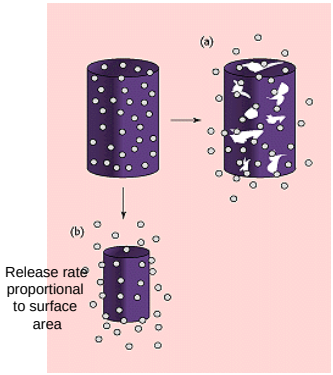
Stimulus	Hydrogel	Mechanism
pH	Acidic or basic hydrogel	Change in pH — swelling — release of drug
Ionic strength	Ionic hydrogel	Change in ionic strength — change in concentration of ions inside gel — change in swelling — release of drug
Chemical species	Hydrogel containing electron-accepting groups	Electron-donating compounds — formation of charge/transfer complex — change in swelling — release of drug
Enzyme-substrate	Hydrogel containing immobilized enzymes	Substrate present — enzymatic conversion — product changes swelling of gel — release of drug
Magnetic	Magnetic particles dispersed in alginate microshperes	Applied magnetic field — change in pores in gel — change in swelling — release of drug
Thermal	Thermoresponsive hydrogel poly(N-isopro-pylacrylamide)	Change in temperature — change in polymer-polymer and water-polymer interactions — change in swelling — release of drug
Electrical	Polyelectrolyte hydrogel	Applied electric field — membrane charging — electrophoresis of charged drug — change in swelling — release of drug
Ultrasound irradiation	Ethylene-vinyl alcohol hydrogel	Ultrasound irradiation — temperature increase — release of drug

« Intelligent » hydrogels

Sensibility to the environment

Reversibility and repetability as a function of the environment

Controlled-released mechanisms. Degradation



These particles will degrade through bulk hydrolysis in water or body fluids, yielding polymer fragments over time.

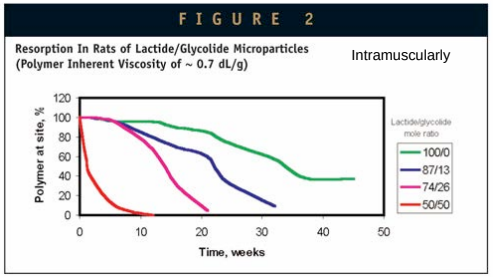


Figure 7. Drug delivery from (a) bulk-eroding and (b) surface-eroding biodegradable systems.

Lisa Branon-Peppas, nov 2007

Dr. Thomas Tice

Factors affecting biodegradation of polymers

Chemical structure
Chemical composition
Distribution of repeat units in multimers
Presence of ionic groups
Presence of unexpected units or chain defects
Configurating structure
Molecular weight
Molecular-weight distribution
Morphology (amorphous/semicrystalline, microstructures, residual stresses)
Presence of low-molecular-weight compounds
Processing conditions
Sterilization process
Storage history
Shape
Site of implantation
Adsorbed and absorbed compounds (water, lipids, ions, etc…)
Physicochemiacal factors (ion exchange, ionic strength, pH)
Physical factors (shape and size changes, variations of diffusion coefficients, mechanical stresses, stress- and solvent-induced cracking…
Mechanism of hydrolysis (enzymes versus water)
…

Lisa Branon-Peppas, nov 2007

4. MUCOADHESION

Definition bioadhesion/mucoadhesion

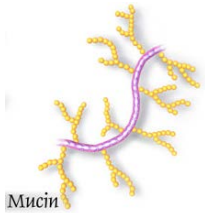
Maximizing the residence of the dosage vehicle in the stomach
- to solve a specific absorption window
- to localize drug delivery
- to facilate intimate contact with the underlying absorption surface

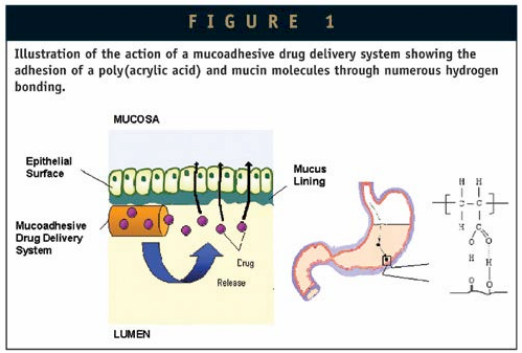
To improve and enhance the **bioavailability** of drugs

Ophtalmic/nasal drug delivery as well

Mucoadhesion itself is based on the intimate contact of a dosage form or individual particles with the mucus layer covering the epithelial surface which acts as the connecting link between the epithelial tissue surface and the adhesive

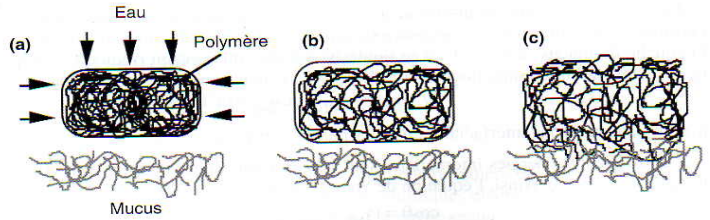
Adhesion





High molecular weight glycosylated protein
Negative charge (pH>2.6, sialic acid and sulfate)
Produced by many epithelial tissues

Adhesive mechanisms



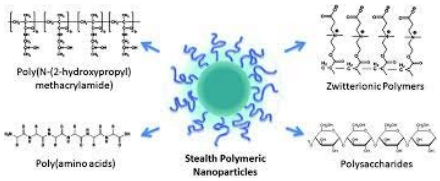
a. Water absorption
b. Swelling of the polymer network
c. Migration of polymer chains and mucus formation of a mixed layer leading to adhesive bond

Diffusion of polymers

Adhesive polymers/coating

Polysaccharides	Alginate Carraghenate Dextran Dextran sulfate Chitosan	Anionic Anionic Neutral Anionic Cationic
Cellulose derivatives	Carboxymethylcellulose* Hydroxypropylcellulose*	Anionic Neutral
Acrylic derivatives	Methyl polymethacrylate * Poly(acide acrylique)*	Neutral Anionic
Proteins	Gelatin Gliadin	Mixed Mixed

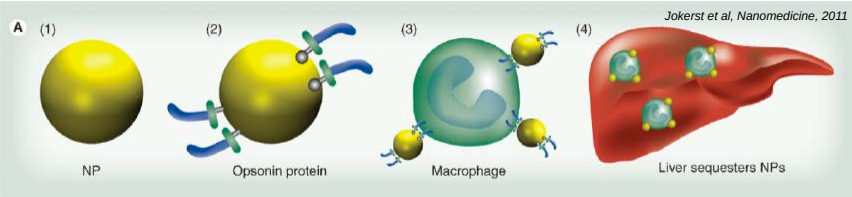
* Non biodegradable



5. STEALTH PARTICLES

Removal of nanoparticles

After IV administration of nanoparticles :



Liver, spleen

- Opsonization of np by blood proteins (binding on the surface np)
- Capture by macrophages
- Rapid concentration in liver and spleen : passive targeting

Opsonization

Prior binding ot complement proteins (opsonins: fibronectin, immunoglobulin…) at the surface of the particles, in particular via hydrophobic interactions.

Opsonin receptors located on the plasma membrane of the macrophages: phagocytosis of the particles by the MPS.

Natural protective mechanism to remove the virus and bacteria

However:

- rapid concentration in liver and spleen
- low circulation half-life
- potential toxicity on the MPS
- how target other organs?

Opsonization

Direct action to overcome opsonization

- Saturation of the macrophages activity by a first administration of empty DDS before drug-loaded particles

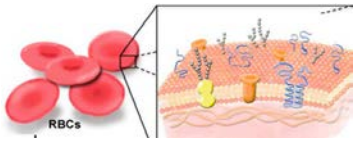
- Injection of phagocytic inhibitors such as gadolinium chloride or dextran sulfate

But it is a transitory effect and new Kuppfer cells are produced.

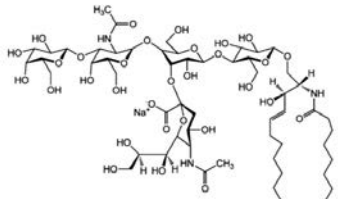
What about the red cells or some of bacteria escaping the RES?

Nature as model

Red cells

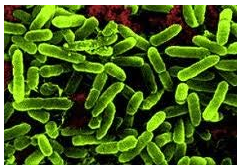


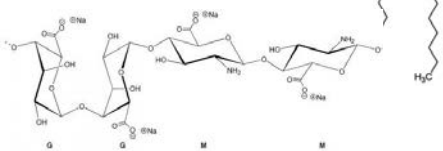
http://physicsworld.com/cws/article/news/2011/jun/24/nanoparticles-play-at-being-red-blood-cells



Monosialogangliosides (GM1)

Pseudomonas aeruginosa





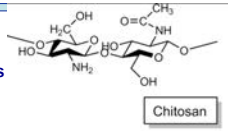
Polysaccharides

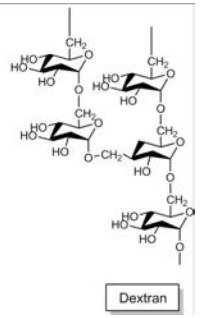
Hydrophilic molecules

Strategies to overcome opsonization

Surface modification: particle decoration
Pathogen-mimetic stealth nanocarriers

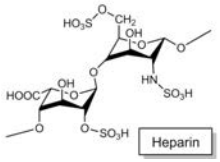
Dextrans, heparin, chitosan $T_{1/2}$: 3 min to 5 h



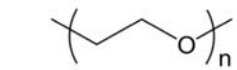


Poly(ethylene glycol) = PEG

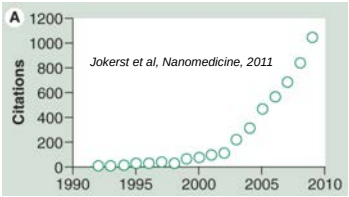
neutral
flexible
hydrophilic



Surface barrier layer
- PEG adsorption
- Covalent bound through PEG derivatives of biodegradable polymers (PLA, PLA/GA)
- Covalent conjugation to preformed nanoparticles through surface functional groups



Polyethylene glycol (PEG)



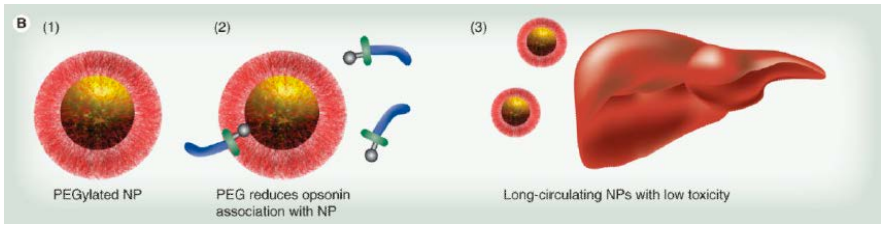
Stealth nanoparticles

Poloxamine (tetrablock) and poloxamers (triblock)

Block co-polymers: Hydrophilic blocks of ethylene oxyde (EO) and hydrophobic blocks of propylene oxide (PO) monomer units

Adsorption

After IV administration of stealth nanoparticles



50

Jokerst et al, Nanomedicine, 2011

Stealth versus non-stealth particles: SLN

Solid lipid nanoparticles

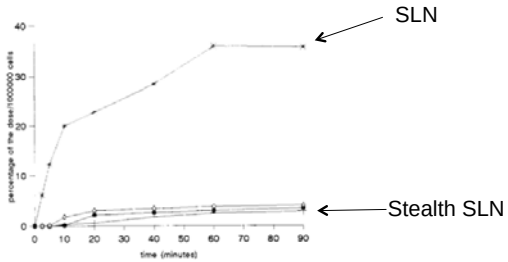


Fig. 2. Uptake of non-stealth SLN G and stealth SLN by macrophages. *, non-stealth SLN G; ◆, SLN BG with 0.15% of stearate-PEG 2000; +, SLN BG with 0.30% of stearate-PEG 2000; ■, SLN BG with 0.60% of stearate-PEG 2000. The percentages refer to the warm microemulsion.

Bocca et al, IJP, 1998

51

Stealth versus non-stealth particles: PLGA nanoparticles

Pharmacokinetic parameters of PLGA nanoparticles derived using the mean ($n = 3$) nanoparticle levels in blood versus time data[a]

Dose (μg polymer per mouse)	β (s^{-1})	$T^{\beta}_{1/2}$ (s)	V_{β} (ml)	AUC (μg s/ml)	Body clearance (ml/s)
63	0.05	13.00	2.33	506.4	0.12
125	0.04	17.24	3.02	1029.8	0.12
250	0.03	22.50	2.45	3316.3	0.08
750	0.02	35.00	3.03	12 487.6	0.06

[a] $T^{\beta}_{1/2} = 0.693/\beta$, V_{β} = dose/β AUC, Body clearance = dose AUC, AUC = (area of trapezoids from $t = 0$ to 60 s) + ($C_{60\ s}/\beta$).

Pharmacokinetic parameters of PLGA(32)–mPEG(5) nanoparticles derived using the mean ($n = 3$) nanoparticle levels in blood versus time data[a]

Dose (μg polymer per mouse)	k_{el} (h^{-1})	$T_{1/2}$ (h)	V_{darea} (ml)	AUC (μg h/ml)	Body clearance (ml/h)
150	0.10	7.08	1.79	845.5	0.18
300	0.11	6.45	1.52	1856.7	0.16
600	0.09	7.83	1.84	3618.5	0.16
1050	0.09	7.39	1.85	6040.2	0.17

[a] $T_{1/2} = 0.693/k_{el}$, V_{darea} = dose/k_{el} AUC, Body clearance = dose AUC, AUC = (area of trapezoids from $t = 0$ to 24 h) + ($C_{24\ h}/k_{el}$).

Circulation time is increased!

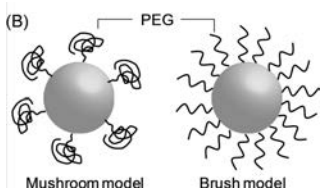
Panagi et al, IJP, 2001

PEG properties

Adsorption/Covalent bound

Density

Chain length
- Stability
- Drug release
- Half-life time



Amoozgar, Wiley Onterdiscip Rev Nanomed Naobiotechnol, 2013

6. TARGETING

Passive targeting

Passive targeting DDS uses the physicochemical properties (particle diameter, hydrophilic properties, etc.) of a carrier (transporter of the drug) to control behavior inside the body. Physiology.

Pathophysiological factors:	Inflammation/infection
	EPR effect
Physicochemical factors:	**Size**
	Molecular weight
Anatomical opportunities:	Catheterization
	Direct injection
Chemical approaches:	Prodrugs
	Chemical delivery systems

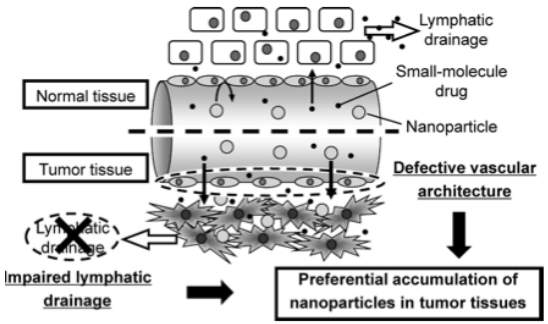
Tumor passive targeting: EPR effect

Tumor pathophysiology ≠ than that of normal tissues

- Extensive angiogenesis (to feed the tumor)
- Defective vascular architecture (fenestration)
- Impaired lymphatic drainage/recovery system

Passive drug targeting takes advantage of these properties

Passive targeting. EPR effect.

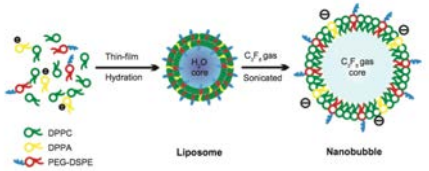


Enhanced Permeability and Retention effect

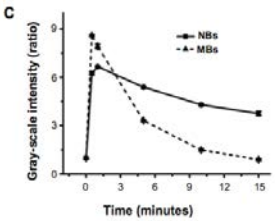
Passive targeting can therefore result in increase in drug concentrations in solid tumors of several-fold relative to those obtained with free drugs.

Ogawara et al, Biol. Pharm. Bull, 2013

Passive targeting for tumor imaging

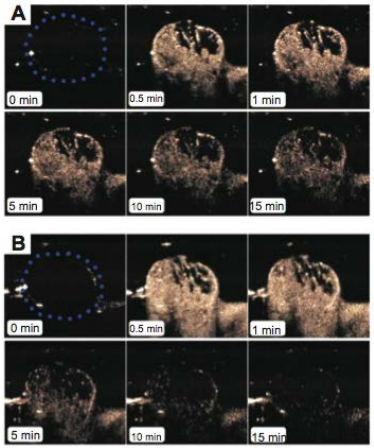


Formation of nanobubbles 400 nm (and microbubbles 1200 nm)-containing gas



Small diameter:
- permeation of the tumor vasculature
- absorbtion of high-frequency ultrasound

Ultrasonic imaging



Nano

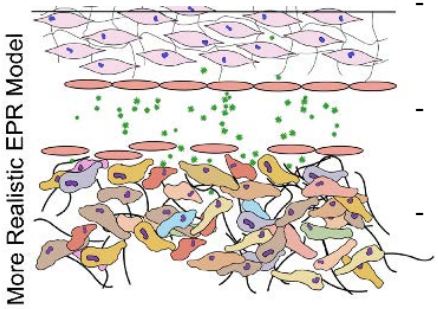
Micro

Ultrasound imaging of tumor

Nanoparticles are retained in the tumor for a longer period

Yin et al, Int. J. Nanomedicine, 2012

Limitations of EPR effect.



- low vascular permeability (pancreatic cancer)
- production of anti-PEG IgM induced by first dose
- Animal tumor models differed from clinical tumors
- Heterogenous tumors

Need for actively targeted DDS

Nichols and Bae, JCR, 2014

Active targeting

Active targeting DDS adds special mechanisms to the passive type to tightly control the directionality toward the target tissue.

For active targeting we use carriers combining ligands, e.g. antibodies, peptides, sugar chains, etc., that have specific molecular recognition features that can find target molecules of certain cells.

Specificity.

Active targeting

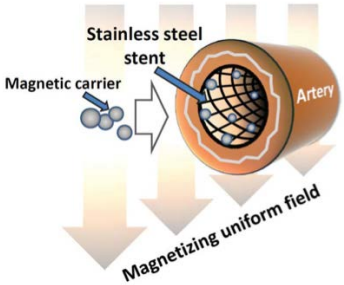
Physical/External Stimuli : Ultrasound
Magnetic field

Biochemical targets : Organs
Cellular
Intracellular

Jaspreet K et al, Current Nanoscience, 2005
Nicolas et al, Chem Soc Rev, 2013 for a review

Active targeting through magnetic field

Stent-targeted delivery



Prevention of arterial reobstruction

Magnetically responsive particles.
Magnetizable implant (stent): small mesh tube to « open » the artery
Externally generated uniform magnetic field
Increase the concentration of the carriers inside the stent
Drug release: restenosis

Chorny et al, Life, 2011

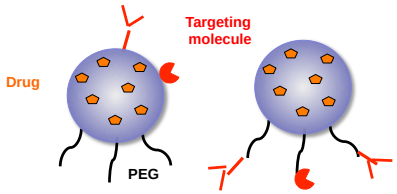
Active targeting

Physical/External Stimuli : Ultrasound
Magnetic field

Biochemical targets : Organs
Cellular
Intracellular

Jaspreet K et al, Current Nanoscience, 2005
Nicolas et al, Chem Soc Rev, 2013 for a review

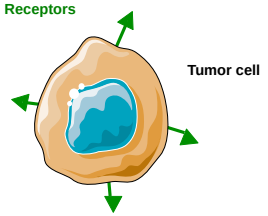
Biochemical targets



Stealth particles.

Ligand(s) at the nanoparticle surface.

Ligand link to the polymer prior to formulation or coupled at the surface of preformed nanocarriers.



Multivalent nanoparticles.

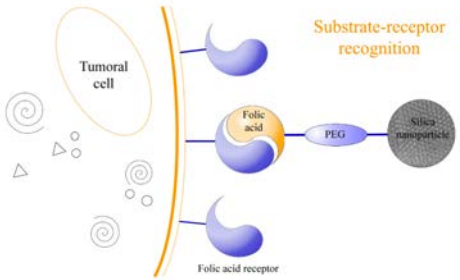
Molecular recognition by « receptors »

Ligands:
- small molecules
- carbohydrates
- peptides and proteins
- antibodies

Small molecules as ligands (1): vitamins

Vitamins (folic acid and biotin) overexpressed in tumor cells (100-300 x).

Tumor cell need folic acid and biotin and their receptors are overexpressed.

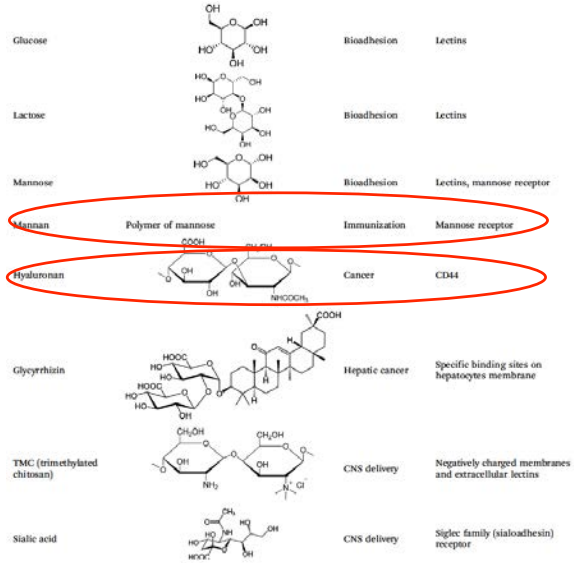


Lebret et al, McKimmon Conference, 2008

Treatment
Diagnostic

Relative nonselective interaction
Free vitamins in competition with targeted nanoparticles
…

Carbohydrates as ligands

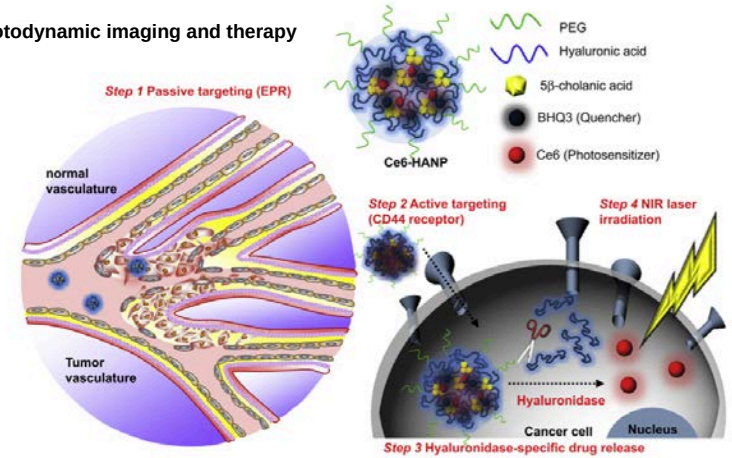


Readily available
Inexpensive to manufacture

Nicolas et al, Chem Soc Rev, 2013

Carbohydrates as ligands: hyaluronan

Photodynamic imaging and therapy

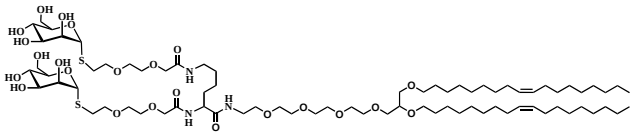


Photosensitizer generates :
- fluorescence (imaging)
- singlet oxygen upon irradiation (therapy) inhibiting the tumor growth

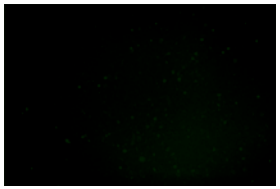
Yoon et al, Biomaterials, 2012

Carbohydrates as ligands: mannan/mannose

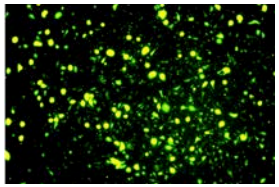
Vaccination field
Mannosylated liposomes
Dendritic cell targeting
Fluorescent liposomes



Immature human dendritic cells
In vitro



500 nanomoles
Unmannosylated liposomes



500 nanomoles
Mannosylated liposomes

Mannosylated derivative allows dendritic cell targeting

Peptides and proteins as ligands:

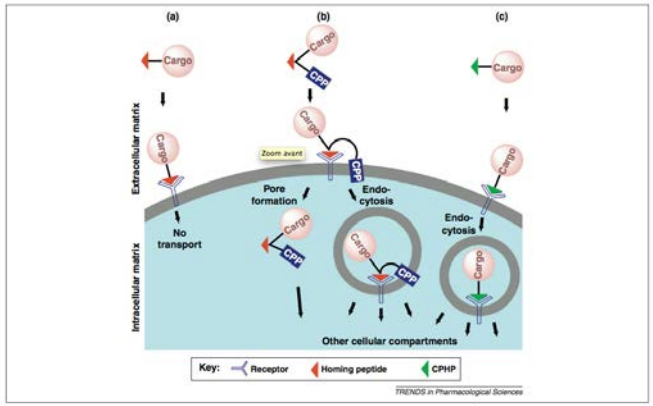
Peptides: production cost low
- high activity
- stable: long-term storage
- easy manipulation
- small size: no or low modification of the nanocarriers
- modification for introduction of functional groups

Glutathione: promotes cell adhesion and permeation-enhancing effect
RGD: tri-peptide, tumor and vascular targeting
cRGD: 170 more active than RGD
Cell penetrating peptides: TAT, PTD, mHph1 and mAP
do not recognize a specific R but able in membrane translocation

.....

Nicolas et al, Chem Soc Rev, 2013

Peptides and proteins as ligands: CPP



Cell penetrating peptides

a. **Homing peptides**: specific targeting

b. **Homing peptides conjugated to CPP**: specific targeting and internalization

c. **CP homing peptide**: internalization without the aid of external agents

Svensen et al, Trends Pharm Scie, 2012

Peptides and proteins as ligands: CPP

Table 1. Homing peptides (HPs) conjugated to cell-penetrating peptides (CPPs)

Sequence (no. of amino acids)	Name	Mode of action	Target tissue	Screening method
GRKKRRQRRRPPQ (13)	TAT	CPP	All cells	Direct targeting
LLIILRRRIRKQAHAHSK (18)	pVEC	CPP	All cells	Direct targeting
FCDGFYACYKDV (12)	ANHP	HP	Breast, ovarian and colon cancers	Phage display
lgaswhrpdkcclgyqkrplp (21)	DIV1	HP	Lymphoma cells	Phage display
lgaswhrpdk (10)	DV3	HP	Lymphoma cells	Phage display
CPGPEGAGC (9)	PEGA	HP	Breast vasculature and tumors, premalignant breast tissue	Phage display
CREKA (9)		HP	Breast adenocarcinoma cells (MCF7)	Phage display

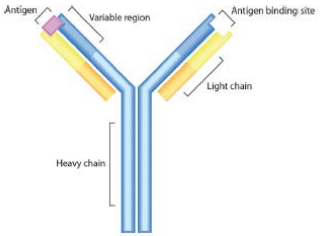
Table 2. Cell-penetrating homing peptides (CPHPs)

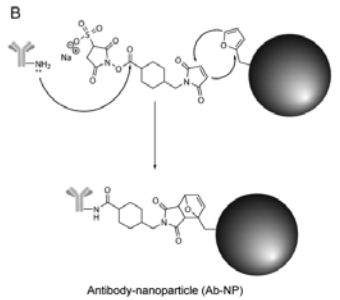
Sequence (no. of amino acids)	Name	Target tissue	Screening method
CTPSPFSHC (9)	TCP-1	Colorectal cancer	Phage display
SFHQFARATLAS (12)	HAP-1	Synovial cells	Phage display
HIQLSPFQSWR (11)	HAP-2	Synovial cells	Phage display
LKKP (4)		Myeloid leukemia cells (K562)	Synthetic peptide library
EPKK* (4)		Embryonic stem cells	Synthetic peptide library
ELK*K* (4)		Primary monocytes	Synthetic peptide library
PYEE (4)		Amelanotic melanoma cells (ARN8)	Synthetic peptide library
HMGN2-N F3 (31)	F3	Lymphatic endothelial cells (HL-60 and MDA-MB-435)	Phage display
PFSSTKT (7)	BMHP1	Neural stem cells	Phage display
CTVALPGGYVRVC (13)	Pep42	Melanomas	Phage display
DWRVIIPPRPSA (12)	CAP	Chondrocytes	Phage display
CDCRGDCFC (9)	RGD-4C	Angiogenic blood vasculature	Phage display
CRGDK/RGPD/EC (11)	iRGD	Various tumors	Direct targeting
cRGDf(NMeV) (5)	cRGD	Angiogenic blood vessels	Direct targeting
NGR (3)	NGR	Angiogenic blood vessels	Phage display

K* = *N*-alkyl glycine lysine-like peptoid.

Svensen et al, Trends Pharm Scie, 2012

Antibodies as ligands





Cancer EGFRMab
Anti-CD3 Mab
Anti-HER2 Mab (herceptin/trastuzumab)
Alzeimer's disease

Brain delivery
OX26
anti-CD44 mAB
Autoimmune diseases

Expensive
Time-consuming to produce
Use of fragments but less stable and lower binding avidity

Nicolas et al, Chem Soc Rev, 2013

Owen et al, JCR, 2013

Targeting limitations

Binding site on the target (specificity)

Heterogeneity of the tumor and its vasculature.

- cells not expressing the same epitopes
- necrotic and vascularized regions

Shedding of the target receptors and downregulation

Sometimes no additional benefits in vivo!