NANOMEDICAMENTS: MATERIAUX ET METHODES DE PREPARATION

P. COUVREUR
Professeur à l'Université Paris-Sud
Professeur au Collège de France
Chaire d'Innovation Technologique
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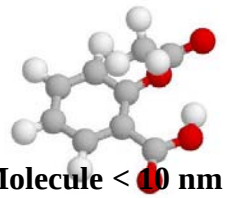
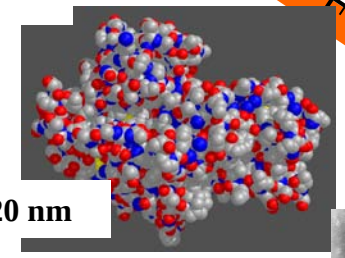
L'ECHELLE DES TAILLES



1 nm

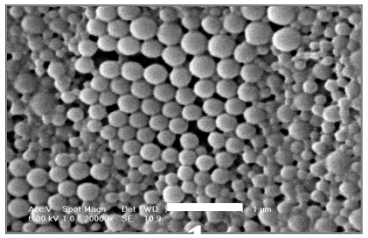
Molecule < 10 nm

10 nm

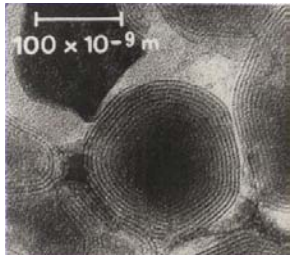


Protein 10 - 20 nm

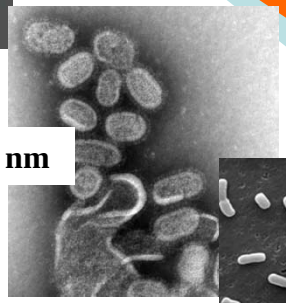
100 nm



nanoparticules

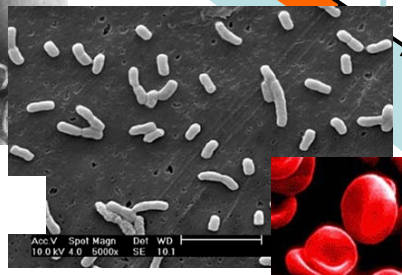


Liposomes



Virus 20 - 400 nm

1 µm



Bacteria 500 nm – 10 µm

10 µm

Erythrocyte

100 µm

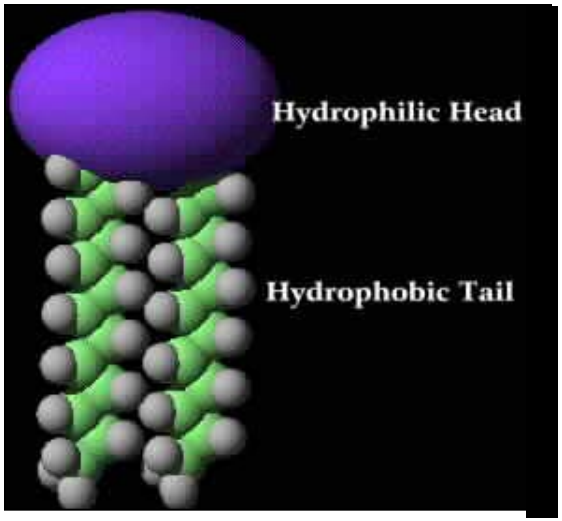
Pollen 10 – 100 µm

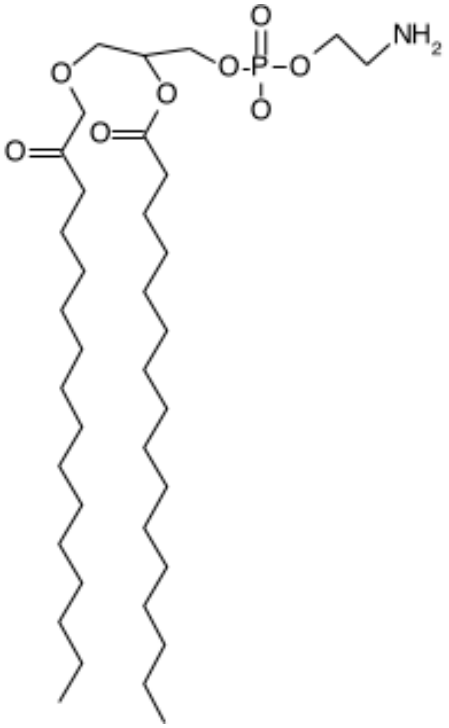
Sand 100 µm – 5 mm

LIPOSOMES

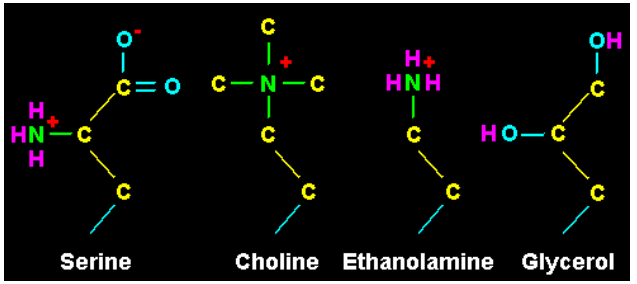
SYSTEMES MOLECULAIRES ORGANISES

Phospholipids

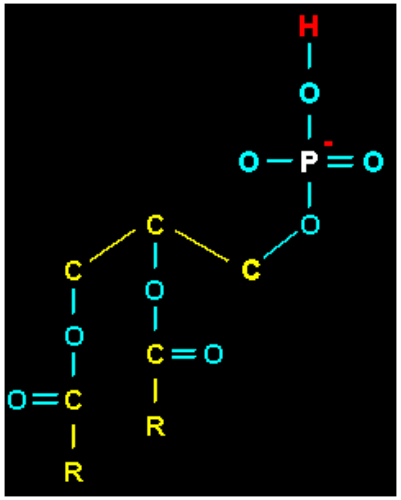




Polar Head Groups



Three carbon glycerol



STRUCTURE DES PHOSPHOLIPIDES

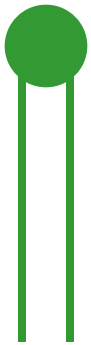
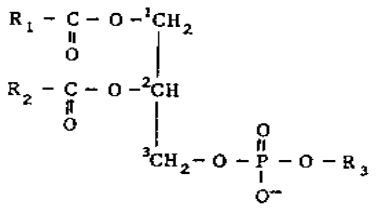


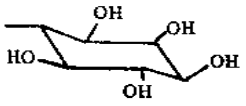
Tableau 1

STRUCTURE CHIMIQUE DES PRINCIPAUX PHOSPHOLIPIDES
(EXTRAIT DE [1])

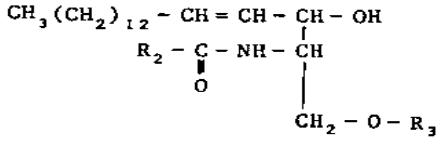
GLYCEROPHOSPHOLIPIDES



R_1, R_2 chaînes aliphatiques

R_3 :		
$-CH_2-CH_2-\overset{+}{N}(CH_3)_3$	phosphatidylcholines	*PC*
$-CH_2-CH_2-\overset{+}{N}H_3$	phosphatidyléthanolamine	*PE*
$-CH_2-\underset{COO^-}{CH}-\overset{+}{N}H_3$	phosphatidylsérine	*PS*
$-CH_2-\underset{OH}{CH}-CH_2OH$	phosphatidylglycérol	*PG*
$-H$	acide phosphatidique	*PA*
	phosphatidylinositol	*PI*

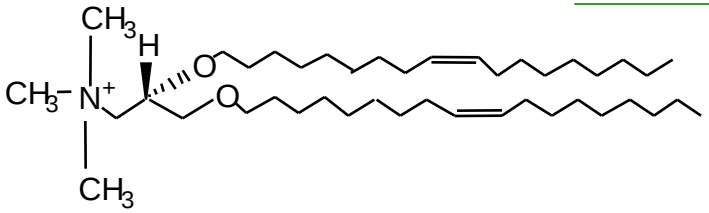
SPHINGOLIPIDES



R_2, chaîne aliphatique

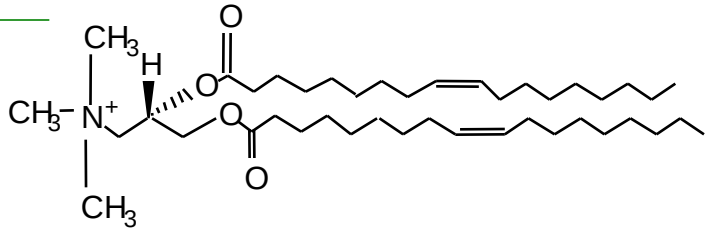
R_3 :		
$\overline{P}O_3-CH_2CH_2-\overset{+}{N}(CH_3)_3$	sphingomyéline	*SM*
βGlc–βGal–βGal–NAc… NeuNAc	ganglioside	*GM*

CATIONIC LIPIDS



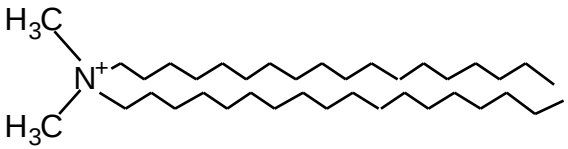
DOTMA

N-(1-(2-3-Dioleoyloxy)propyl)-N,N,N-trimethyl ammonium chloride



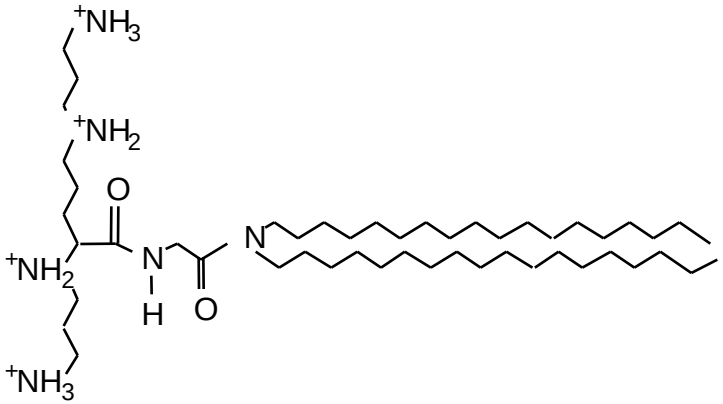
DOTAP

1,2-Dioleoyl-3-trimethylammonium-propane



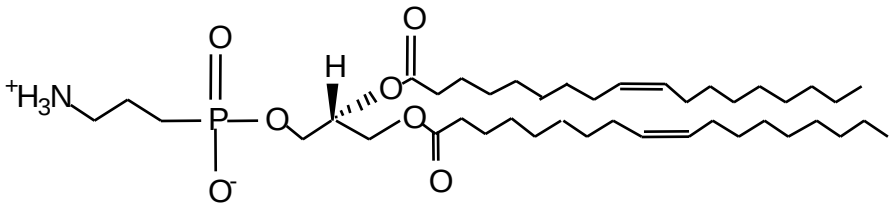
DDAB

Dimethyldioctadecylammonium bromide



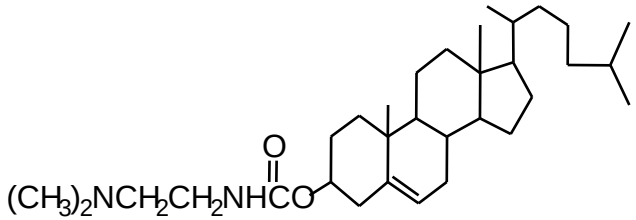
DOGS

Dioctadecylamidoglycyl spermine



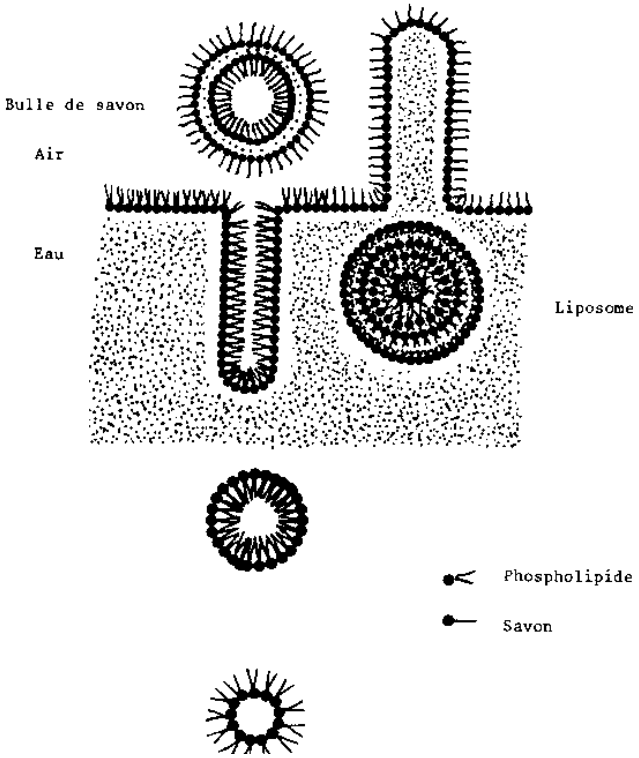
DOPE

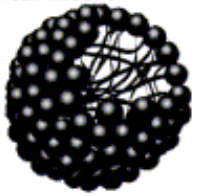
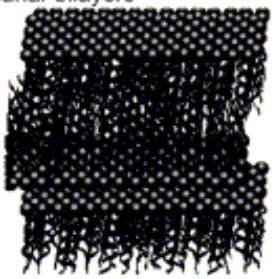
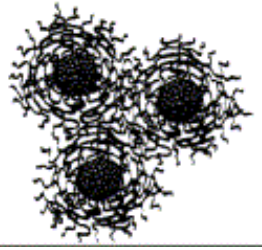
1,2-Dioleoyl-sn-Glycero-3-Phoshoethanolamine



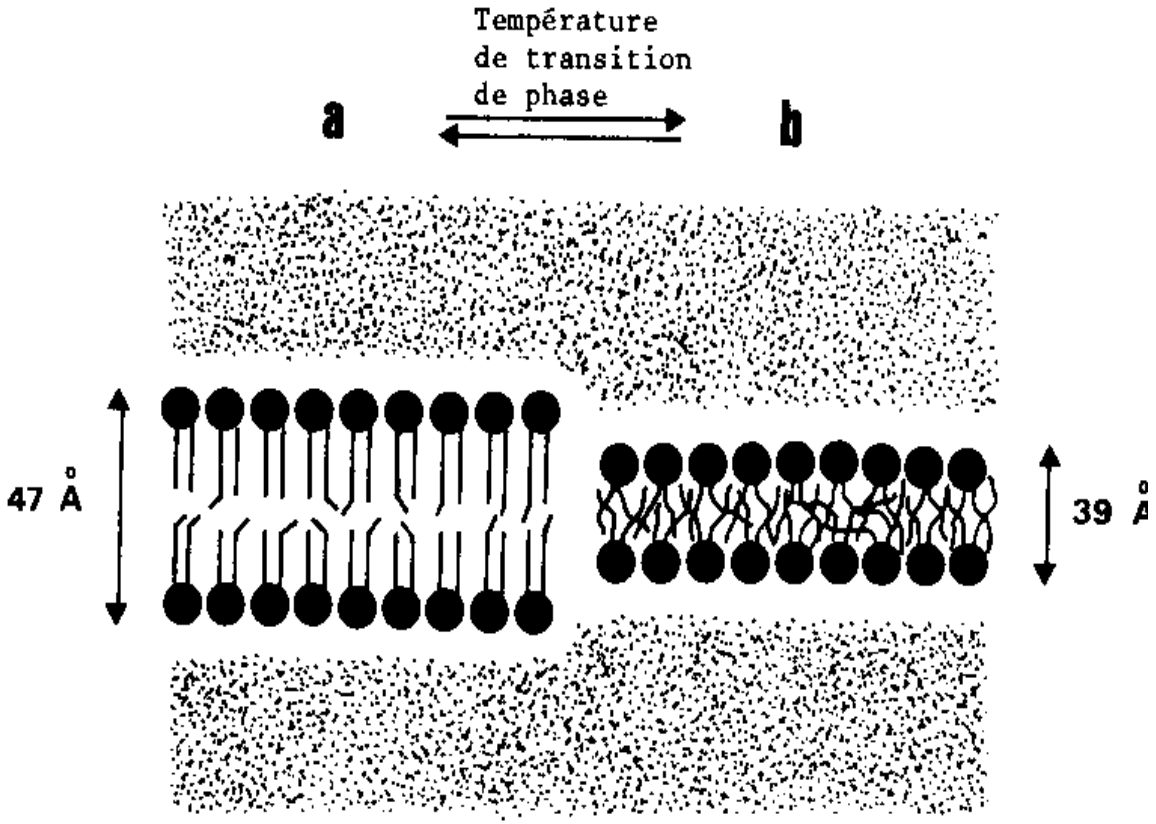
DC-Cholesterol

ORGANISATION SUPRAMOLECULAIRE DES PHOSPHOLIPIDES



Critical Packing parameter ($v/a_o l_c$)	Critical packing shape	Structure Formed
<1/3	Cone 	Spherical micelle 
1/3 – 1/2	Truncated cone 	Cylindrical micelle 
1/2 - 1	Truncated cone 	Flexible bilayers 
~1	Cylinder 	Planar bilayers 
>1	Inverted truncated cone 	Inverted micelles 

TEMPERATURE DE TRANSITION DE PHASE DES PL



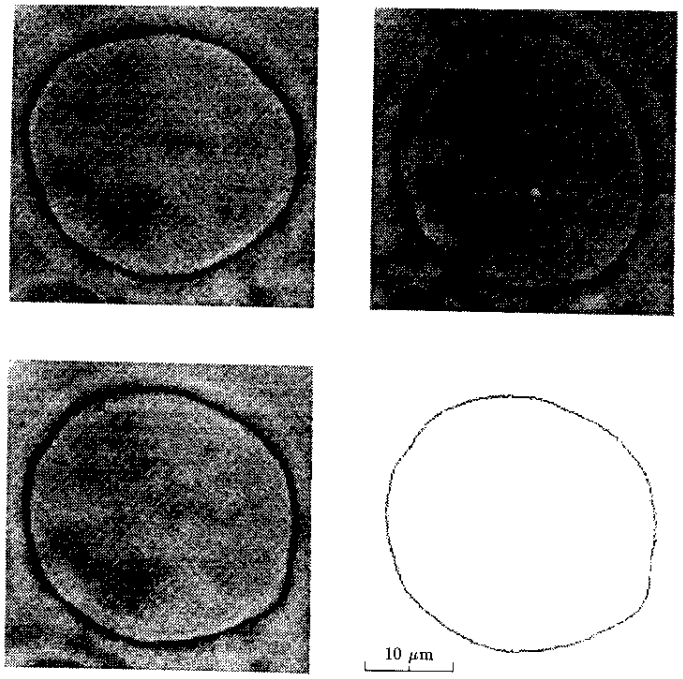
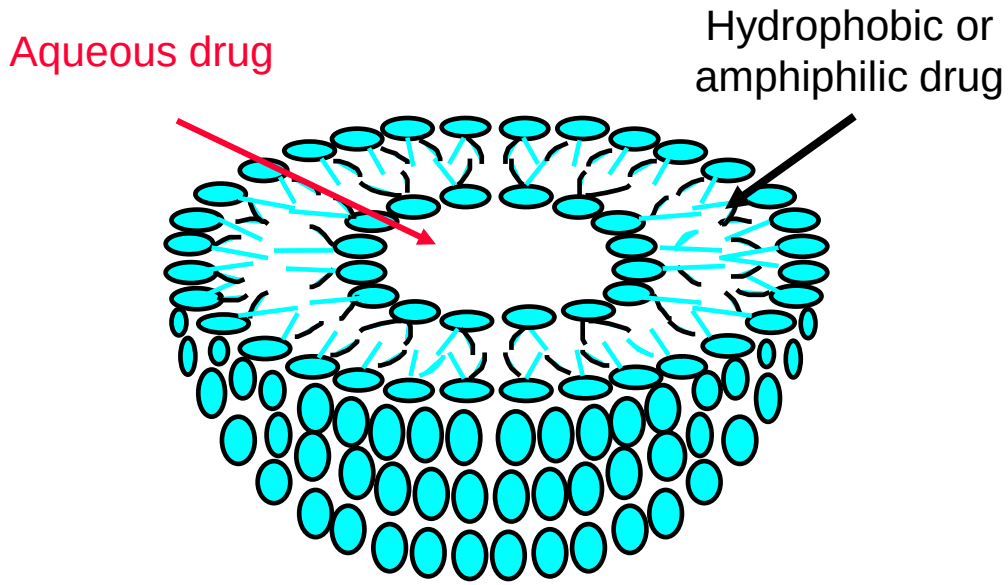
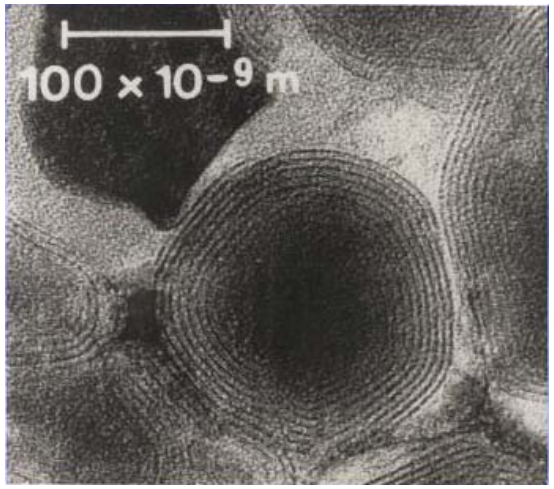


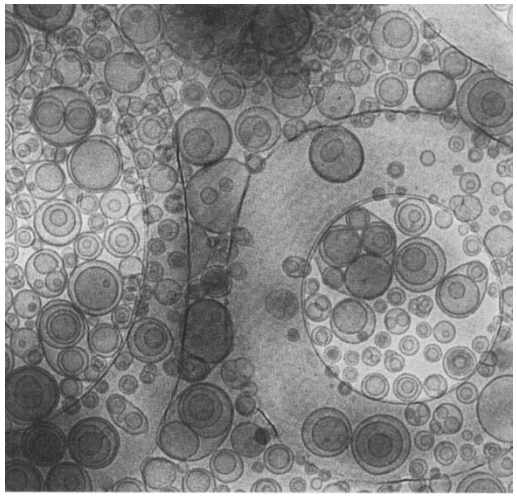
Fig.17 Formes d'un liposome à trois instants différents et contour extrait après l'analyse d'image (adaptée de [17]).

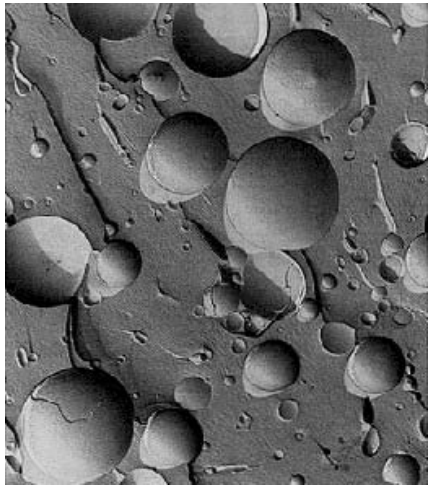
WHAT IS A LIPOSOME ?

- Spherical
- Diameter less than 1, 000 nm
- A vesicle
- An aqueous phase
- Made of amphiphilic molecules
- One or more bilayer membrane

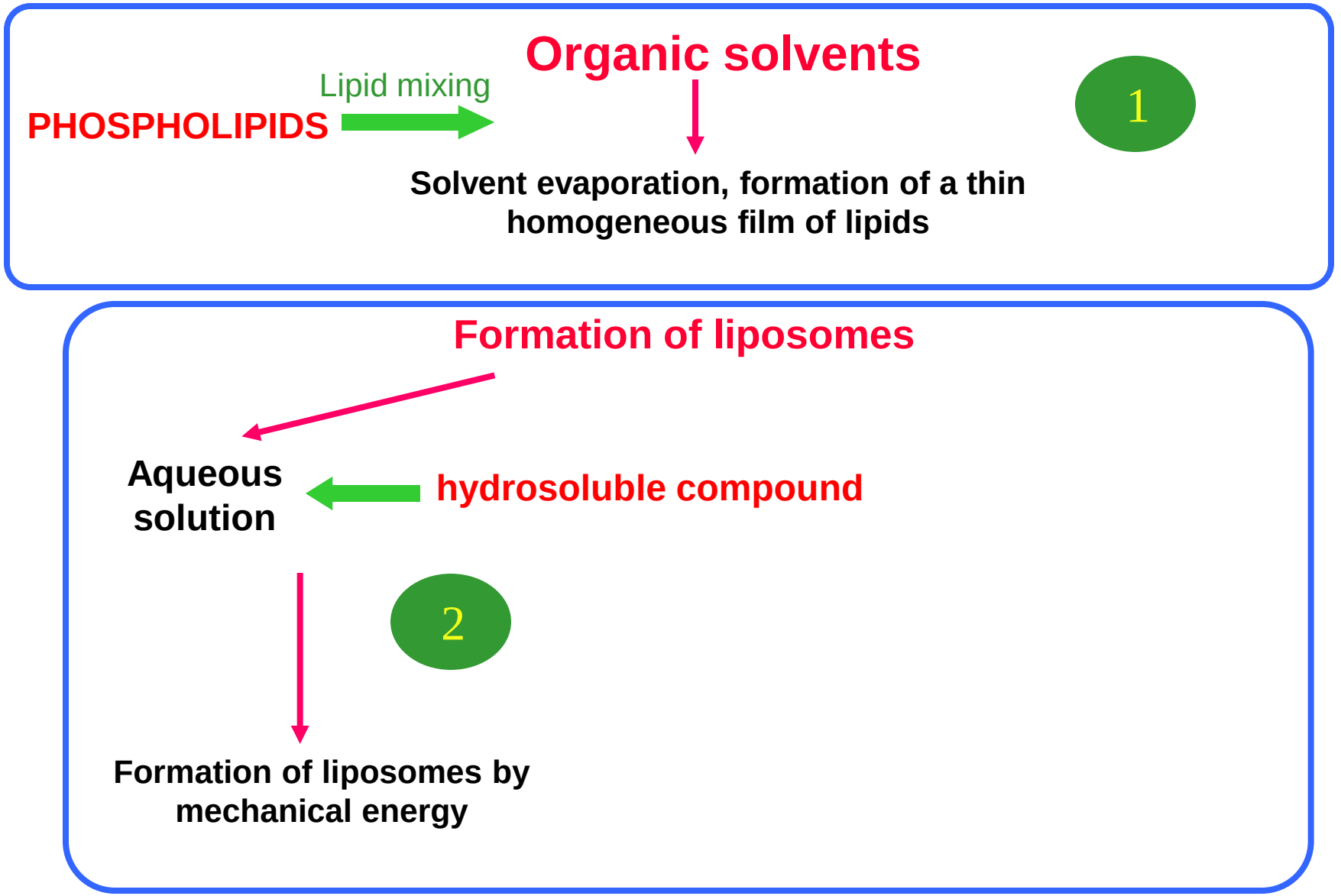




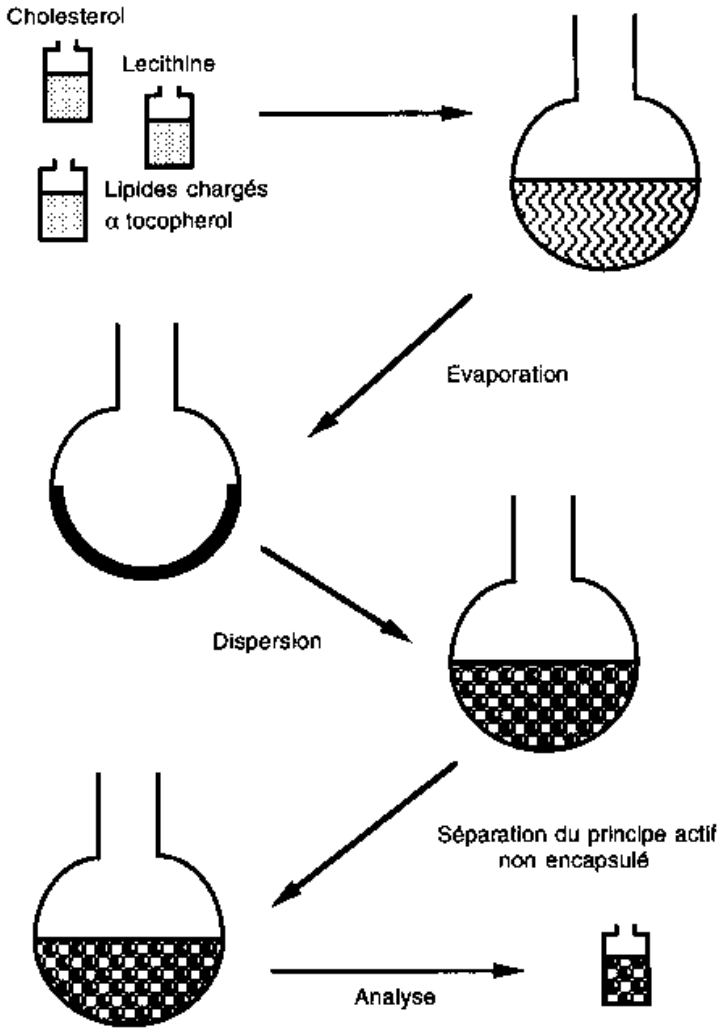


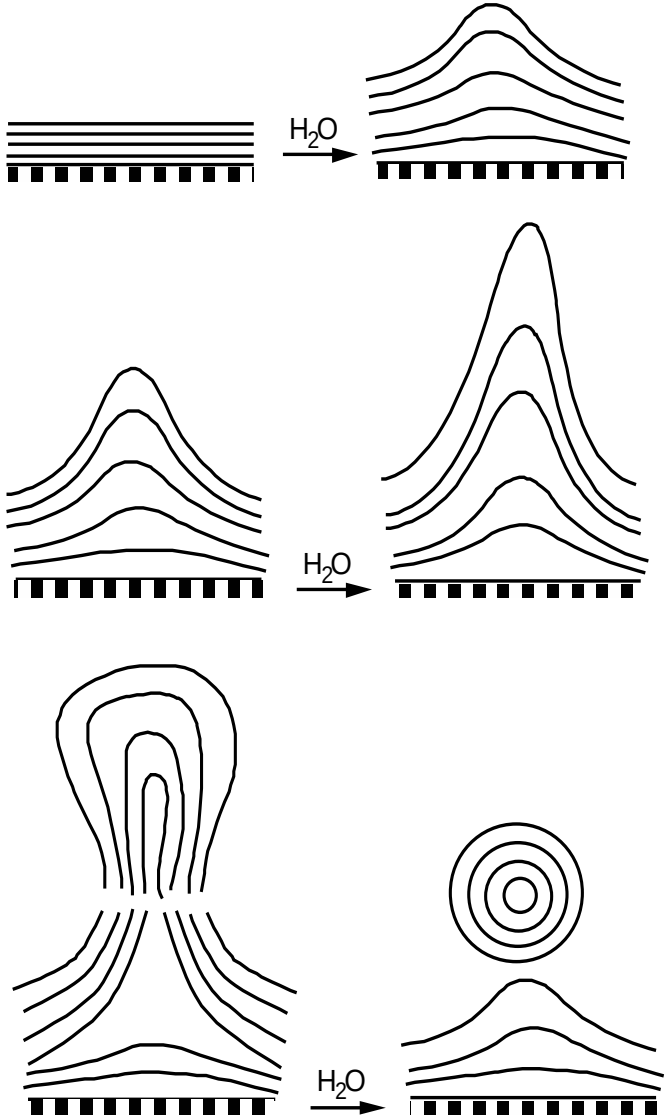


PRINCIPLE OF LIPOSOME FORMATION

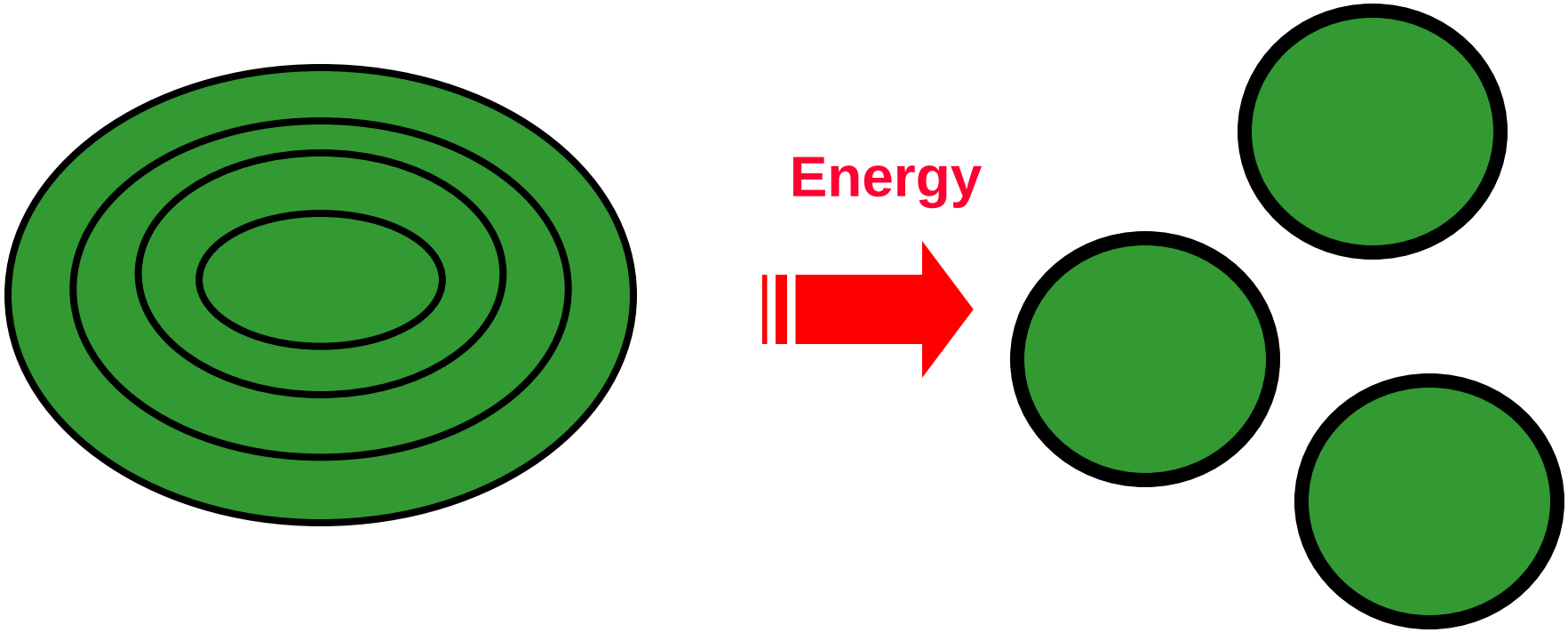


PREPARATION DES LIPOSOME PAR LA METHODE DE BANGHAM





LIPOSOMES HOMOGENEIZATION

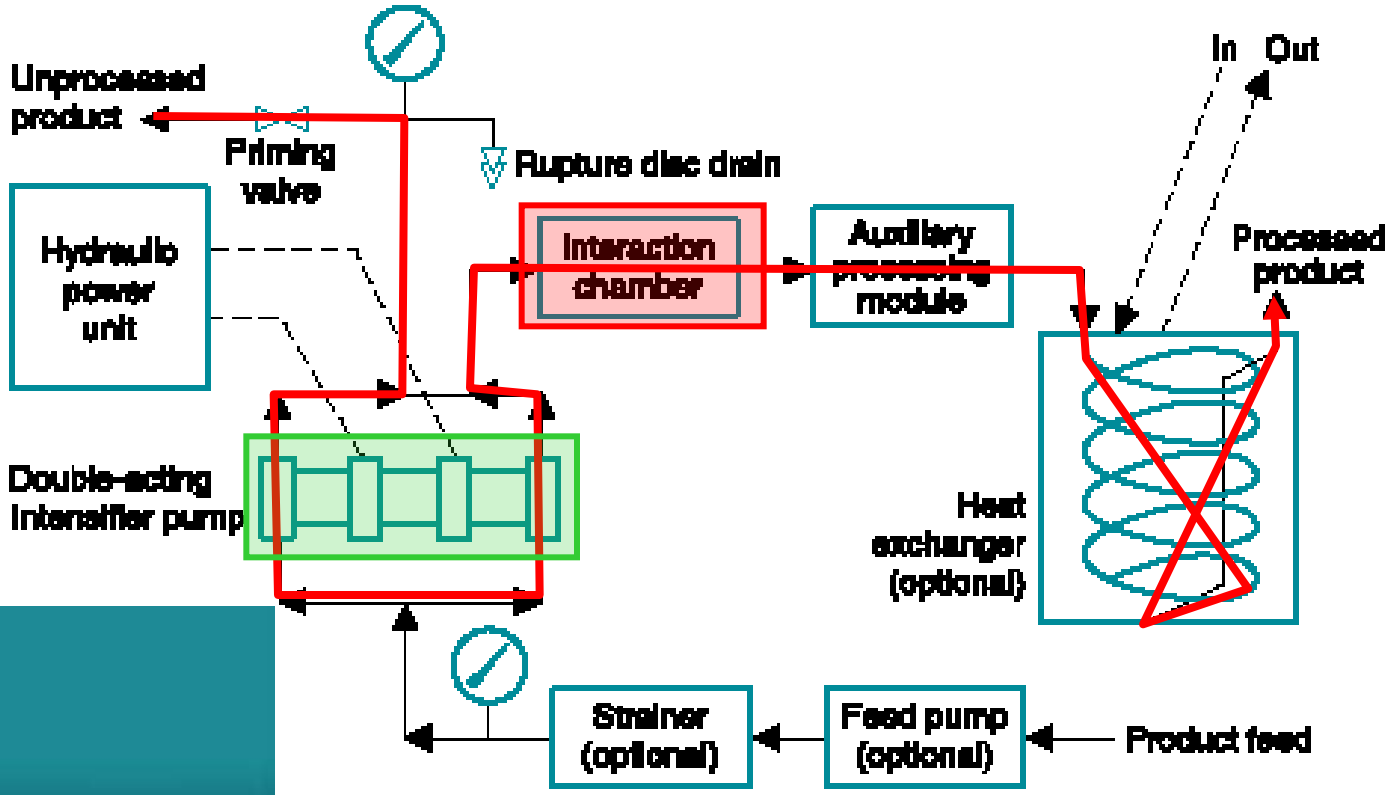


Multilamellar

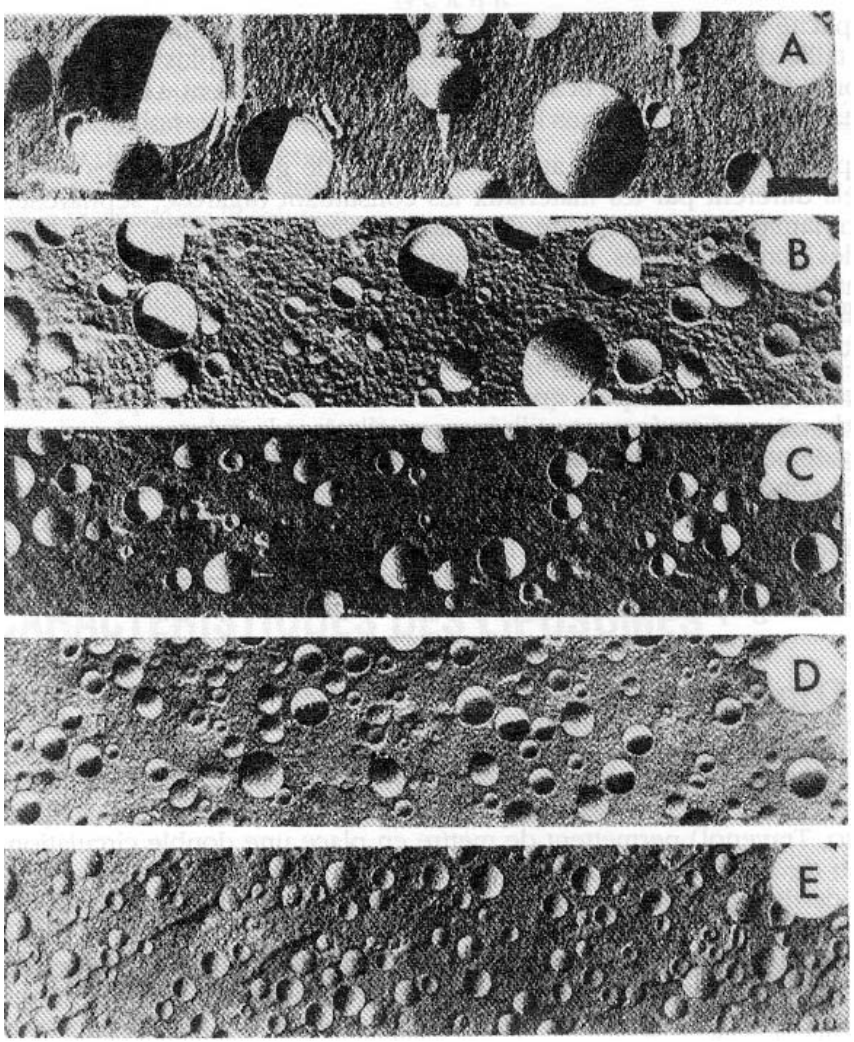
Unilamellar

Sources of Energy: Pressure, Extrusion, Ultrasounds etc.

MICROFLUIDIZER



EXTRUSION



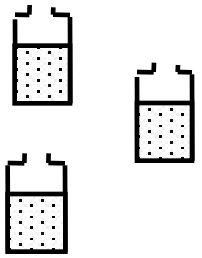
400 nm

200 nm

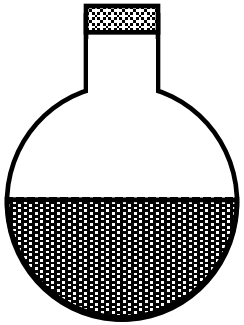
100 nm

30 nm

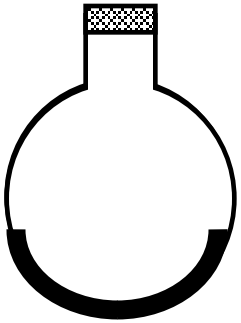
PREPARATION BY REVERSE PHASE EVAPORATION



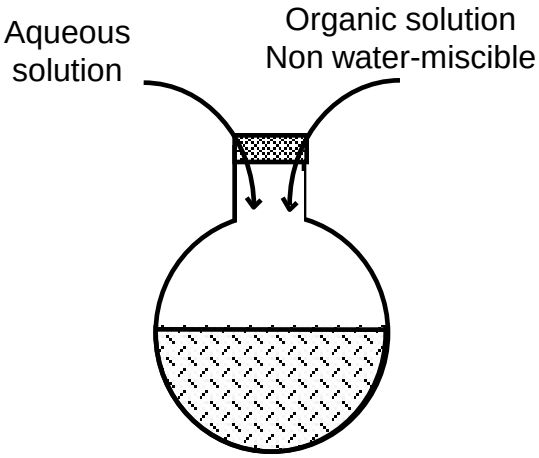
Lipids in organic solvent

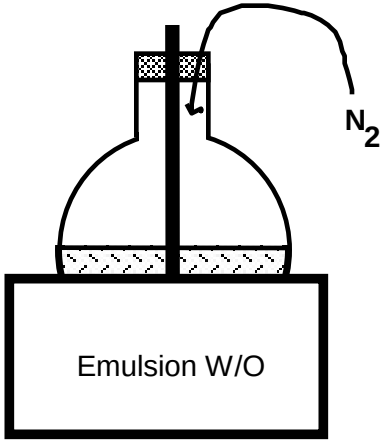


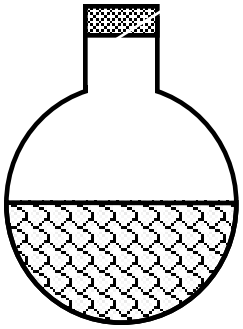
Solvent evaporation



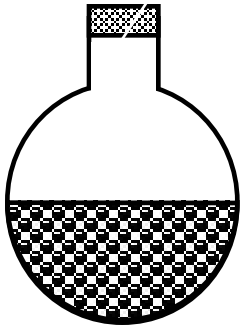
Lipid film formation



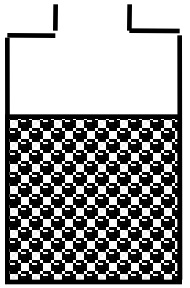




Formation of a gel

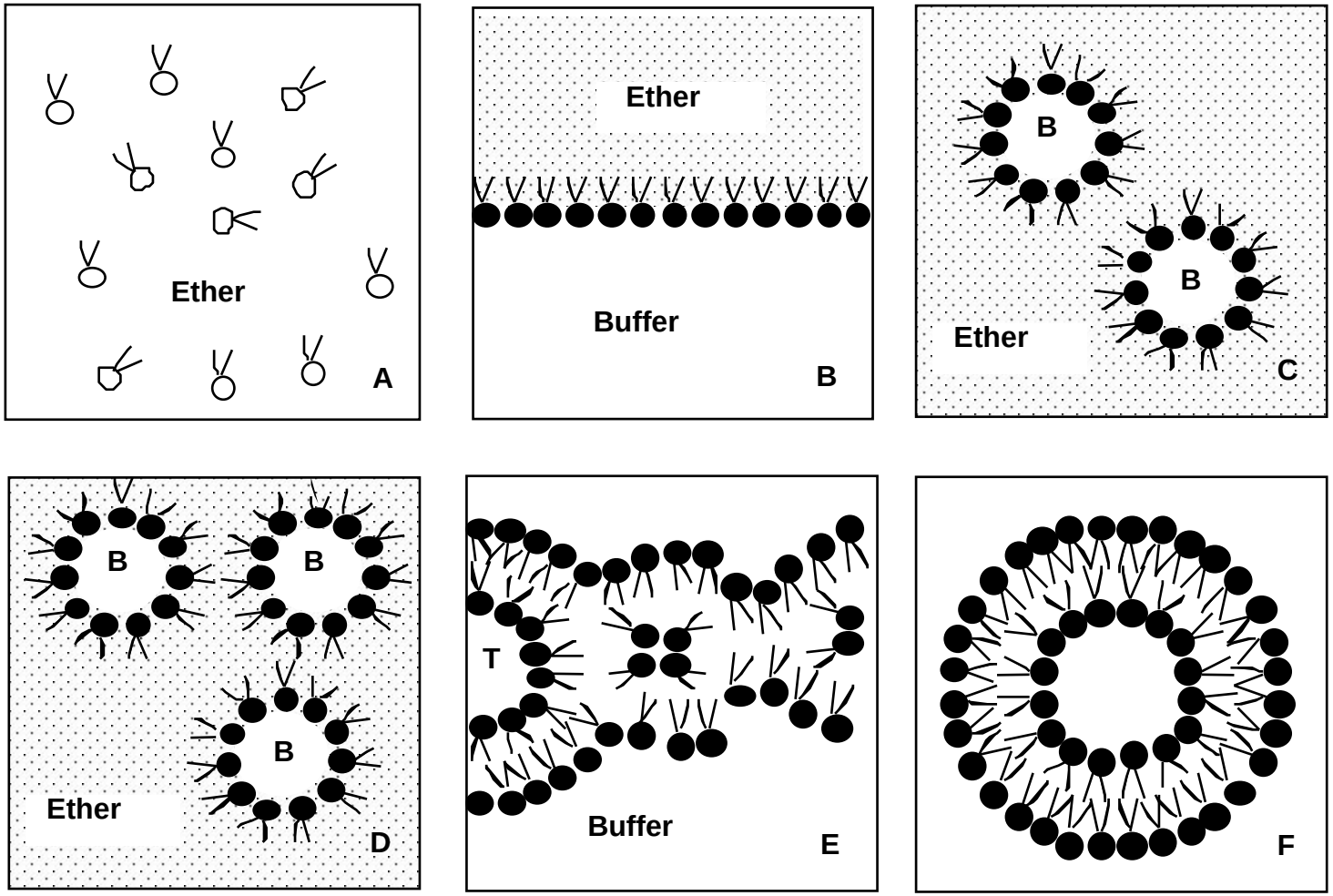


Disruption of the gel

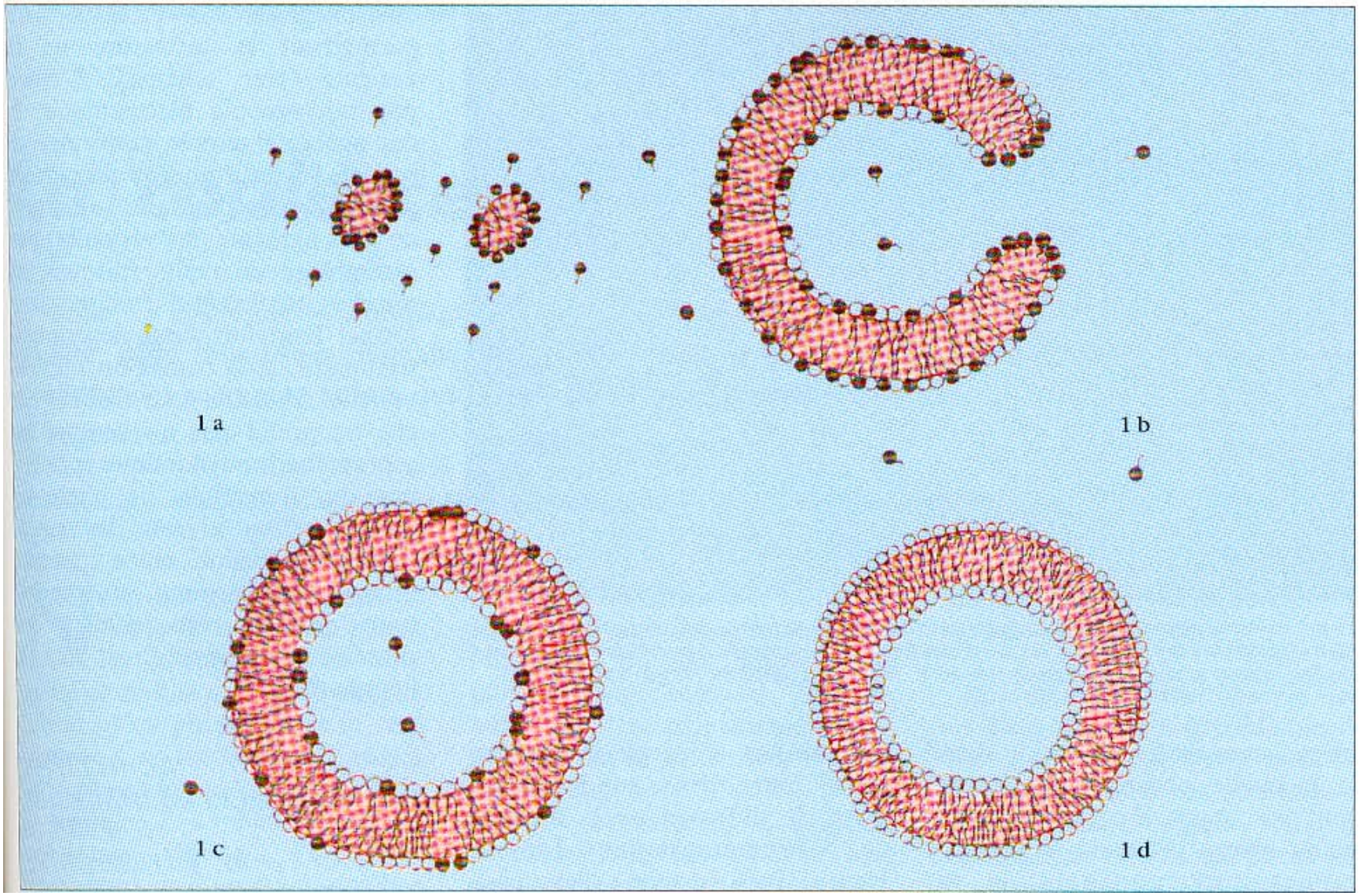


Lliposomes formation

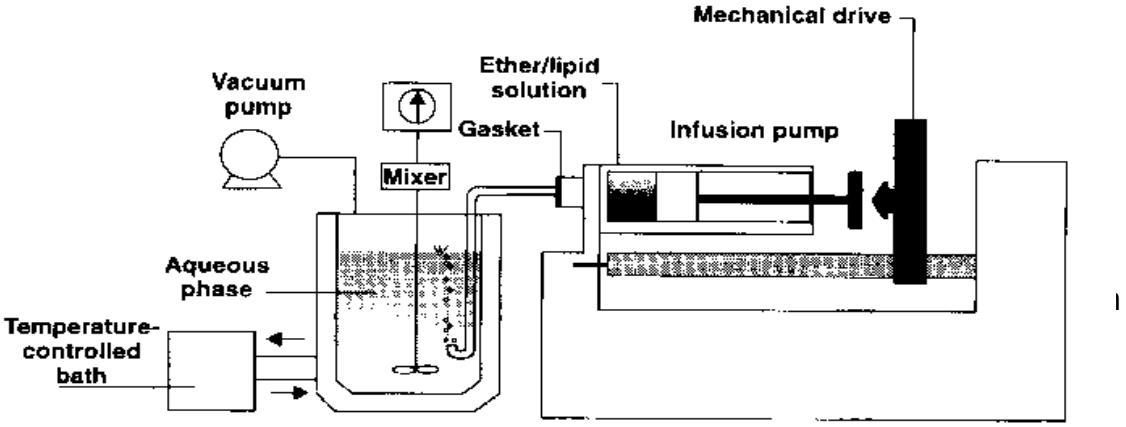
PREPARATION BY REVERSE PHASE EVAPORATION

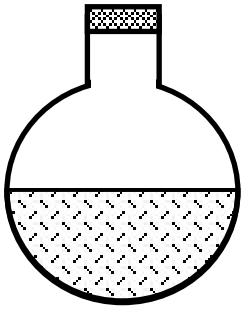


PREPARATION DES LIPOSOMES PAR DIALYSE

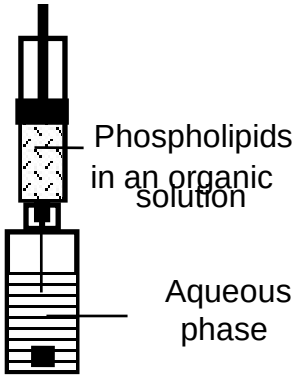


PREPARATION DES LIPOSOMES PAR INJECTION DE SOLVANTS





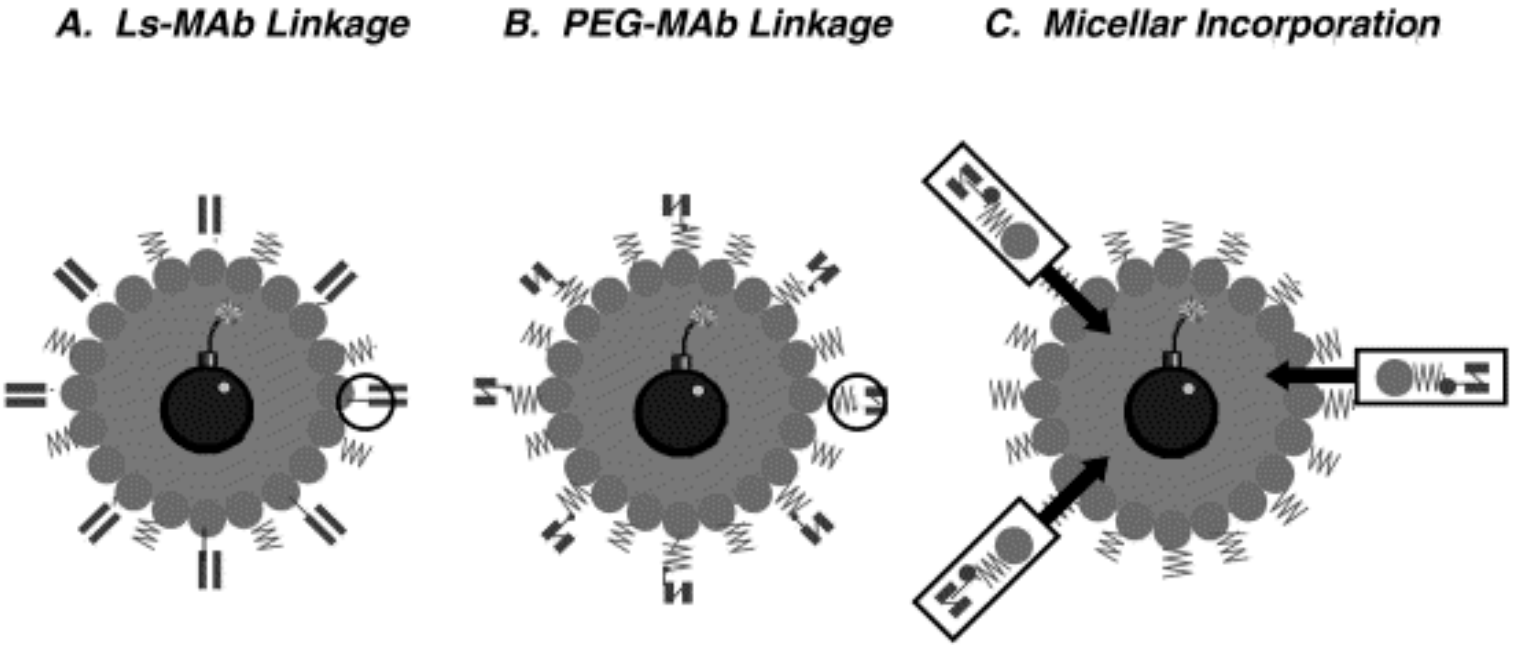
Lipids are dissolved
In a water-miscible solution



Magnetic stirring

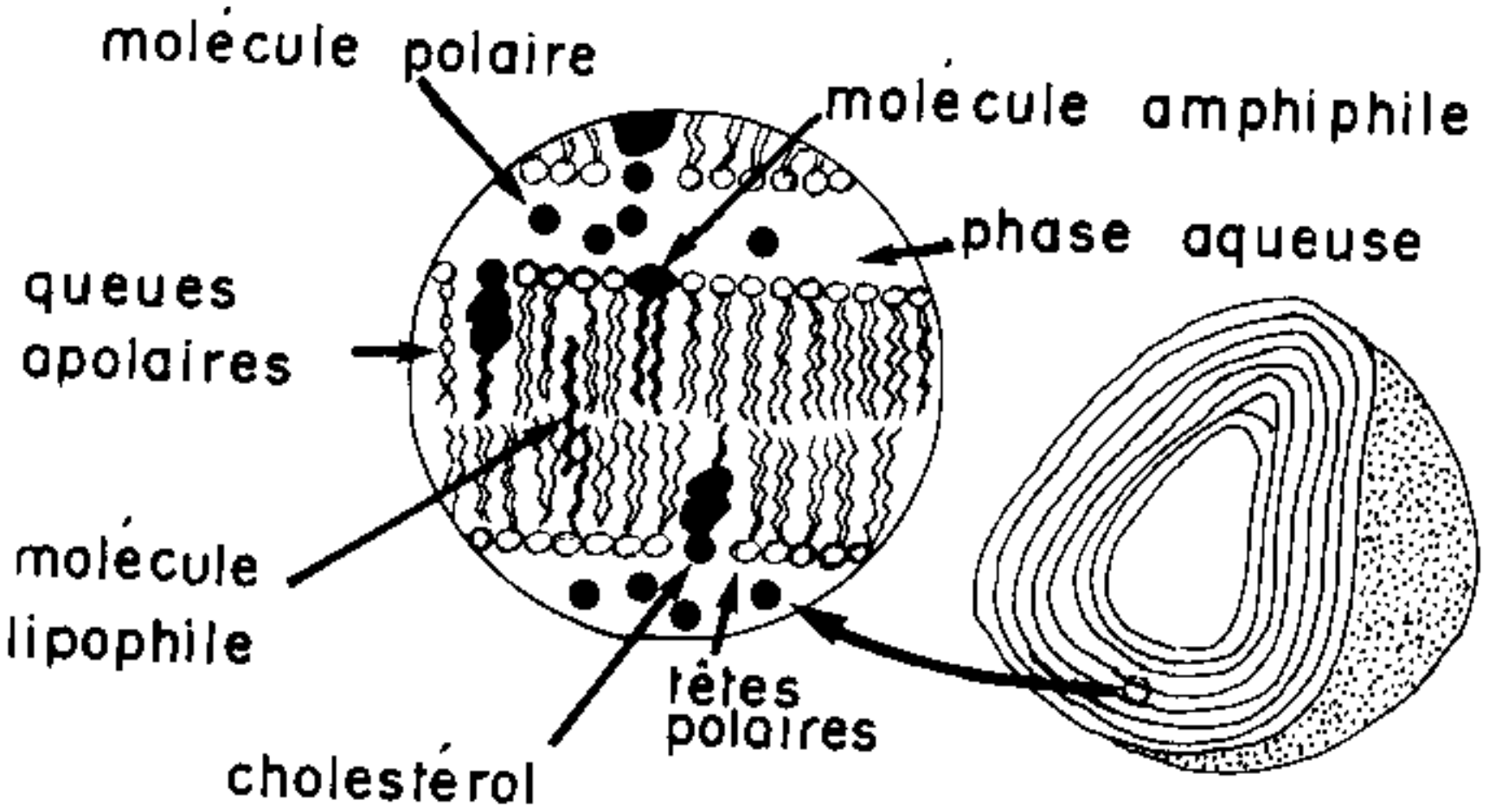
CONJUGATION STRATEGIES USED FOR CONSTRUCTION OF ANTI-HER 2 IMMUNOLIPOSOMES

Park JW et al., Journal of Controlled Release 74, 95-113, 2001

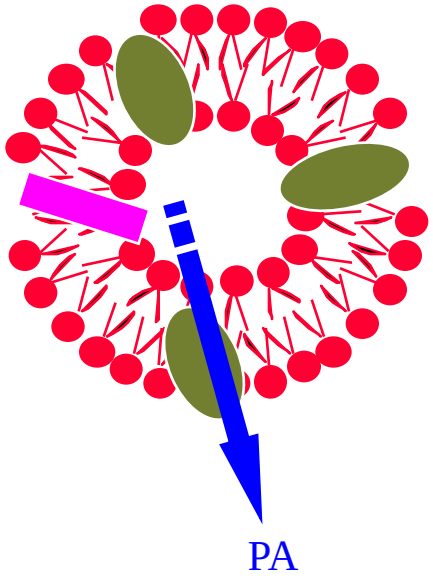


- SUV (70–100 nm) with PEG2000-derivatized disteroylphosphatidylethanolamine (PEG-PE). Anti-HER2 MAb fragments consisted of rhuMAb HER2 (trastuzumab)-Fab′, scFv C6.5, scFv F5, or variants of these. MAb fragments all contained a C-terminal cysteine for covalent conjugation.
- (Left) Ls-MAb linkage. MAb fragments were conjugated to maleimide-terminated phosphatylethanolamine (M-PE) at the liposome surface.
- (Middle) PEG-MAb linkage. MAb fragments were conjugated to maleimide-terminated PEG-PE (M-PEG-PE)
- (Right) Micellar incorporation. Preformed liposomes lacking functional sites for conjugation were converted into immunoliposomes by incorporation of modified MAb fragments coupled to M-PEG-PE, forming micelles which incorporate into liposomes

L' ENCAPSULATION DANS LES LIPOSOMES

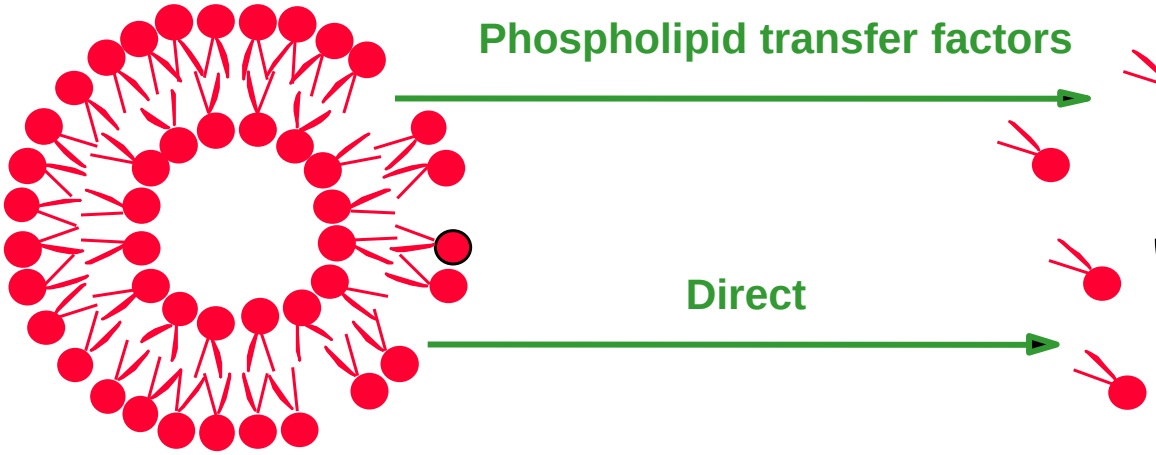


LIPOSOME STABILITY



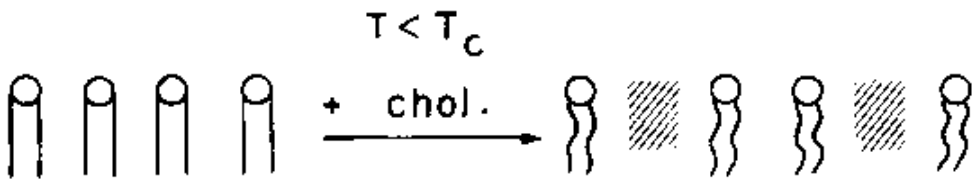
- ✓Fibronectin
- ✓Albumin
- ✓Lipoproteins
- ✓Complement
- ✓Immunoglobulins

DRUG LEAKAGE

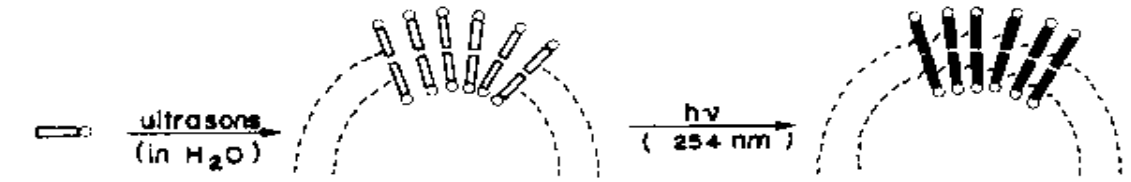


PL EXCHANGE

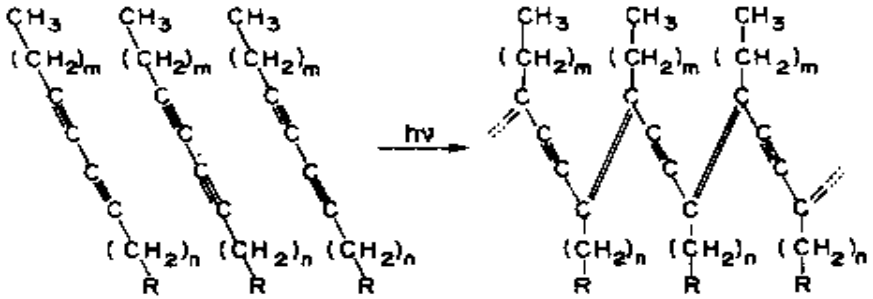
LIPOSOMES STABILIZATION



CHOLESTEROL



POLYMERIZABLE PL



NANOPARTICULES

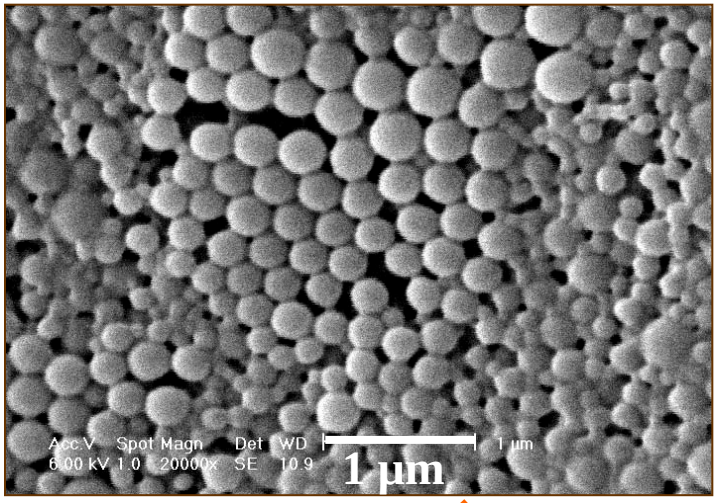
Nanospheres et Nanocapsules

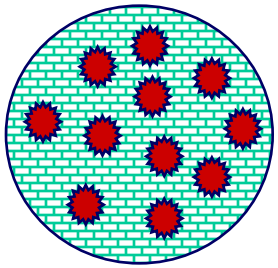
NANOPARTICULES: DEFINITION

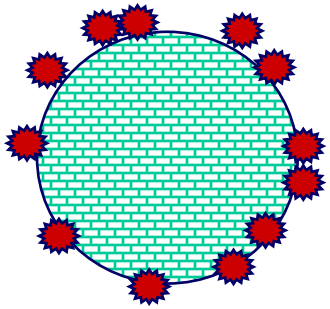
NANOPARTICULES: structures supramoléculaires solides ultradispersées, généralement à base de polymères, ayant une taille inférieure au micron

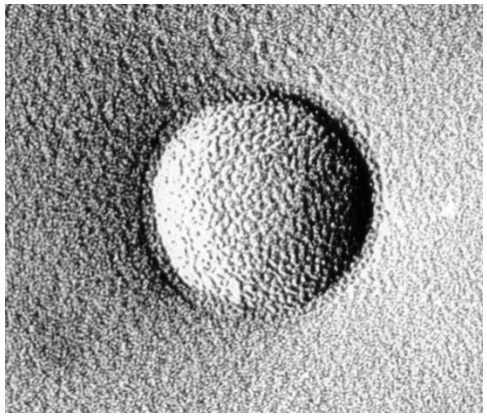
Ces objets peuvent être de type matriciels (NANOSPHERES) ou vésiculaires (NANOCAPSULES)

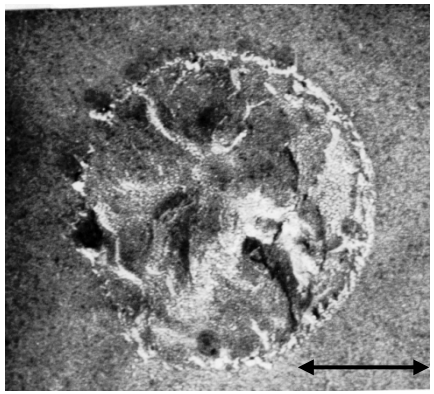
Le médicament vectorisé peut s'associer à ces structures sous différents états physiques







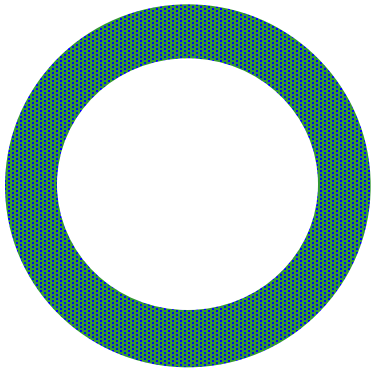


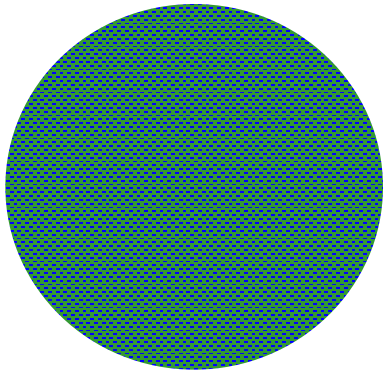


Nanospheres

Nanocapsules

NANOPARTICLES PREPARATION

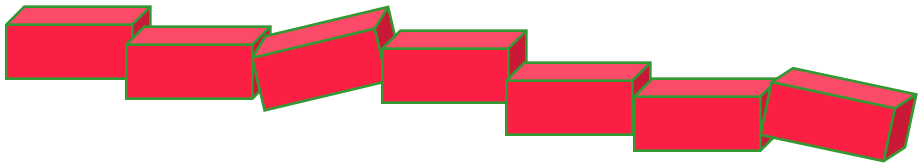




- natural macromolecules
- synthetic polymers

monomers

polymers

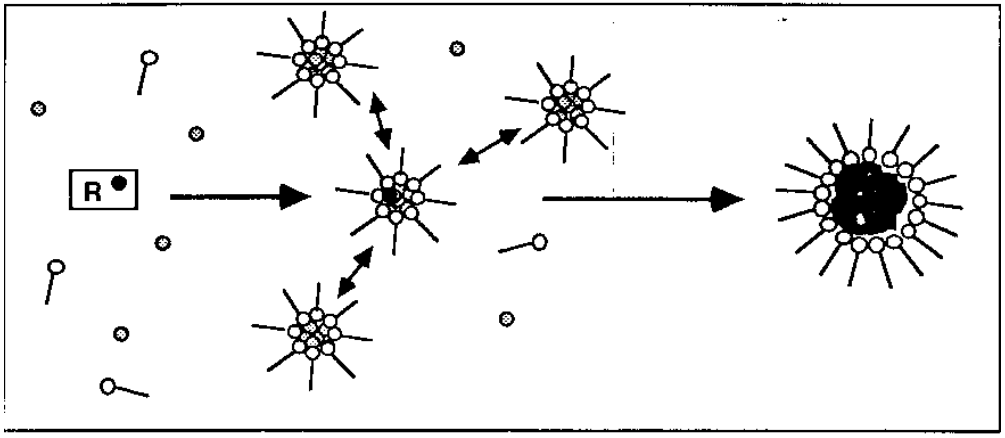


PREPARATION DE NANOPARTICULES PAR POLYMERISATION

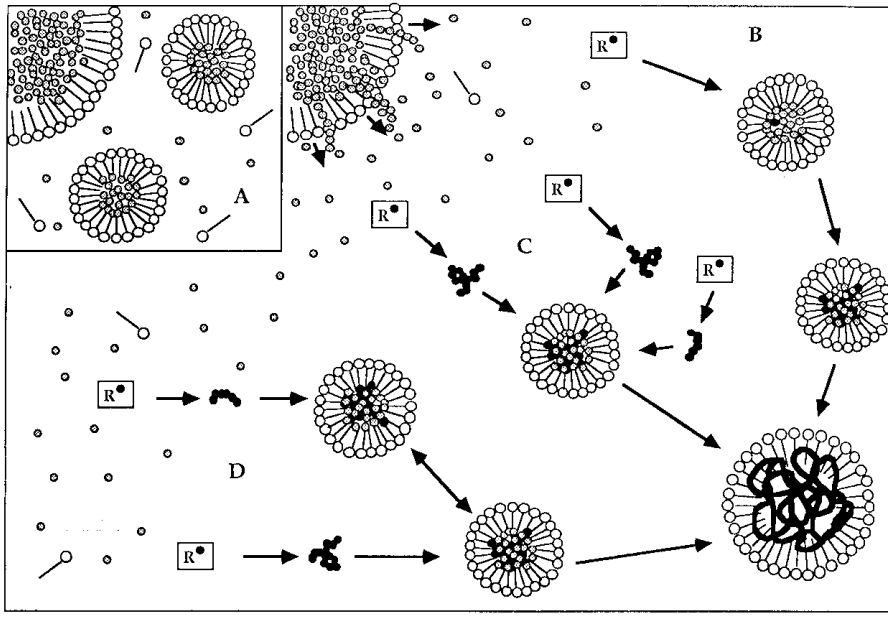
Polymérisation en émulsion et en suspension

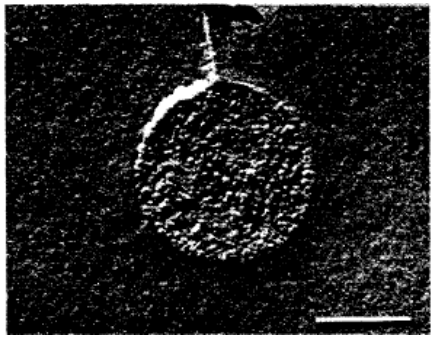
PREPARATION DE NANOSPHERES PAR POLYMERISATION

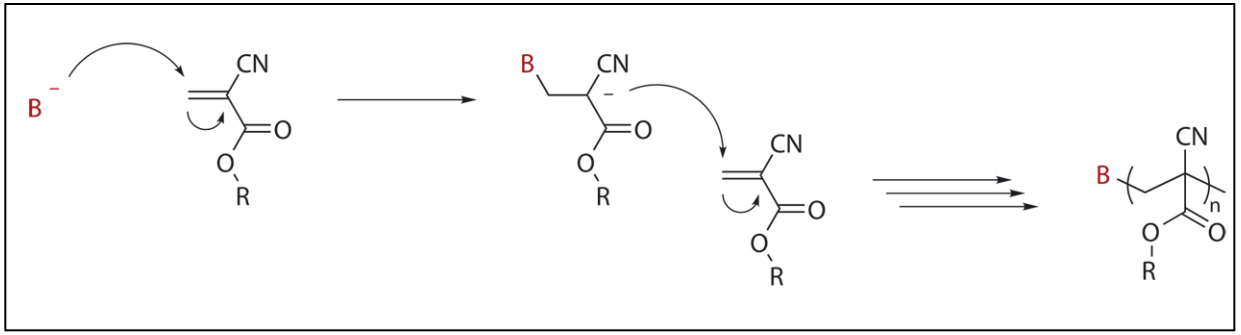
En suspension



En émulsion

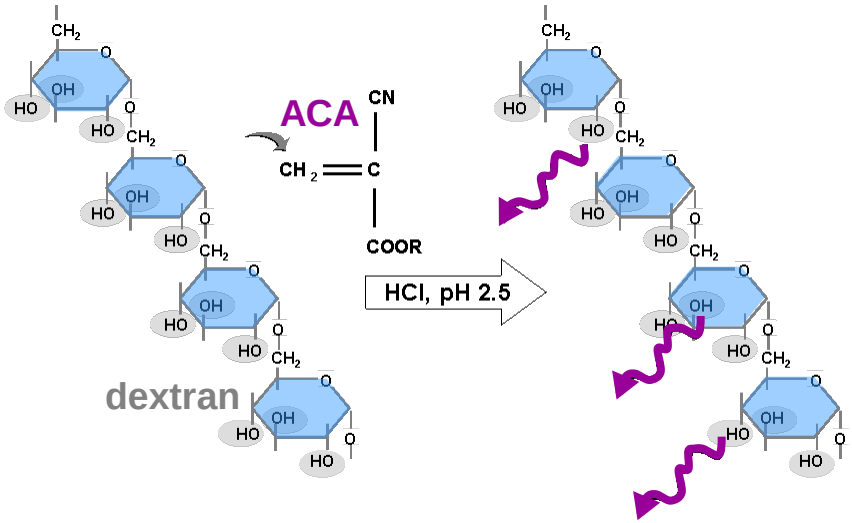


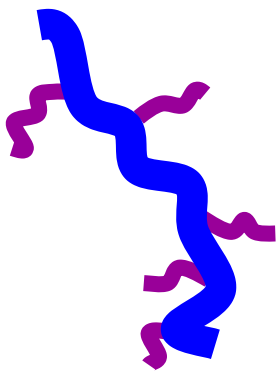


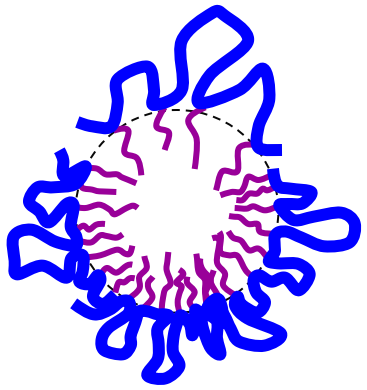


Chauvierre et al. , Macromolecules, 36, 6018-6027 (2003)

A



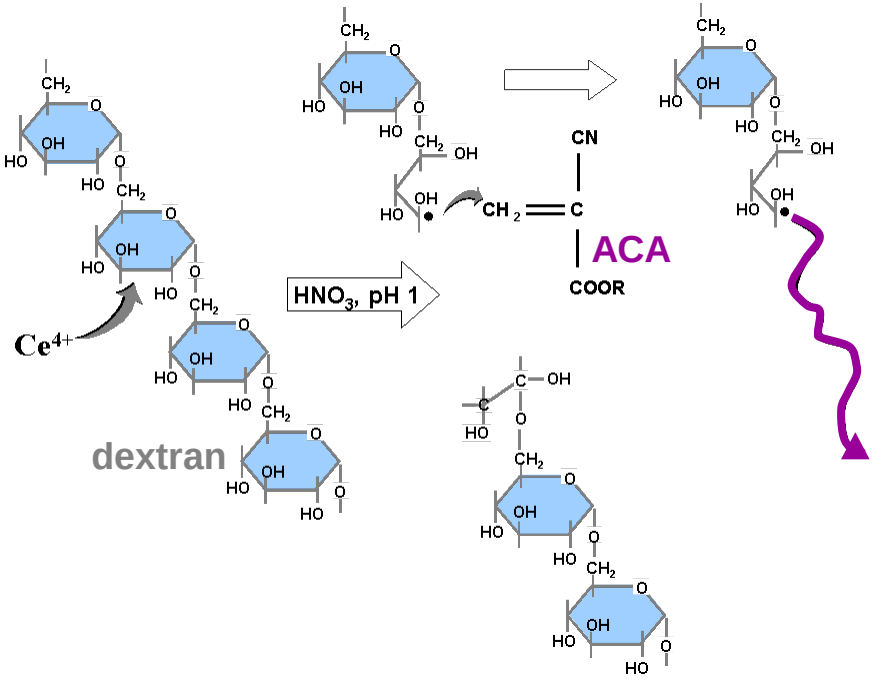




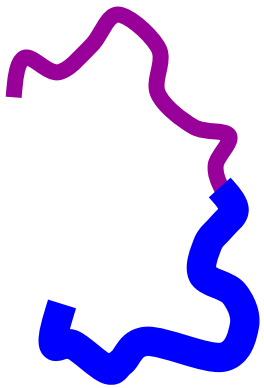
POLYMERIZATION

INITIATION **ELONGATION** **COPOLYMERS** **NANOPARTICLES**

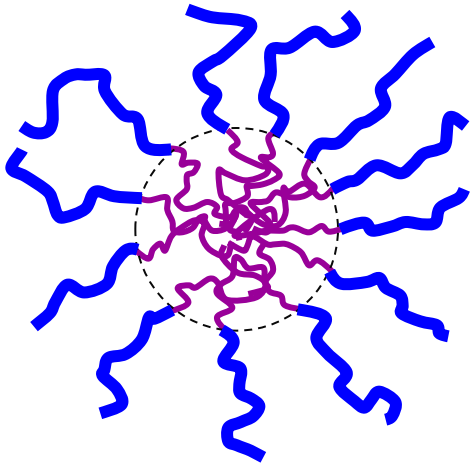
B



Structure ?

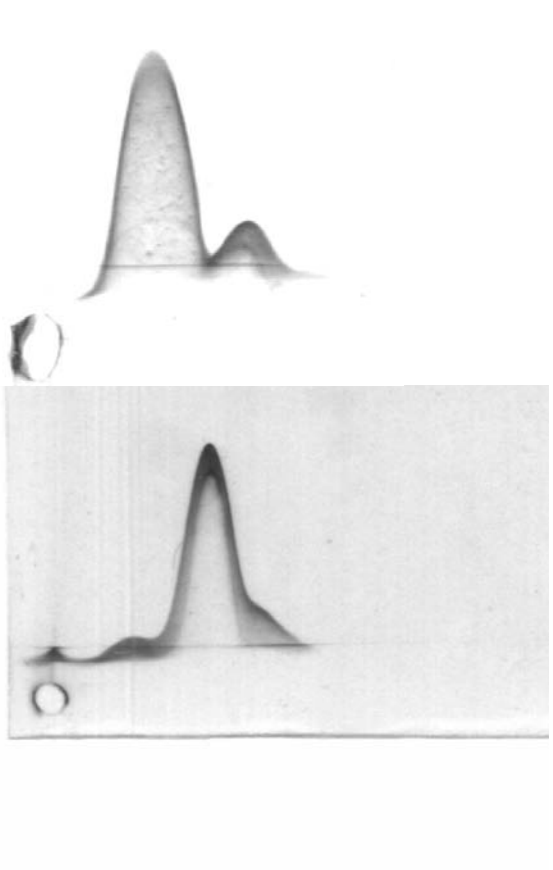


Arrangement des chaînes en surface ?



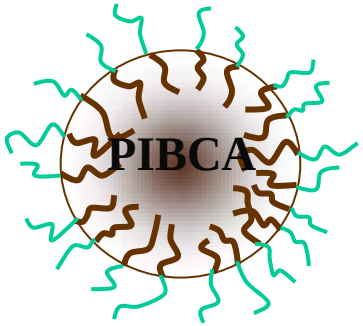
POLYMERISATION ANIONIQUE ET RADICALAIRE ET ACTIVATION DU COMPLEMENT

Chauvierre et al., Cell Mol Biol, 50, 233-239 (2004)



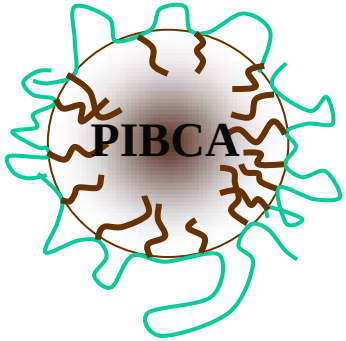
Polymérisation radicalaire

Non activateur



Polymérisation anionique

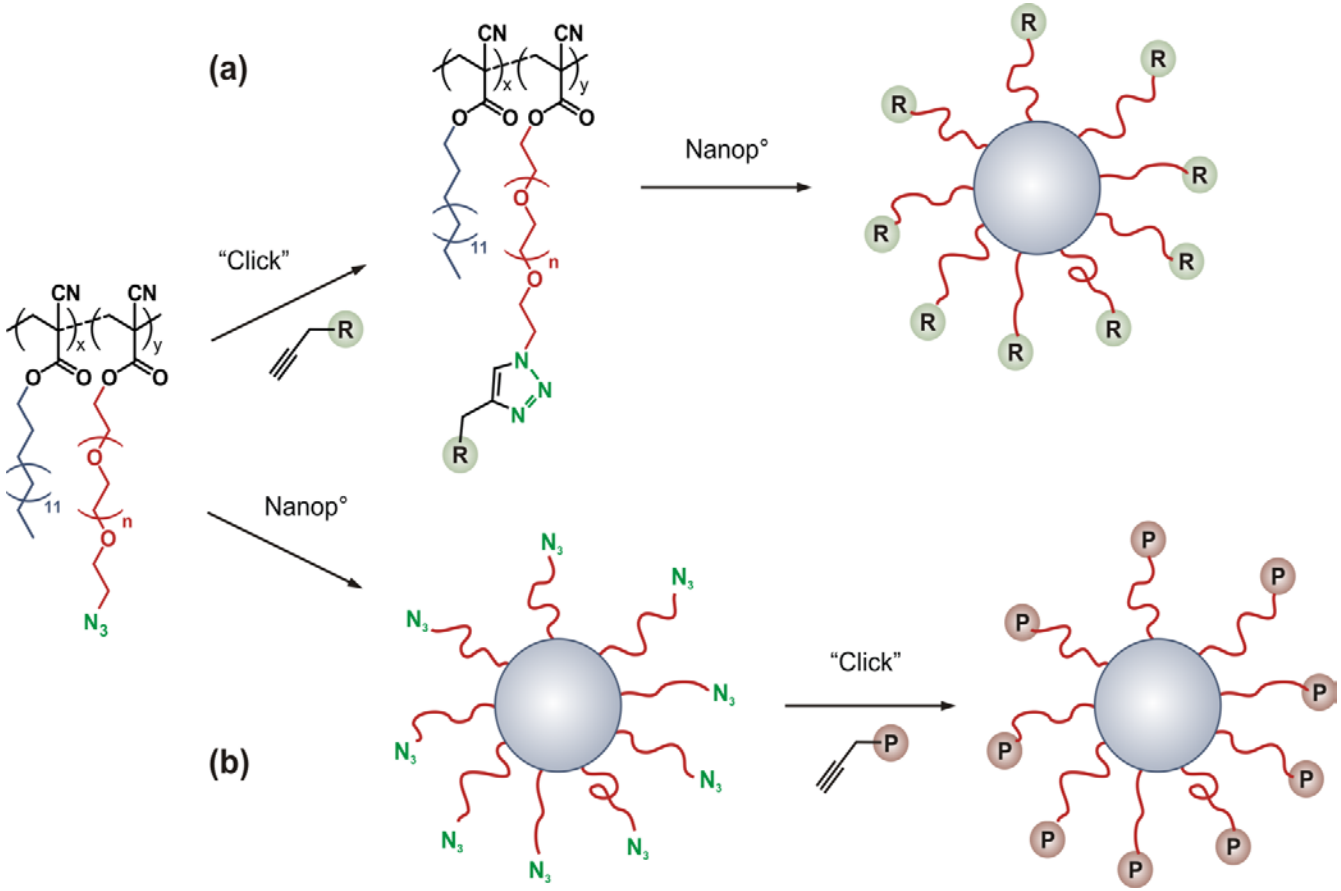
Activateur



Dextrane 71 000KD

« CLICK CHEMISTRY » POUR LE COUPLAGE DE LIGANDS A LA SURFACE DE NANOPARTICULES DE PACA

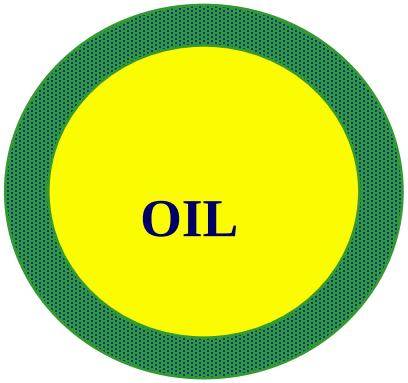
Nicolas J et al., *Macromolecules*, in press, 2008
Nicolas J et al, *Macromolecules*, **41**, 3758-3761 (2008)



PREPARATION DE NANOCAPSULES PAR POLYMERISATION INTERFACIALE

PREPARATION DE NANOCAPSULES PAR POLYMERISATION INTERFACIALE

O/W

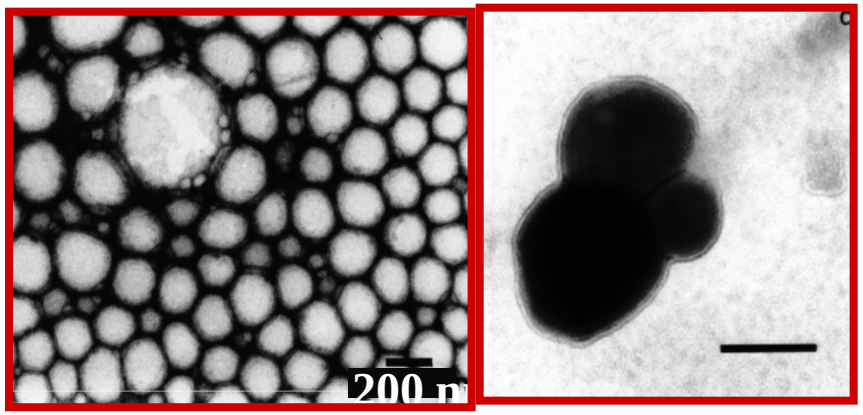


→ PA lipophiles

W/O

→ Oligonucleotides

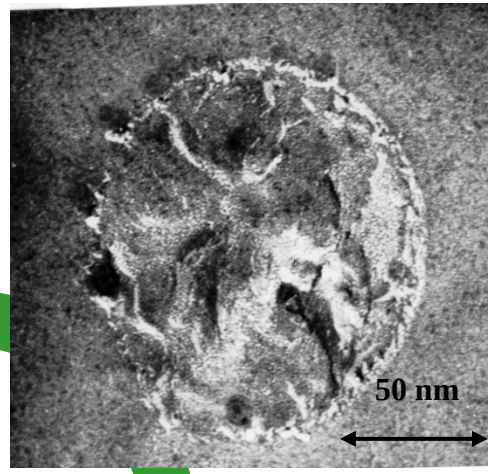
→ Peptides

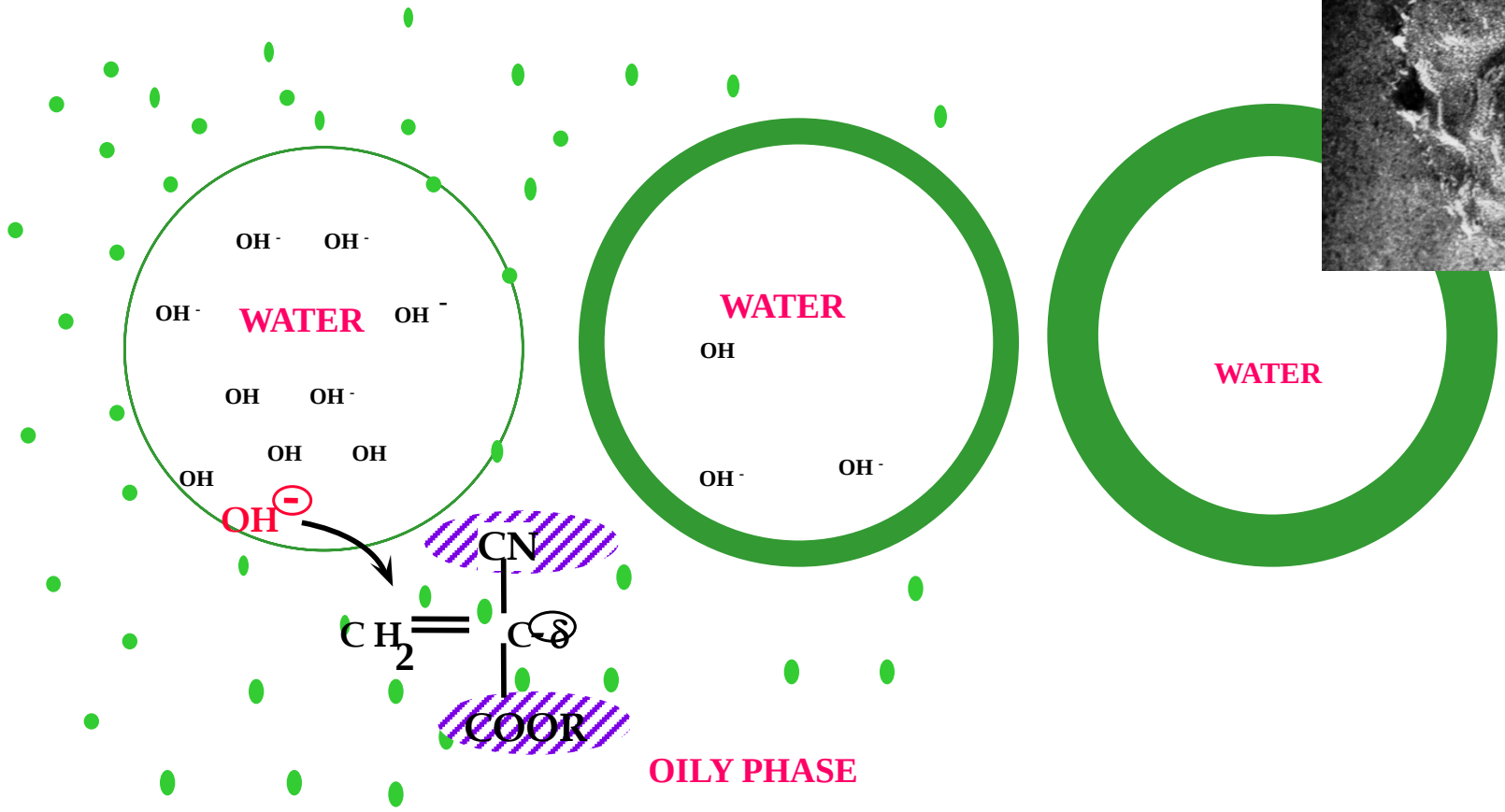


PACA NANOCAPSULE FORMATION

UMR CNRS 8612

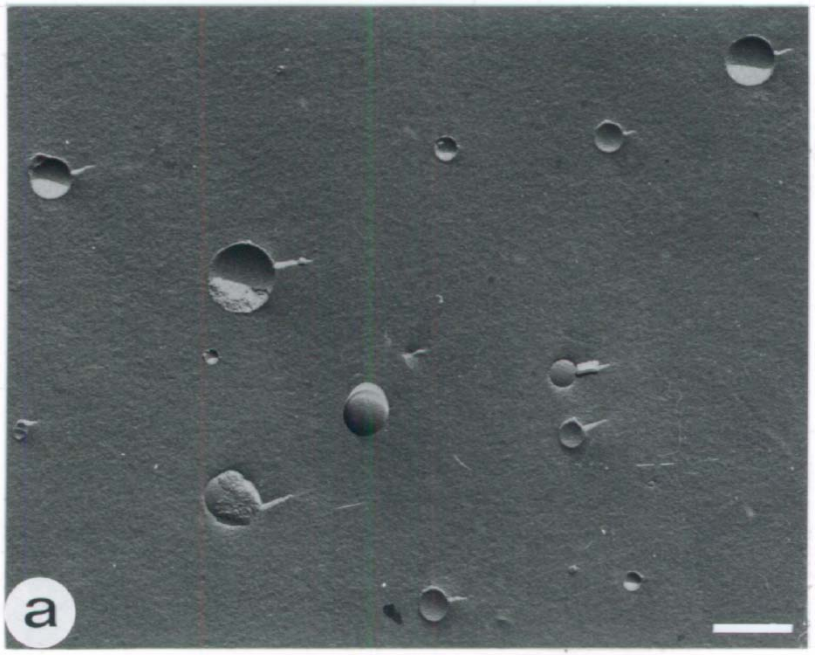
ıbert, J.R. Bertrand, E. Fattal, F. Subra, H. Pinto-Alphandary, C. Malvy,C. Auclair, P. Couvreur, BBRC, 2001



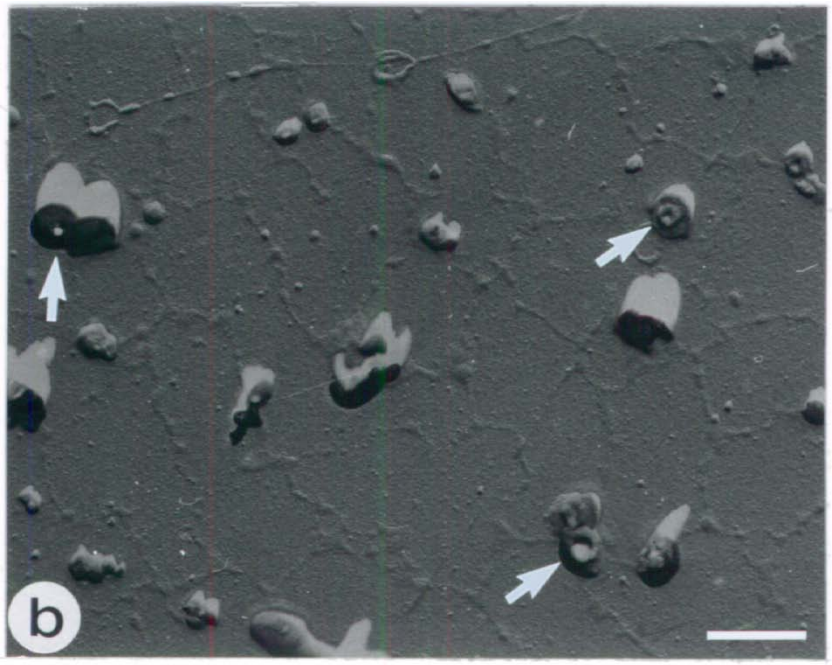


NANOCAPSULES WITH AN AQUEOUS CORE/ FREEZE FRACTURE

Lambert et al., Pharm. Res., 2000



a) Before drying



b) After drying

NANOCAPSULES PREPARATION BY INTERFACIAL POLYCONDENSATION

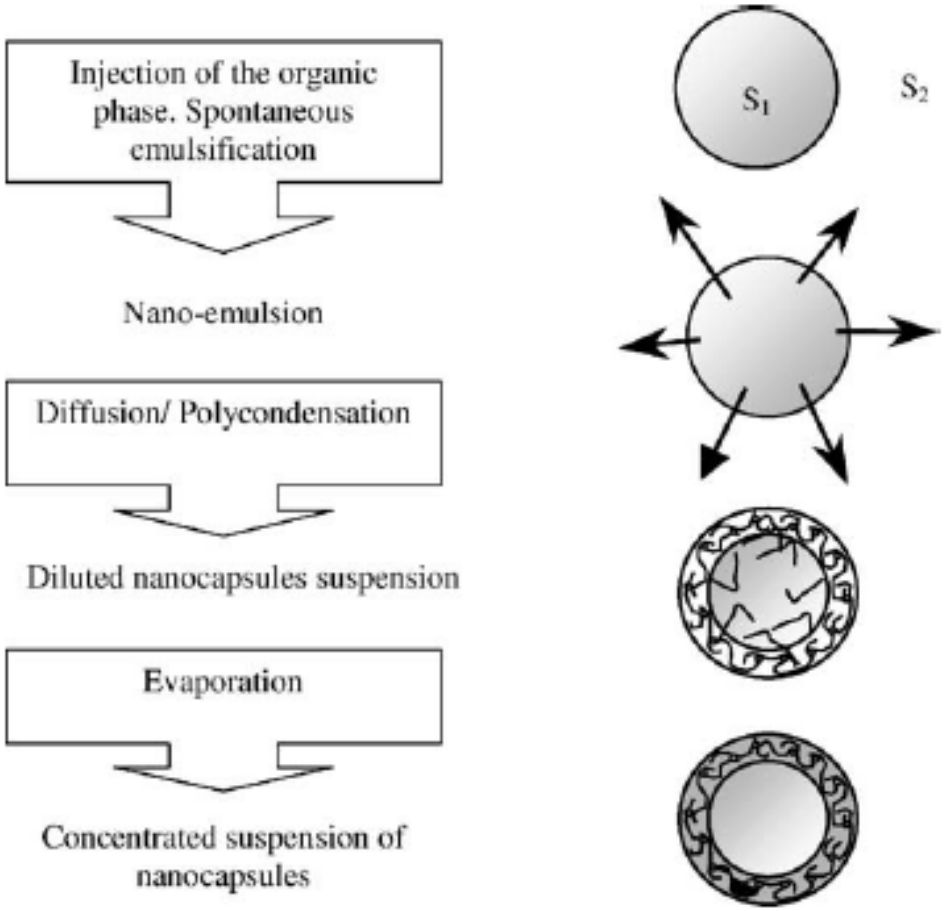
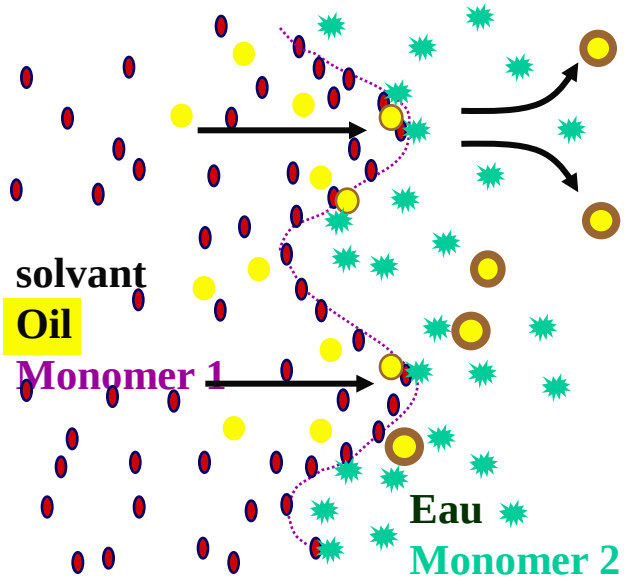
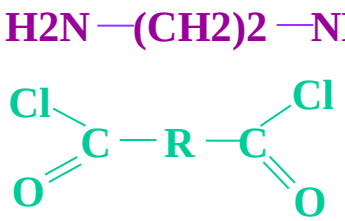


Fig. 1. Mechanism of nanocapsules preparation using the new interfacial polycondensation technique (Montasser et al., 2001).





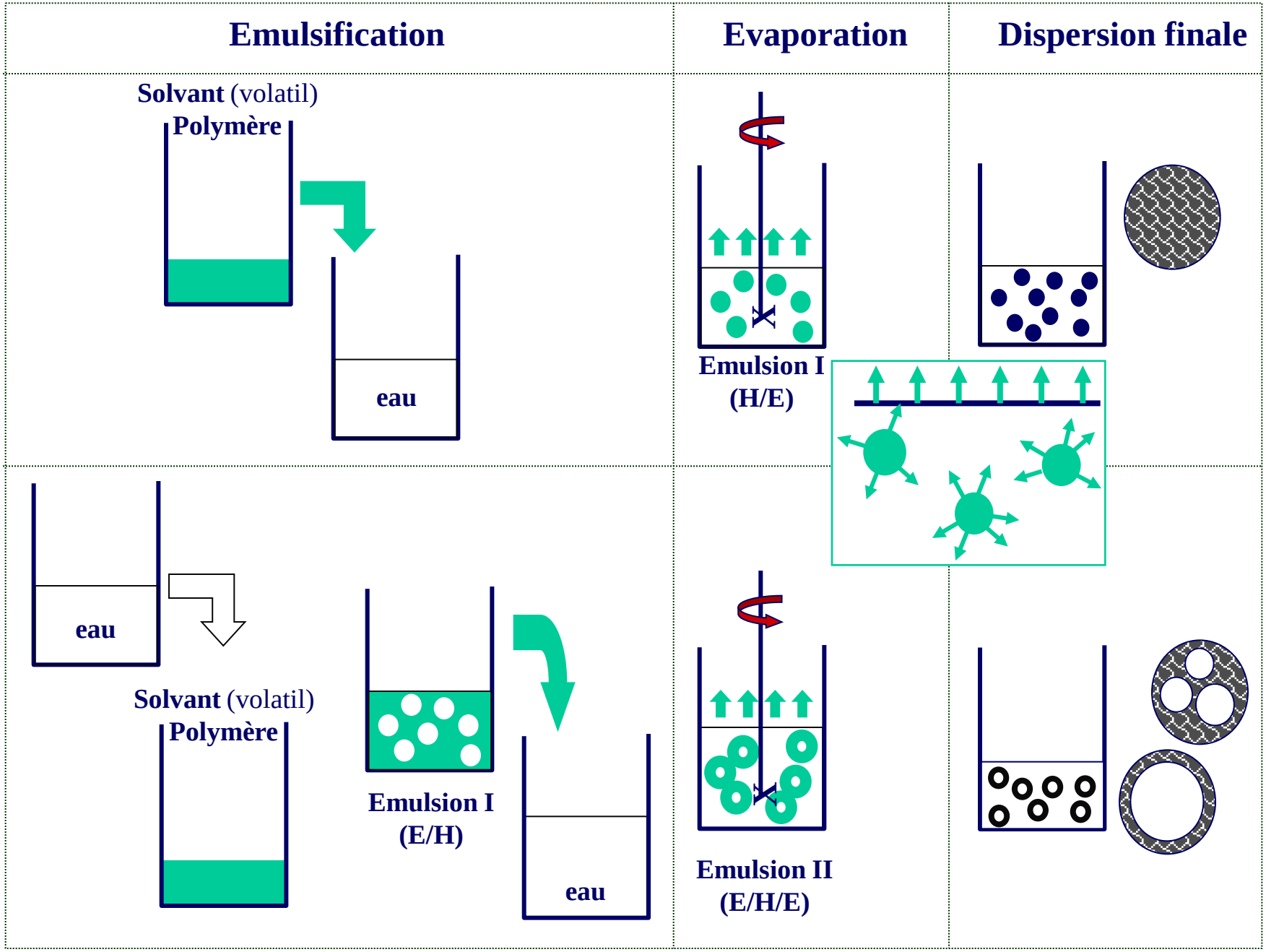
K. Bouchemal et al. / International Journal of Pharmaceutics 269 (2004) 89–100

PREPARATION DE NANOPARTICULES A PARTIR D'UN POLYMERE PREFORME

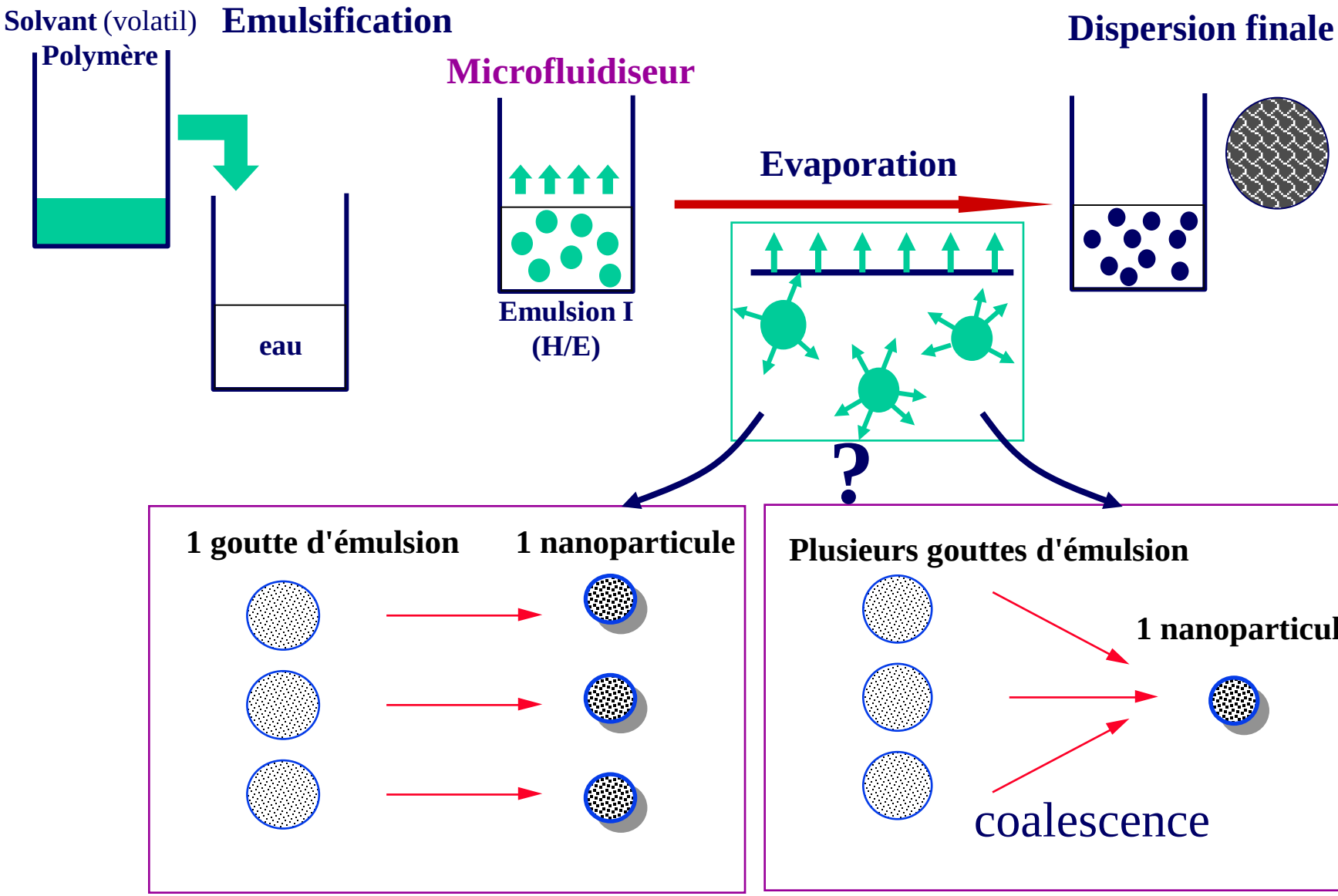
Emulsion évaporation de solvant

Emulsion extraction de solvant

EMULSIFICATION-EVAPORATION DE SOLVANT



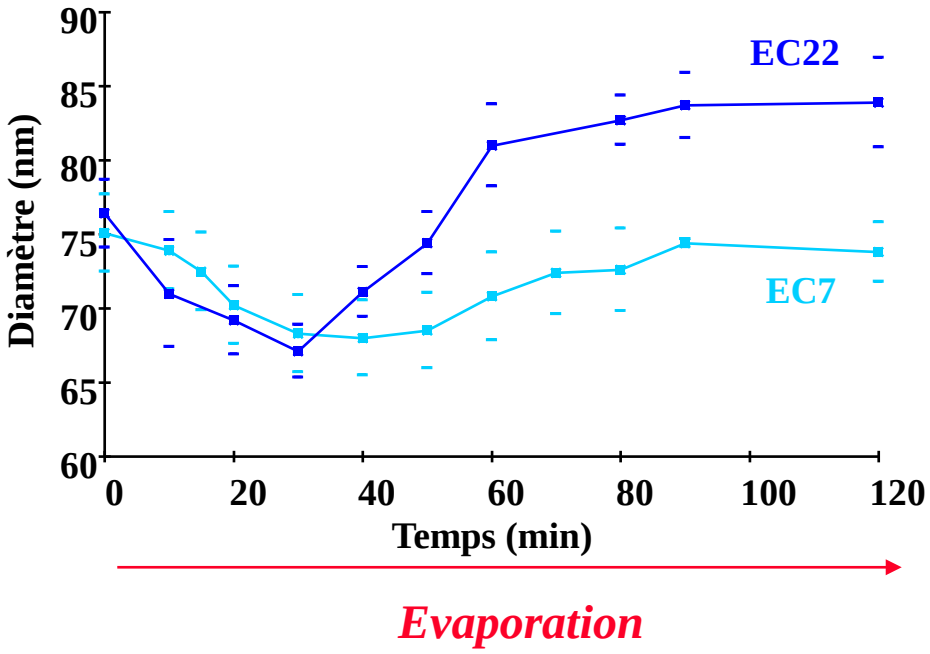
Emulsification – solvent evaporation

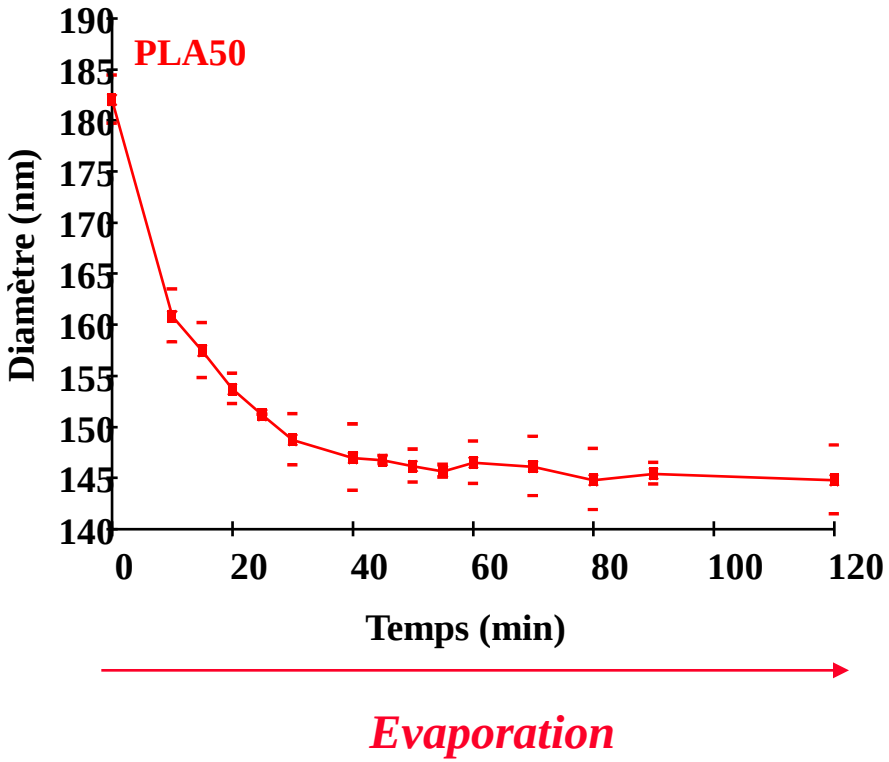


Desgouilles et al., Langmuir 2003

MISE EN EVIDENCE DE LA COALESCENCE

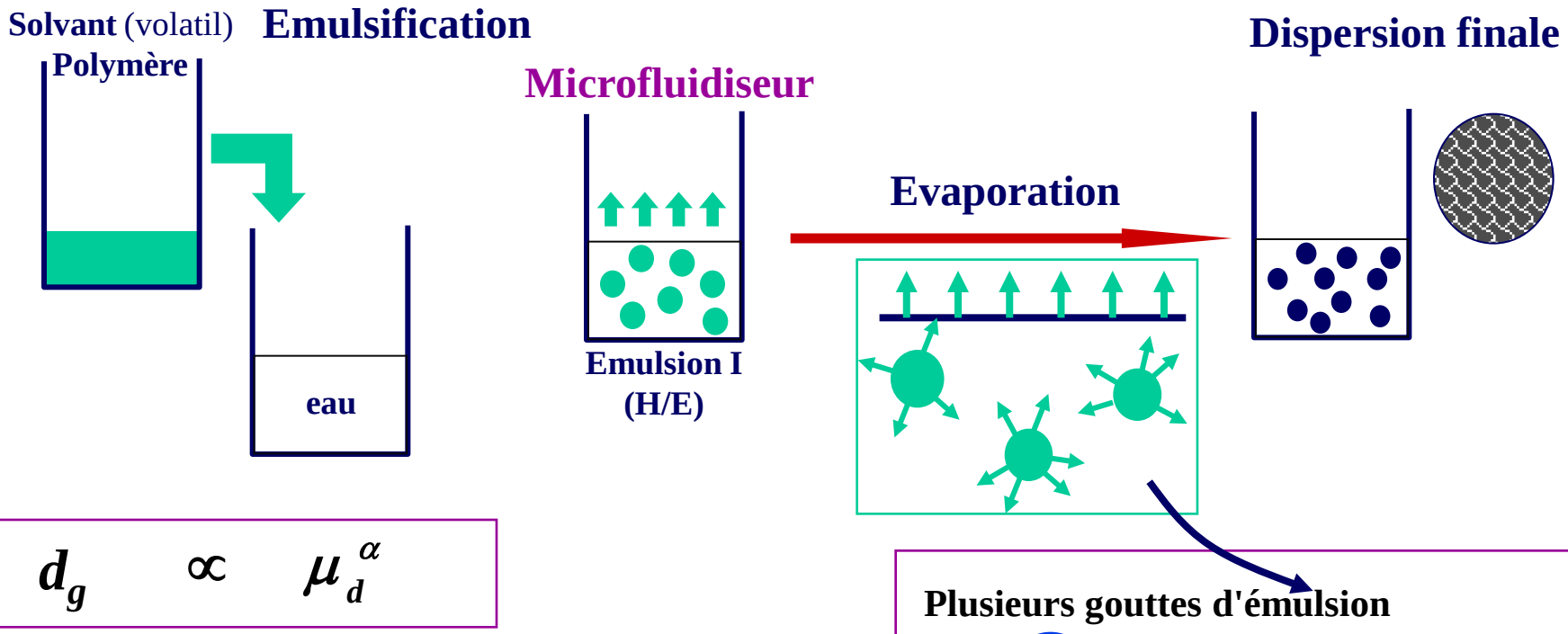
Desgouilles et al., Langmuir 2003





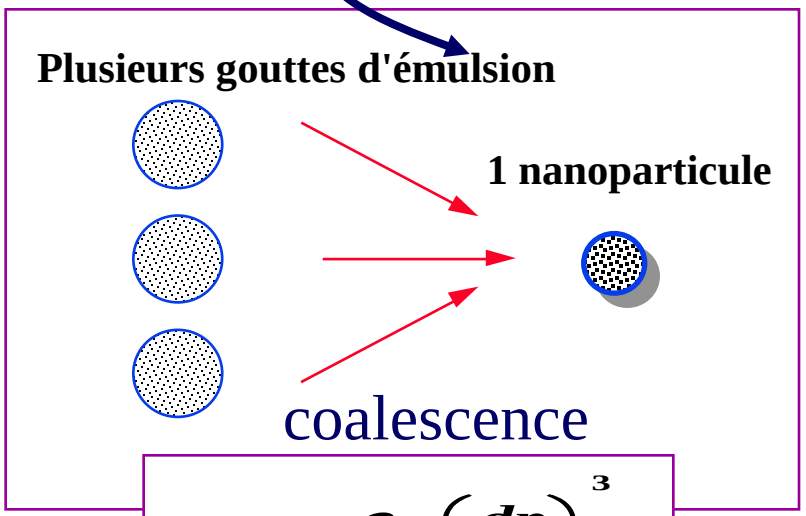
EMULSIFICATION-EVAPORATION DE SOLVANT

Desgouilles et al., Langmuir 2003



$$d_g \propto \mu_d^{\alpha}$$

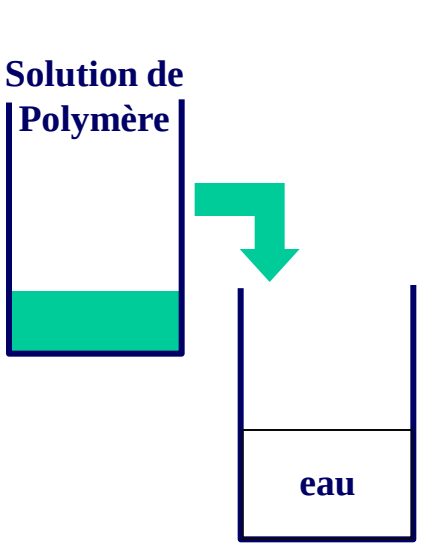
Fc Facteur de coalescence
d g diamère des gouttes
d p diamètre des particules
C concentration massique du polymère
ρ_0 densité
μ facteur de viscosité

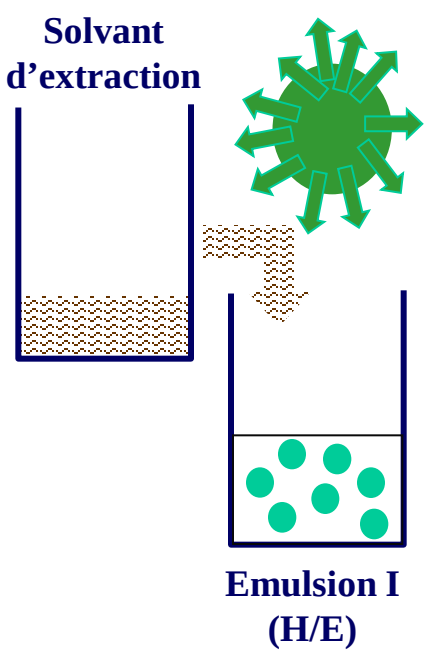


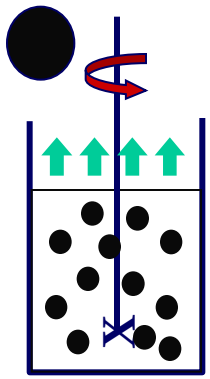
$$Fc = \frac{\rho_0}{C}\left(\frac{dp}{dg}\right)^3$$

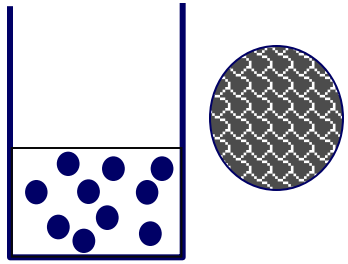
EMULSIFICATION-EXTRACTION DE SOLVANT

Emulsification	Extraction du solvants	Elimination des solvants	Dispersion finale





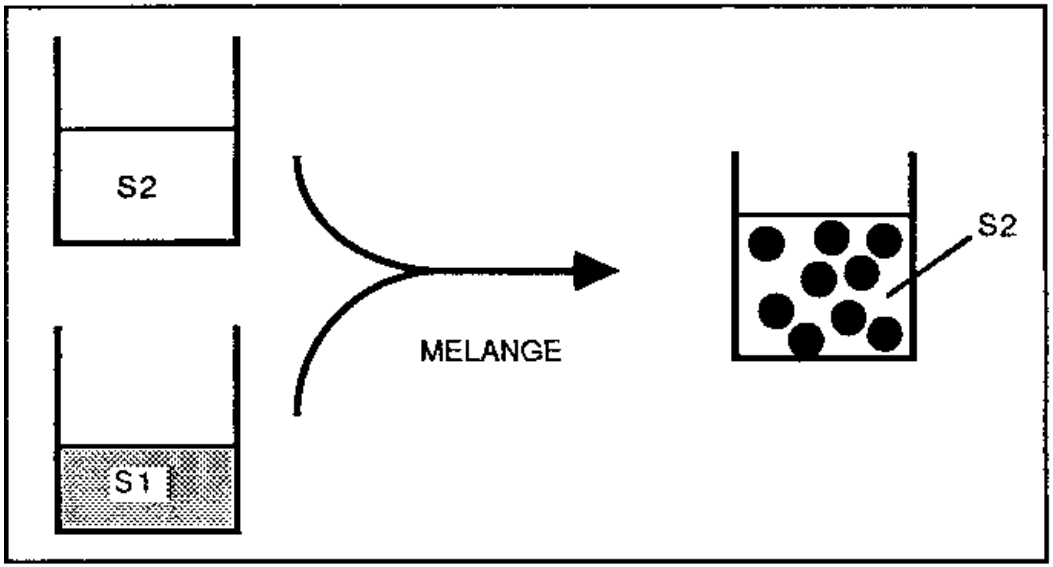


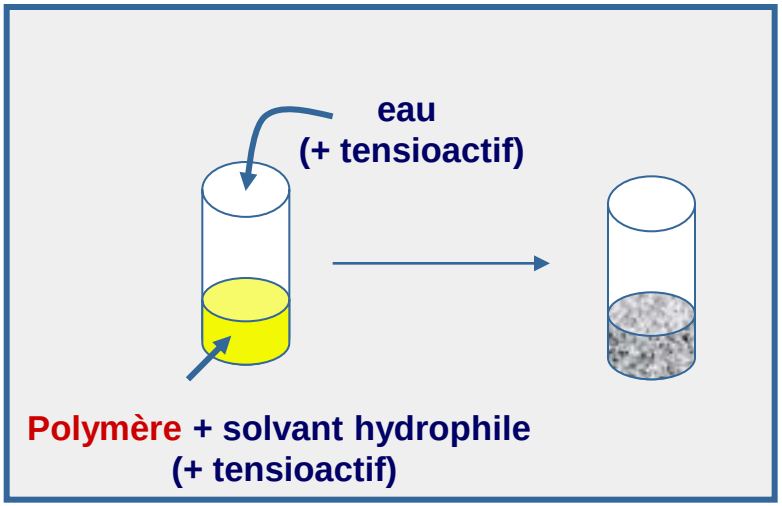


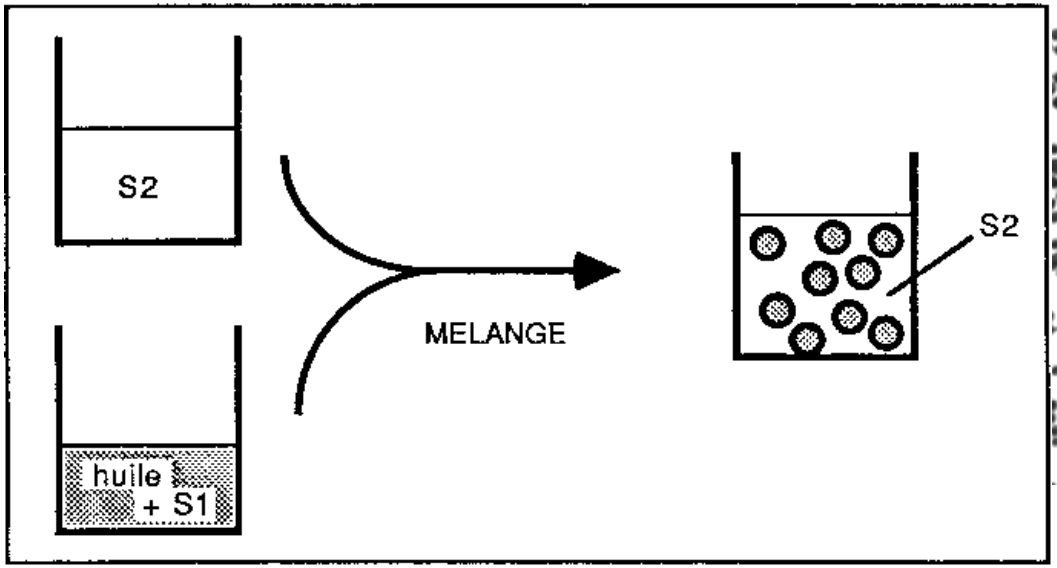
PREPARATION DE NANOPARTICULES A PARTIR D'UN POLYMERE PREFORME

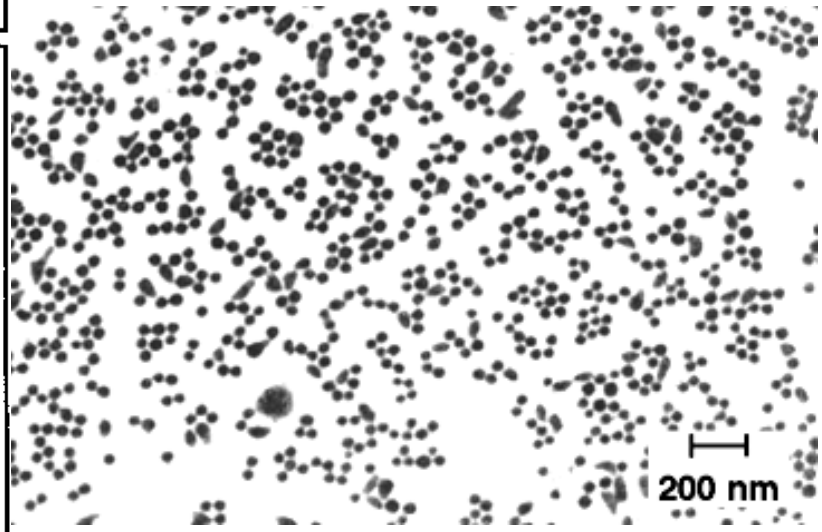
Nanoprécipitation

NANOPRECIPITATION: PRINCIPE GENERAL

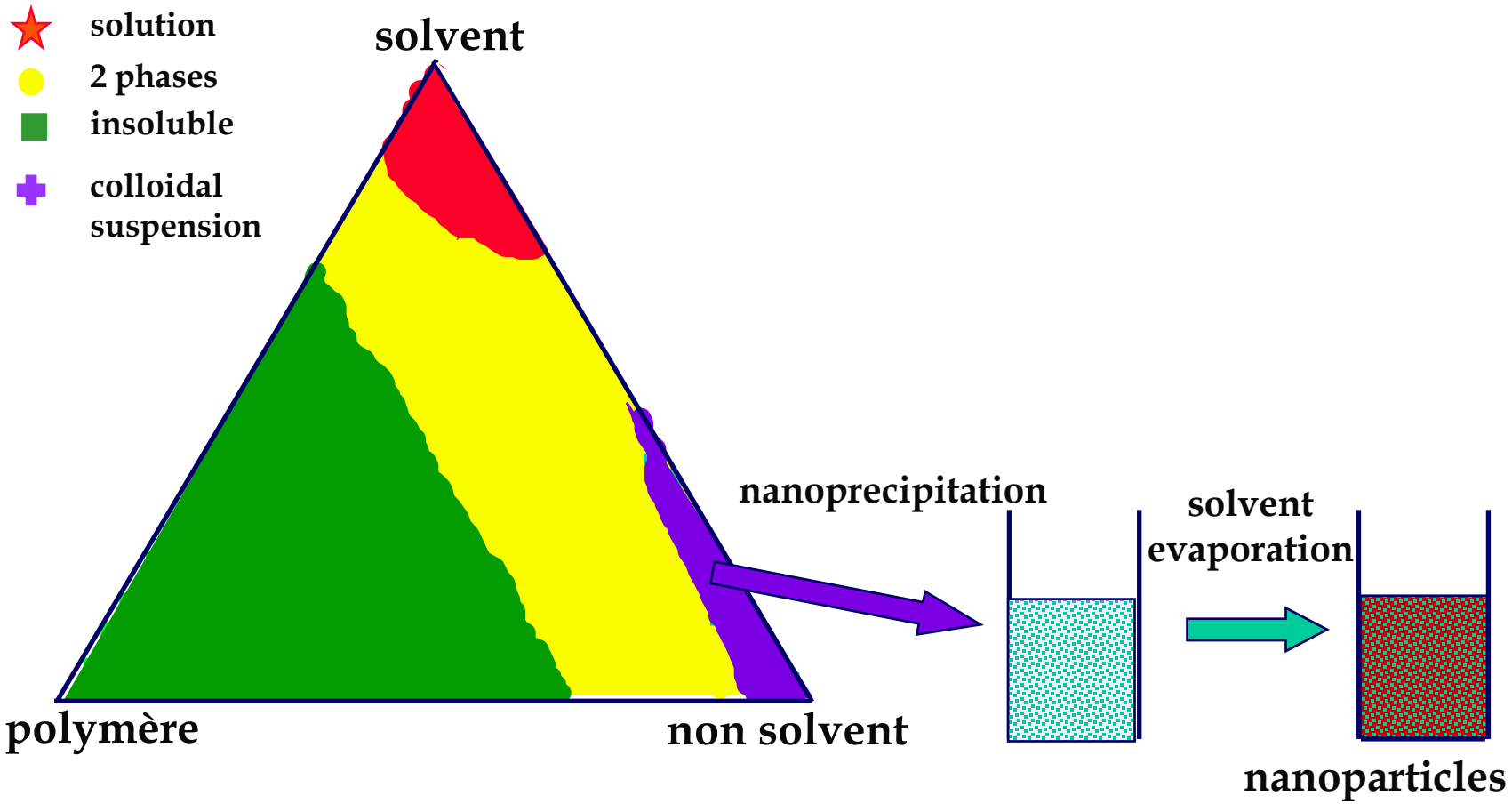






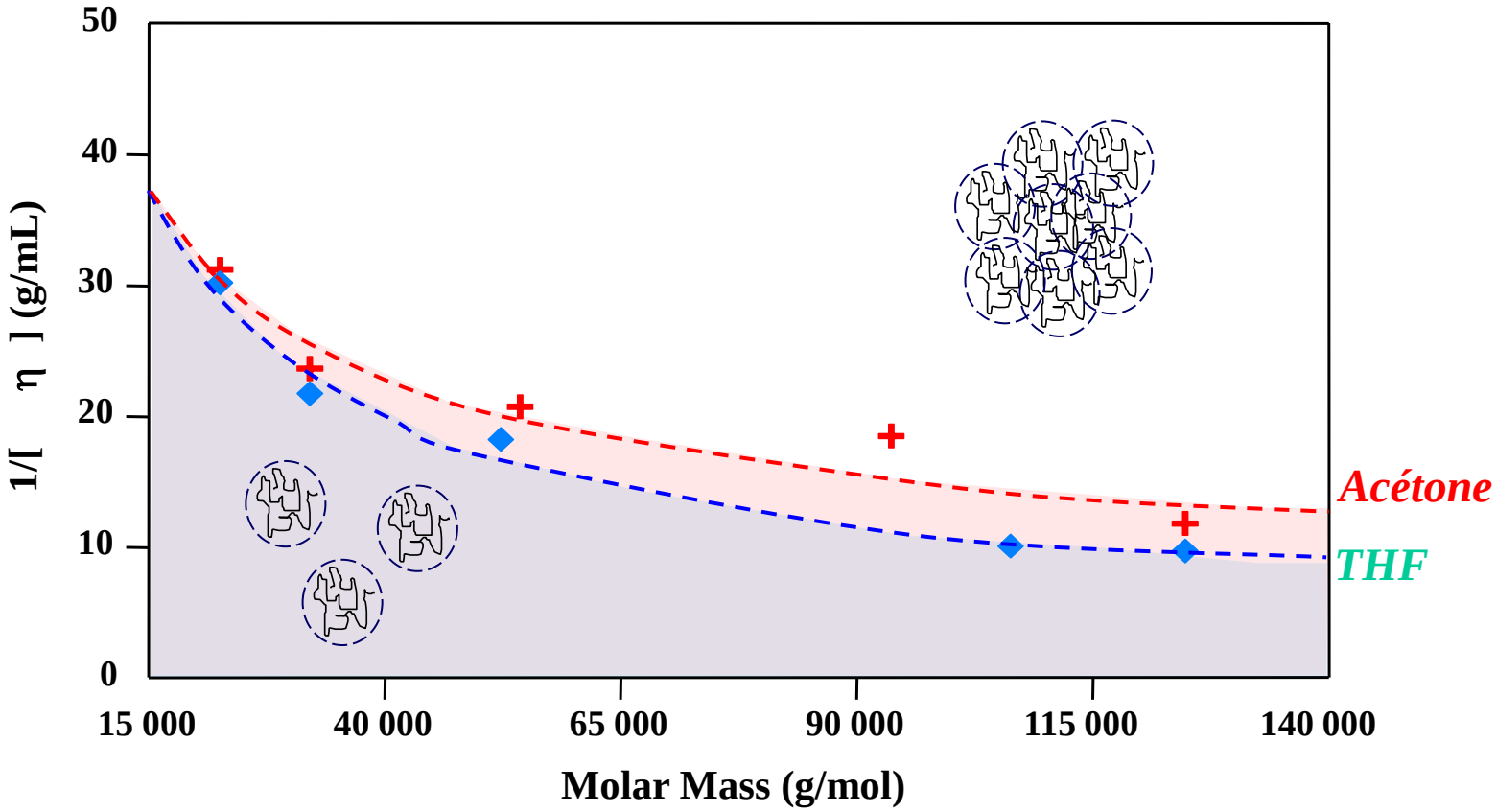


NANOPRECIPITATION ET DIAGRAMME DE PHASE



INFLUENCE DU POIDS MOLECULAIRE ET DE LA VISCOSITE

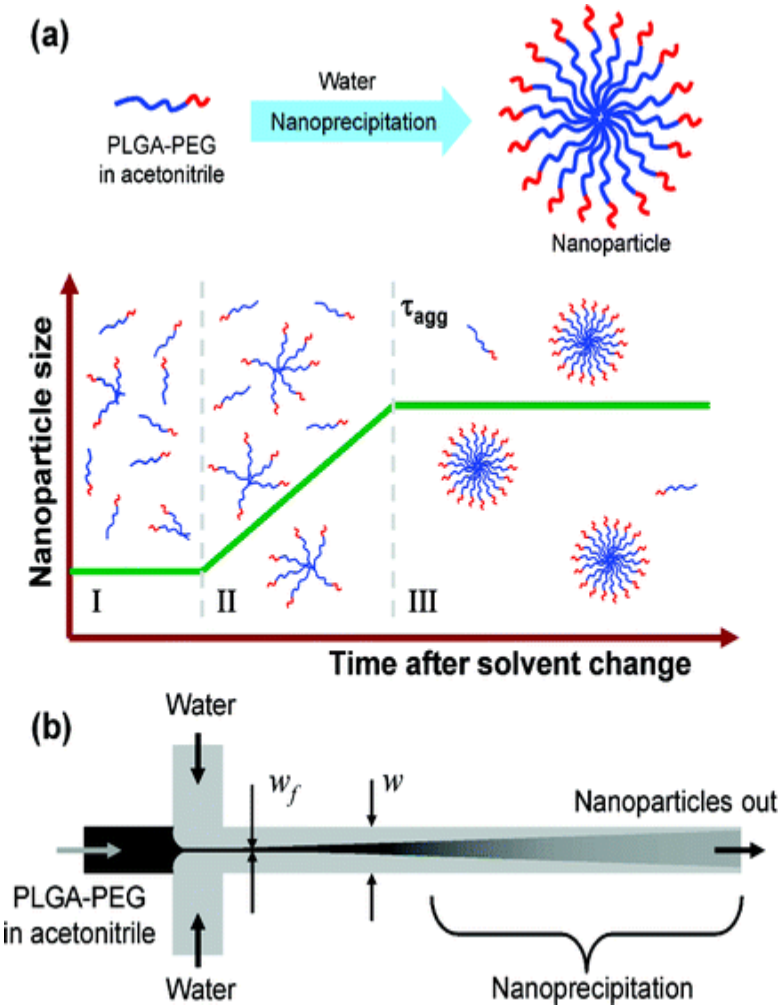
Legrand et al. Int. J. Pharm 2007



Viscosimétrie Capillaire

APPLICATION OF MICROFLUIDICS TO THE PREPARATION OF PLA NANOPARTICLES BY NANOPRECIPITATION

Karnik R et al., Nanoletters, 8, 2906-2912, 2008

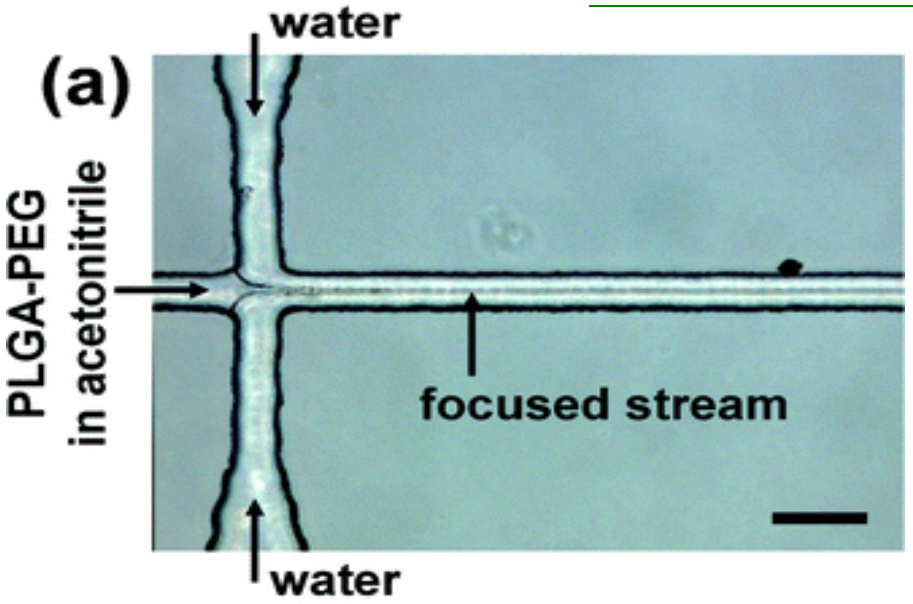


(a) The process occurs in three stages involving nucleation of nanoparticles, growth through aggregation, and results in kinetically locked nanoparticles after a characteristic aggregation time scale τ_{agg}.

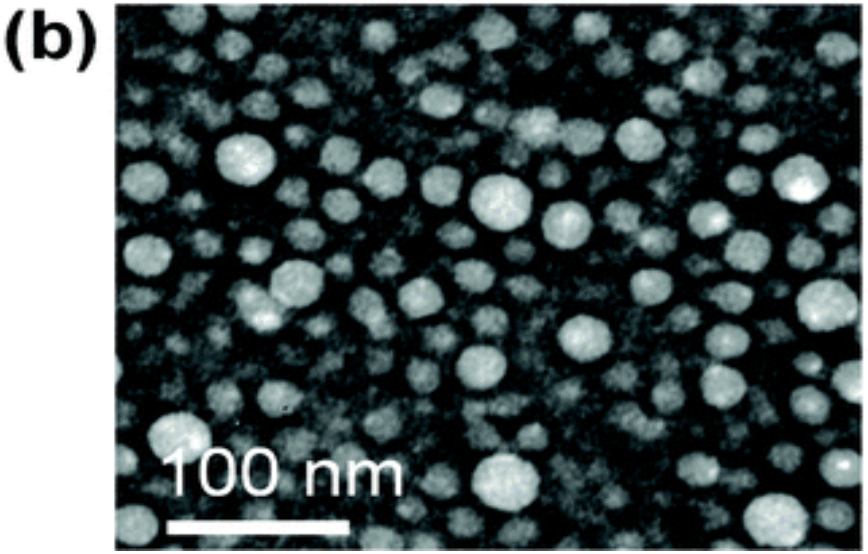
(b) The process of mixing can be carried out in a microfluidic device using hydrodynamic flow focusing, where the polymer stream is focused into a thin stream between two water streams with higher flow rates.

MICROFLUIDICS FOR THE PREPARATION OF PLA NANOPARTICLES BY NANOPRECIPITATION

Karnik R et al., Nanoletters, 8, 2906-2912, 2008



(a) A microfluidic device for hydrodynamic flow focusing of polymeric nanoparticles in water. Scale bar 50 μm.

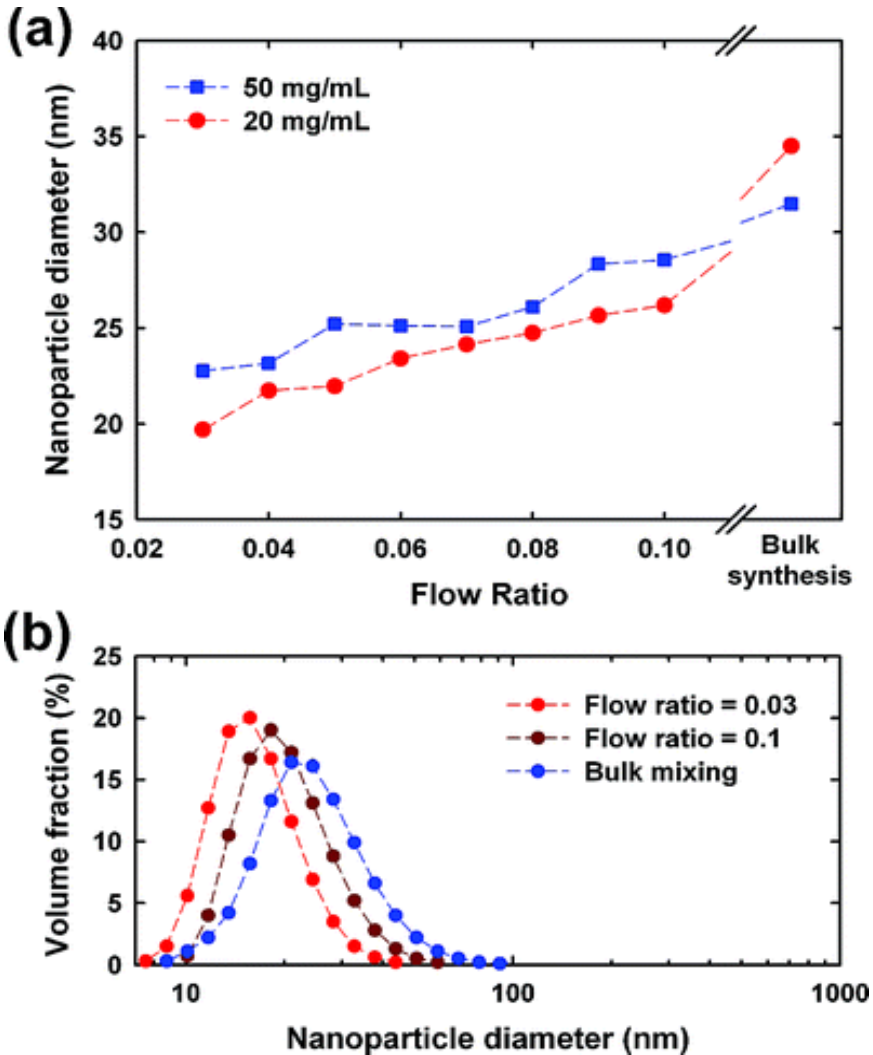


(b) TEM image of nanoparticles synthesized by nanoprecipitation of PLGA15K-PEG3.4K by hydrodynamic flow focusing

EFFECT OF FLOW RATIO ON NANOPARTICLE SIZE

Karnik R et al., Nanoletters, 8, 2906-2912, 2008

$$\textbf{Flow ratio} = \frac{\text{Flow rate of the acetonitrile polymer solution}}{\text{Flow rate of water}}$$



PREPARATION DE NANOPARTICULES A L'AIDE DE METHODES SPECIFIQUES REPOSANT SUR CERTAINES PROPRIETES PARTICULIERES DES POLYMERES

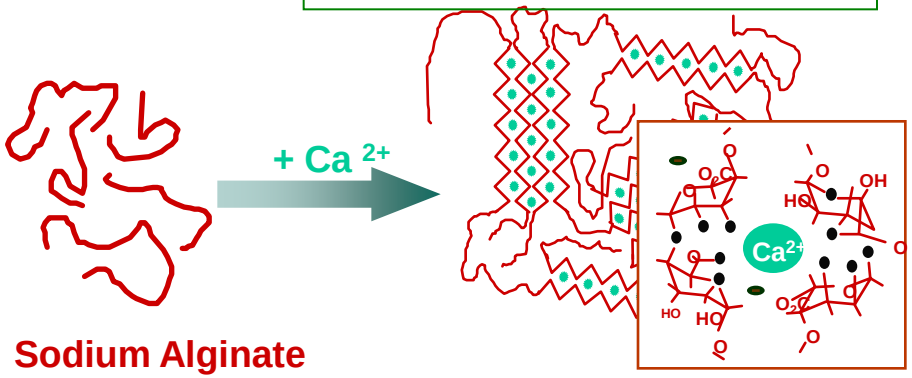
- Gélification
- Complexes d'inclusion
- Formation de complexes électrolytiques (polyplexes)

PREPARATION DE NANOPARTICULES PAR GELIFICATION

Repose sur une propriété particulière de certains polysaccharides

NANOPARTICULES D'ALGINATE

Rajaonarivony et al., J. Pharm. Sci., 1993



Sodium Alginate

Calcium- Alginate GEL

Alginate Na 0.063%

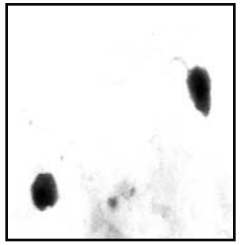
$CaCl_2$ 18mM

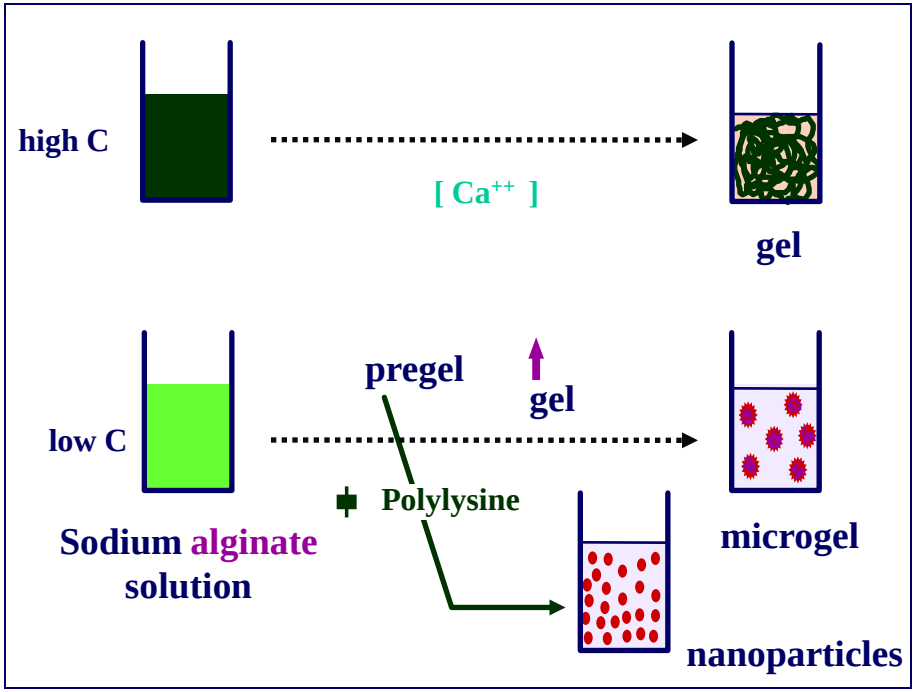
Alginate - Calcium Pregel

Polylysine

NANOPARTICULES

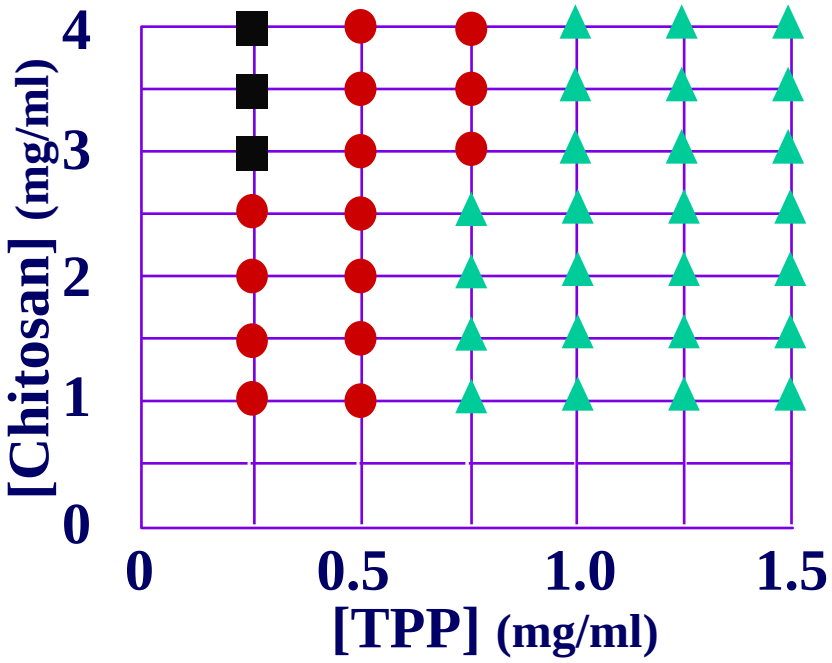
d ~ 350nm

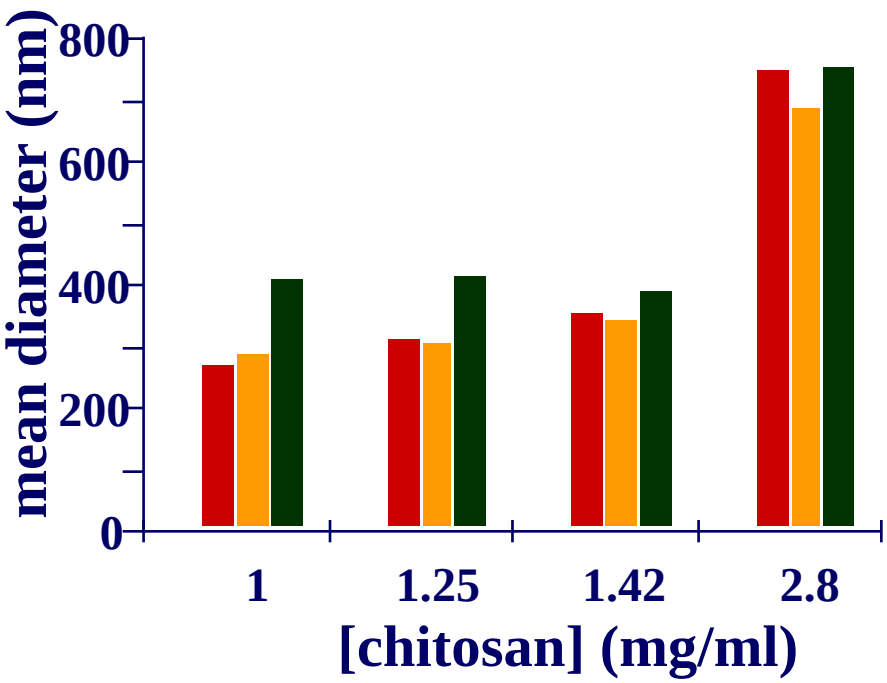




NANOPARTICULES DE CHITOSAN

Calvo et al., J. Applied Polym., 63, 125-132, 1997





solution

nanoparticles

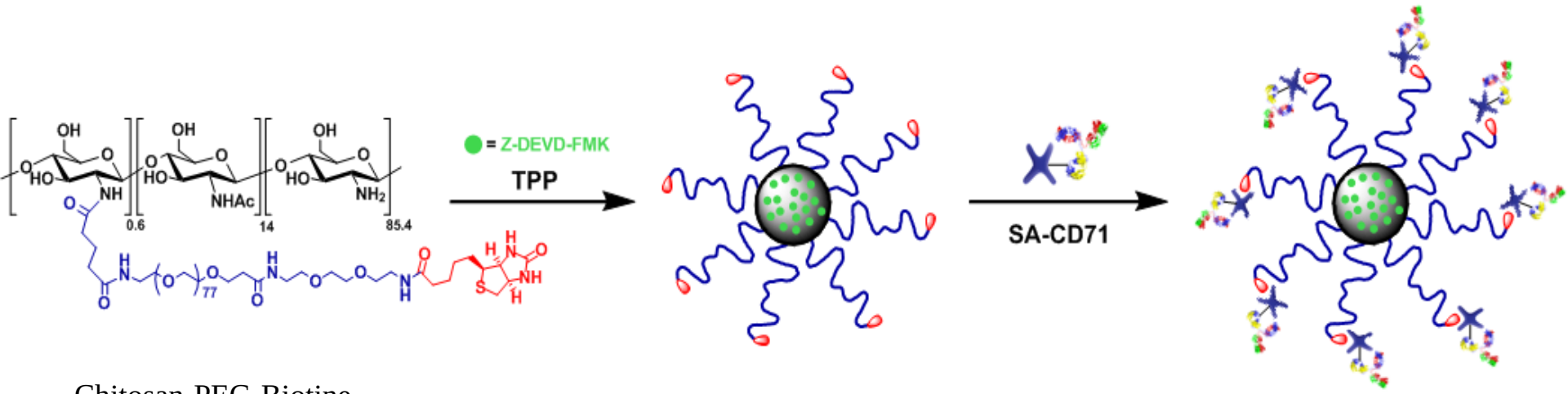
aggregates

polyphosphate (mg/ml)

0.21 0.28 0.43

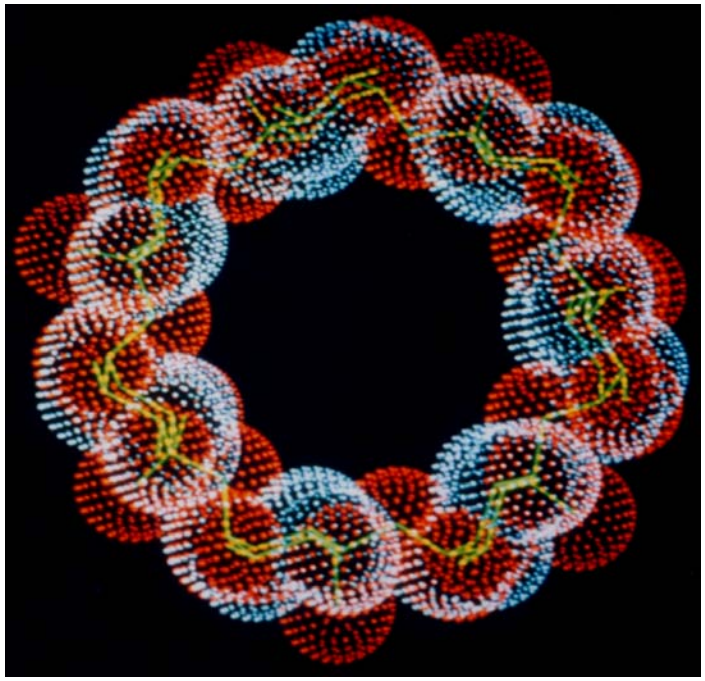
EXEMPLE DE GREFFAGE D'UN ANTICORPS A LA SURFACE DE NANOPARTICULES DE CHITOSAN PAR L'APPROCHE BIOTINE/AVIDINE

Y. Aktaş et al. , Bioconj. Chem., 16, 1503-1511 (2005)



Chitosan-PEG-Biotine

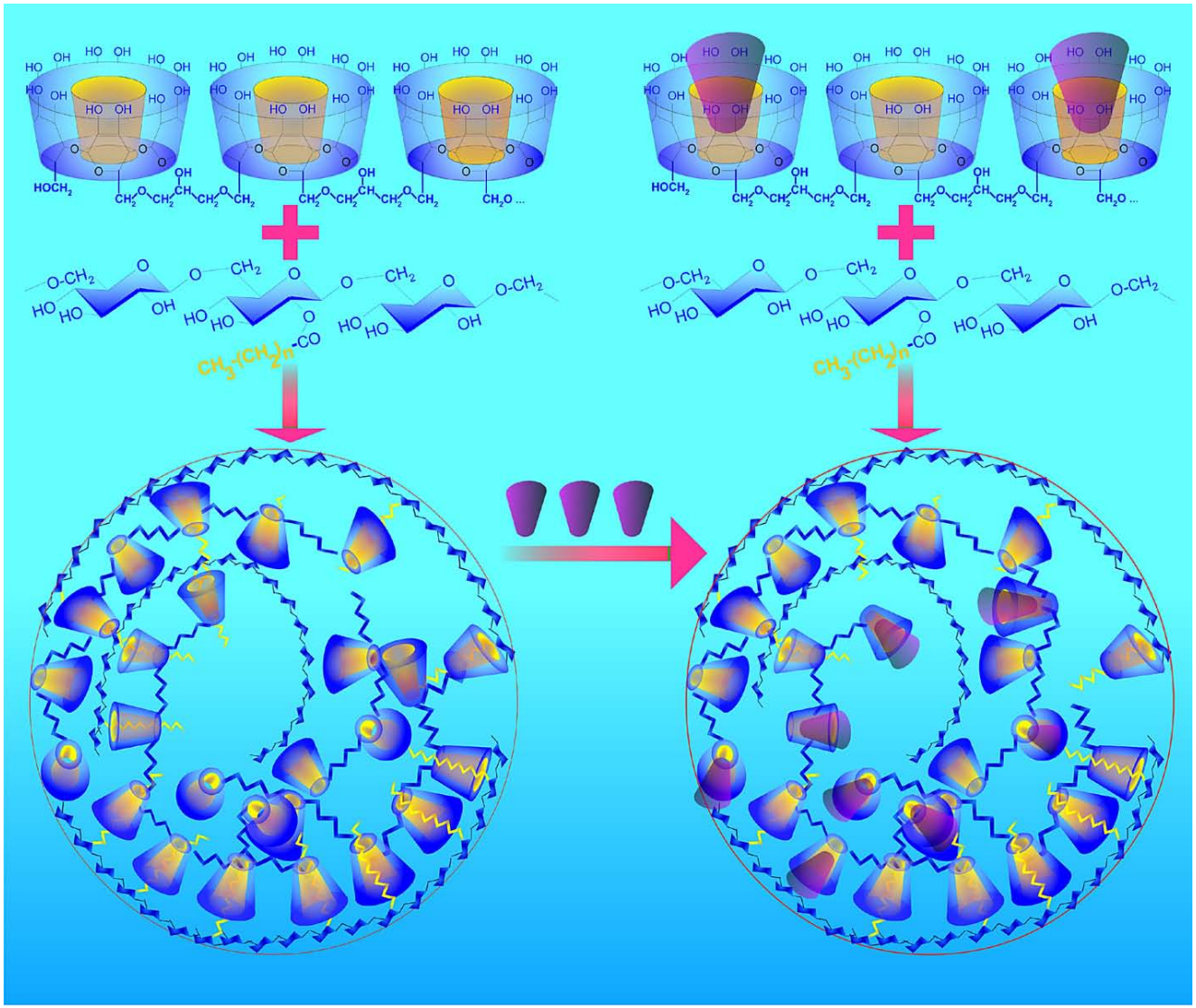
PREPARATION DE NANOPARTICULES PAR AUTO-ASSEMBLAGE

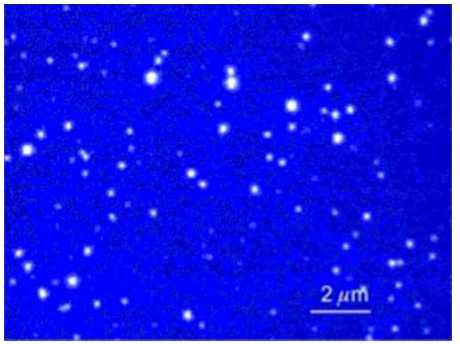


Utilisation des propriétés d'inclusion des Cyclodextrines

NOVEL NANOTECHNOLOGY BASED ON THE “KEY AND LOCK”CONCEPT

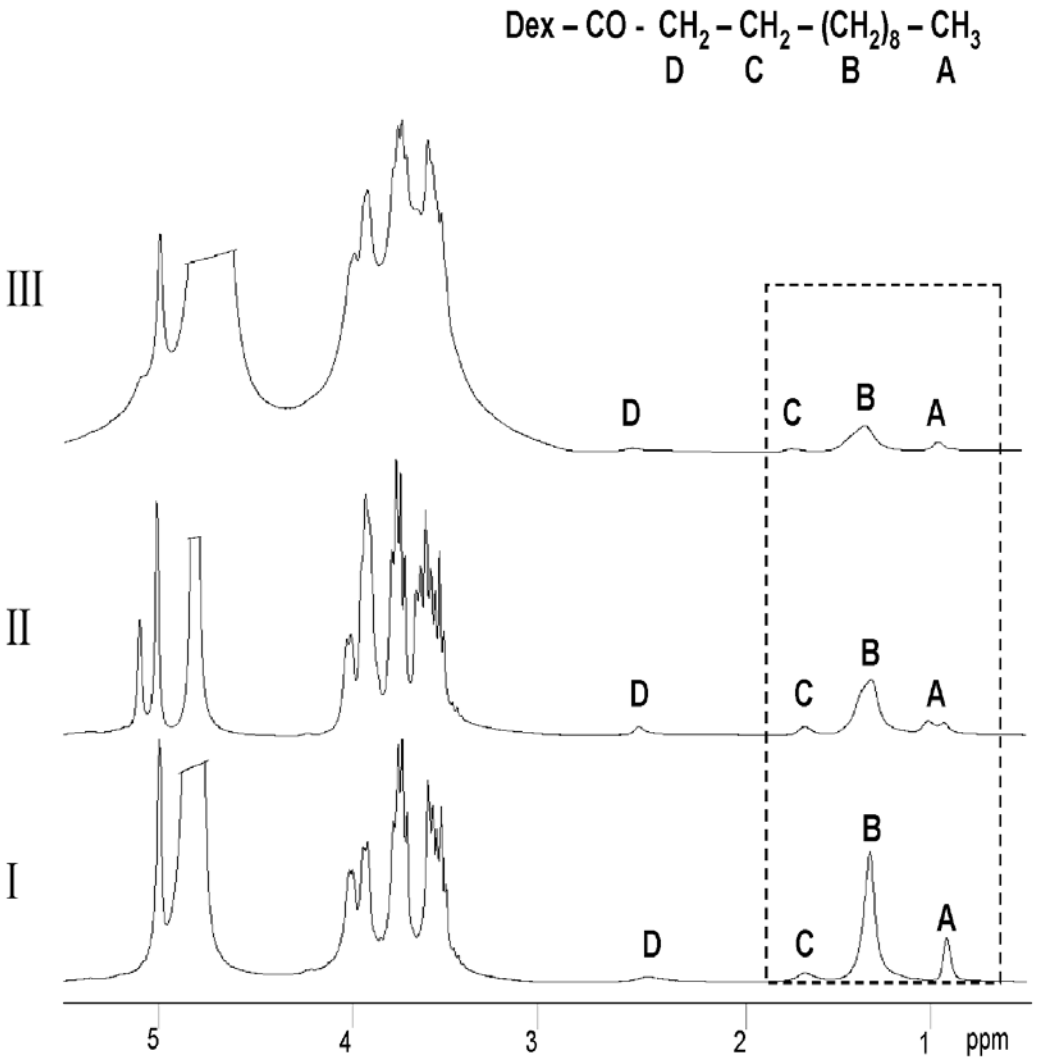
R. Gref et al., French Patent N° 02 08766 (2002)
R. Gref et al., J Control. Rel., 2006

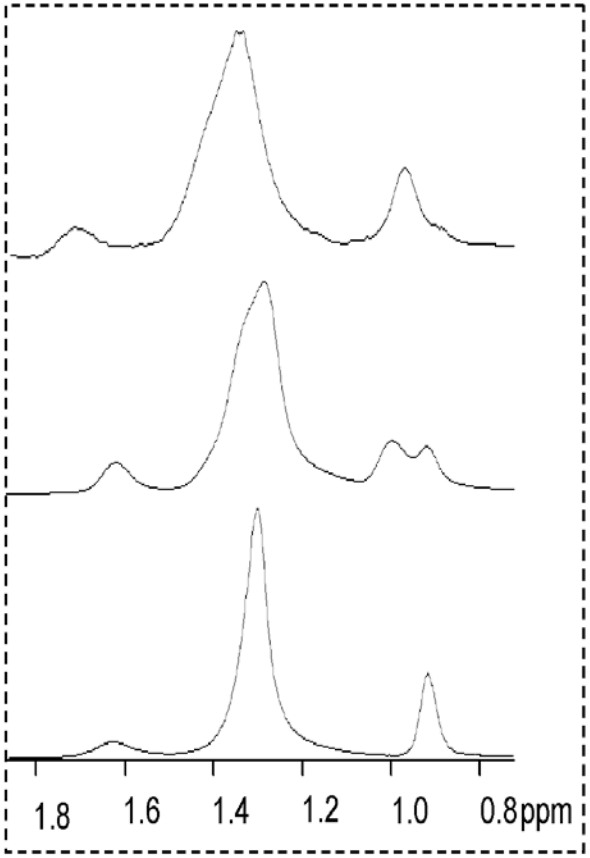


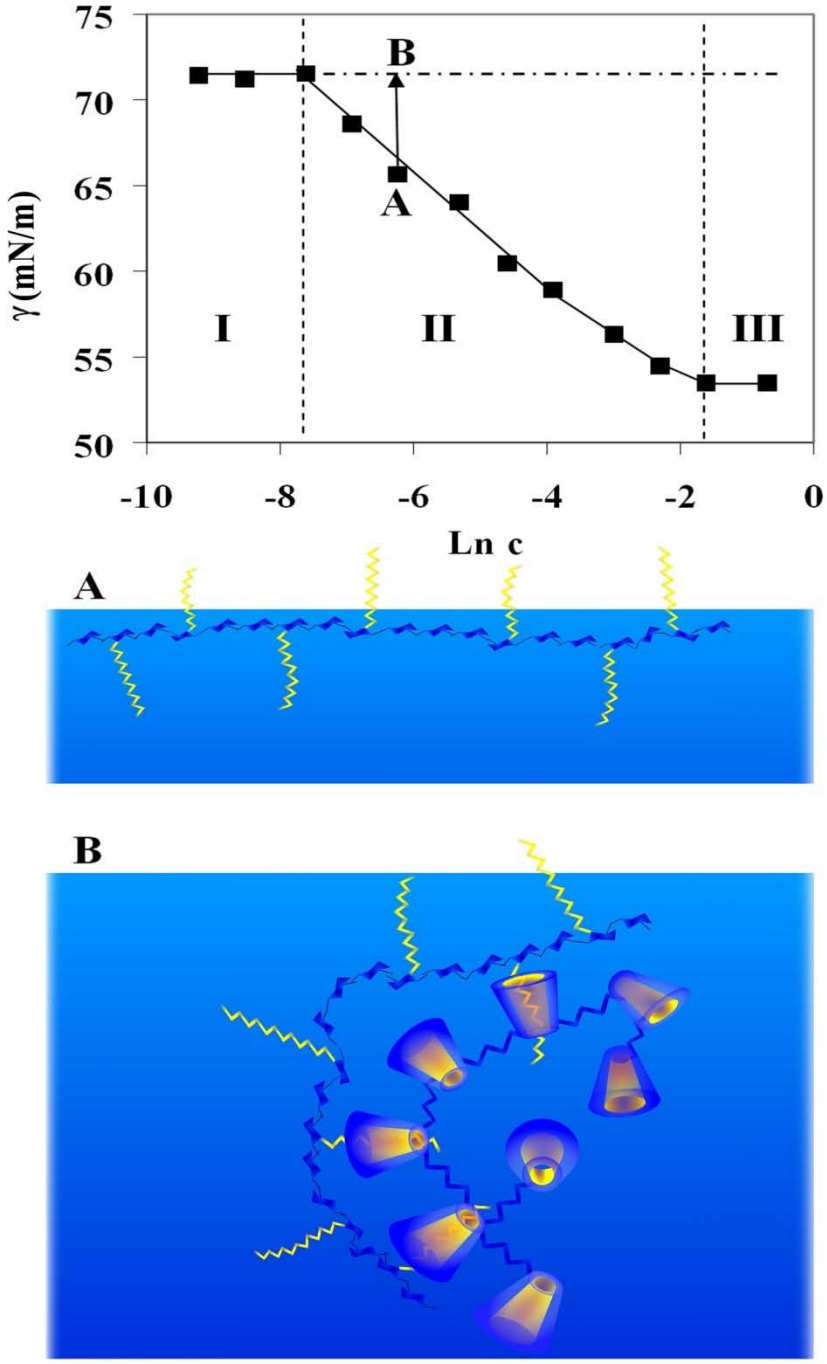


^{3}H-RMN

R. Gref et al., J Control. Rel., 2006







HYDROPHOBIC DEXTRAN REDUCES γ (air/water) BUT THE ADDITION OF POLY-CD RESTAURE THE INITIAL VALUE OF γ (A towards B)

R. Gref et al., J Control. Rel., 2006

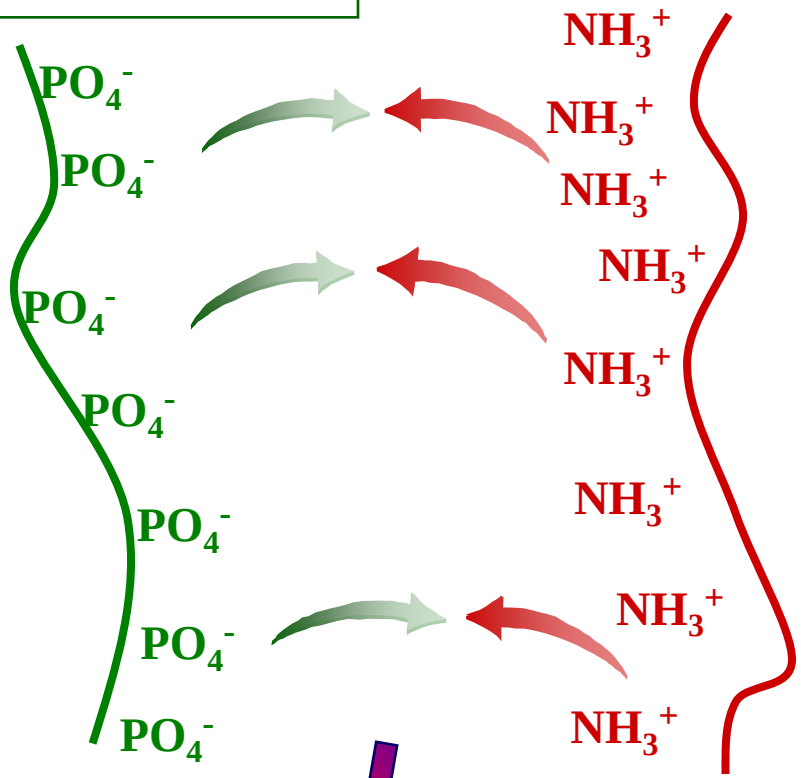
PREPARATION DE NANOPARTICULES PAR INTERACTION ELECTROSTATIQUE

DNA POLYPLEXES

Dunlap et al., Nucleic. Acid. Res. 25: 3095-3101, 1997

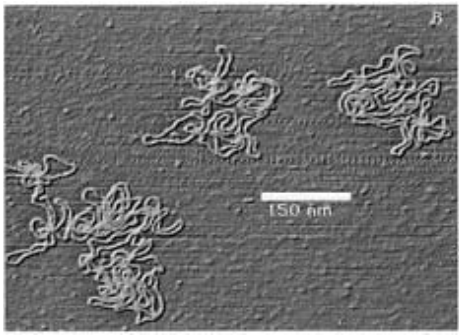
Nucleic acids

Chitosan
Poly(ethyleneimine)
Poly(aminoacid)

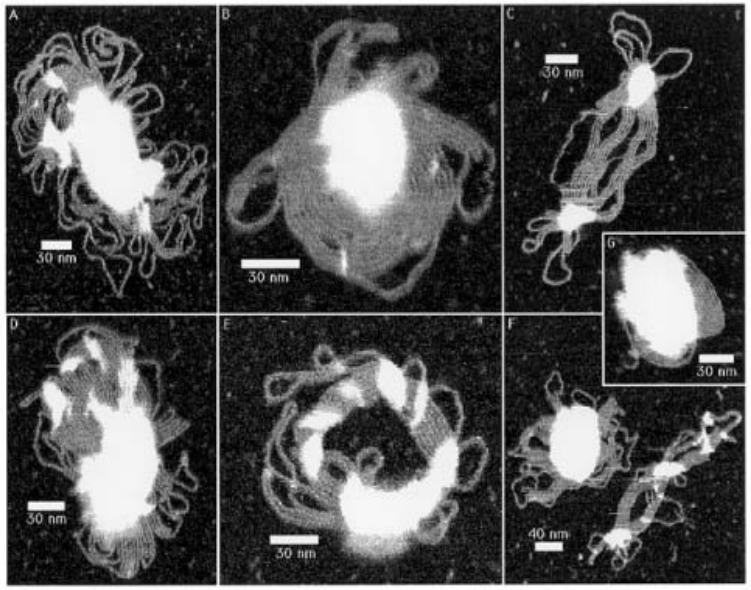


DNA condensation
Colloidal particles
(polyplexes) d_H~20-80nm

AFM



Plasmid



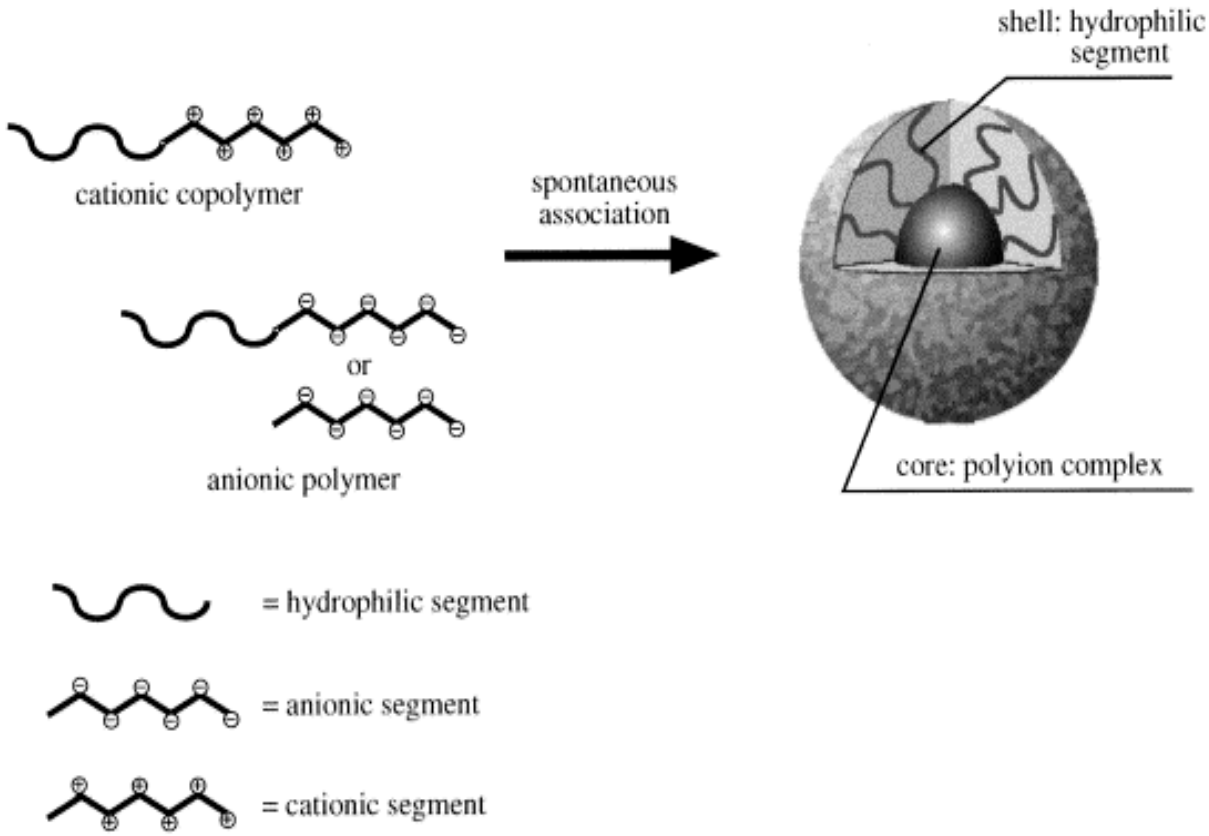
Plasmid condensation with PEI

MICELLES

Concentration Micellaire Critique
CMC

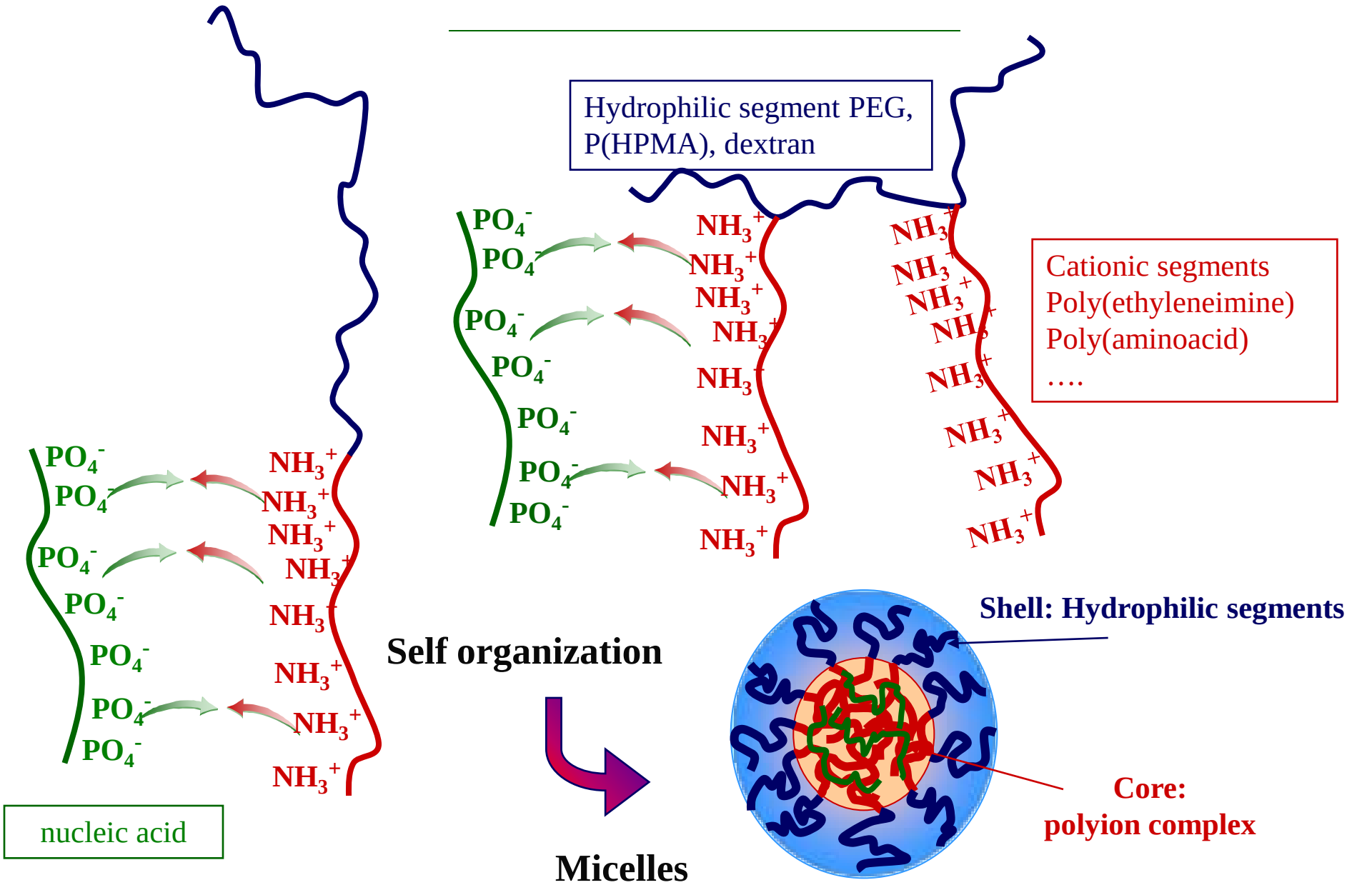
AMPHIPHILIC BLOCK COPOLYMER MICELLES

Kakizawa and Kataoka, Adv. Drug. Deliv. Rev. 54: 203-222, 2002

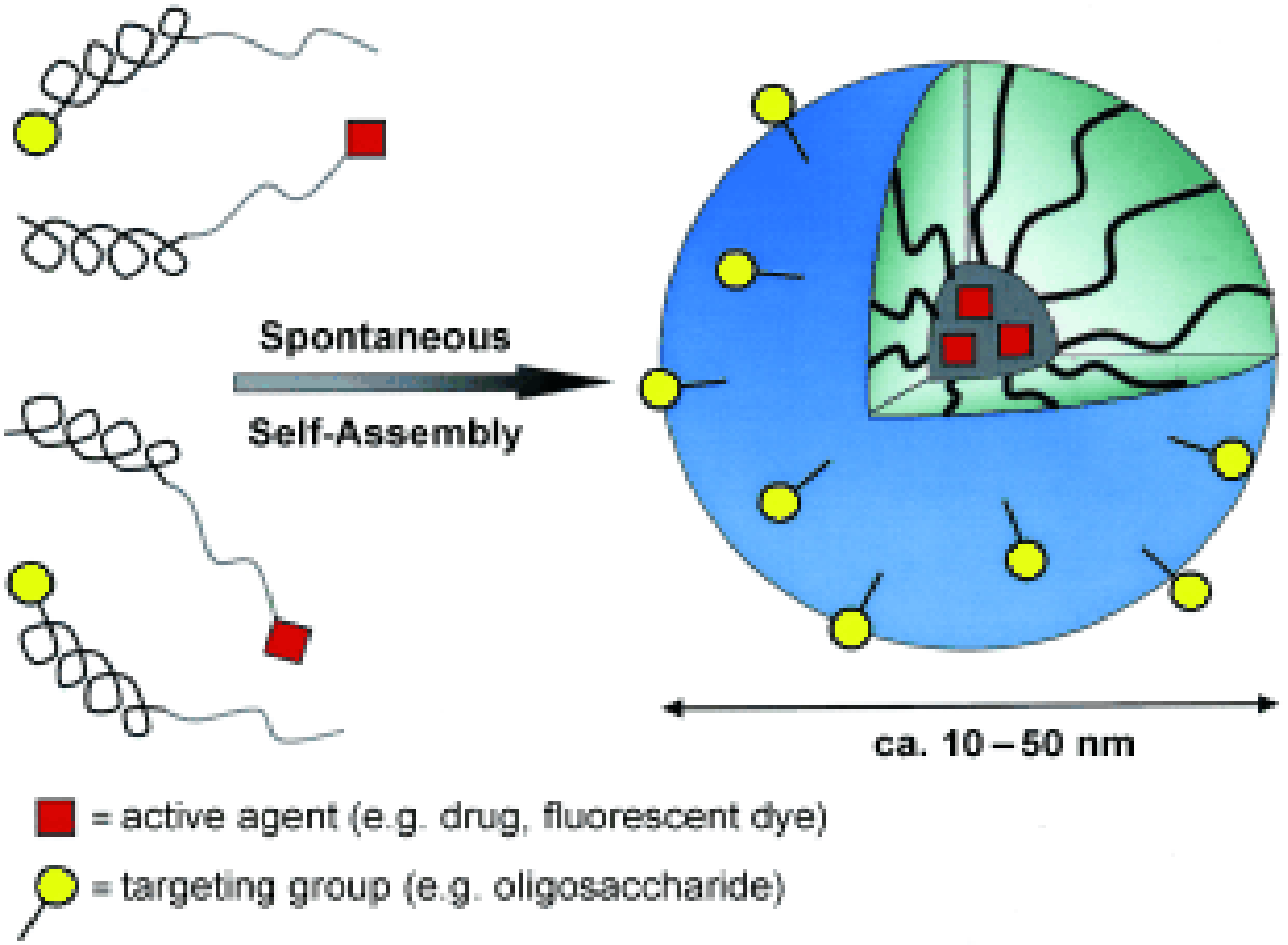


Formation of polyion complex micelles

BLOCK CO-POLYMER MICELLES



ADRESSED BLOCK CO-POLYMER MICELLES



Typical examples of block copolymers are PEO-*b*-PPO, PEO-*b*-PCl, and PEO-*b*-PAsp.