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NPRC Nanostructured Polymers Research Center

Manufacturing of Polymeric Nanomaterials for Biomedical applications

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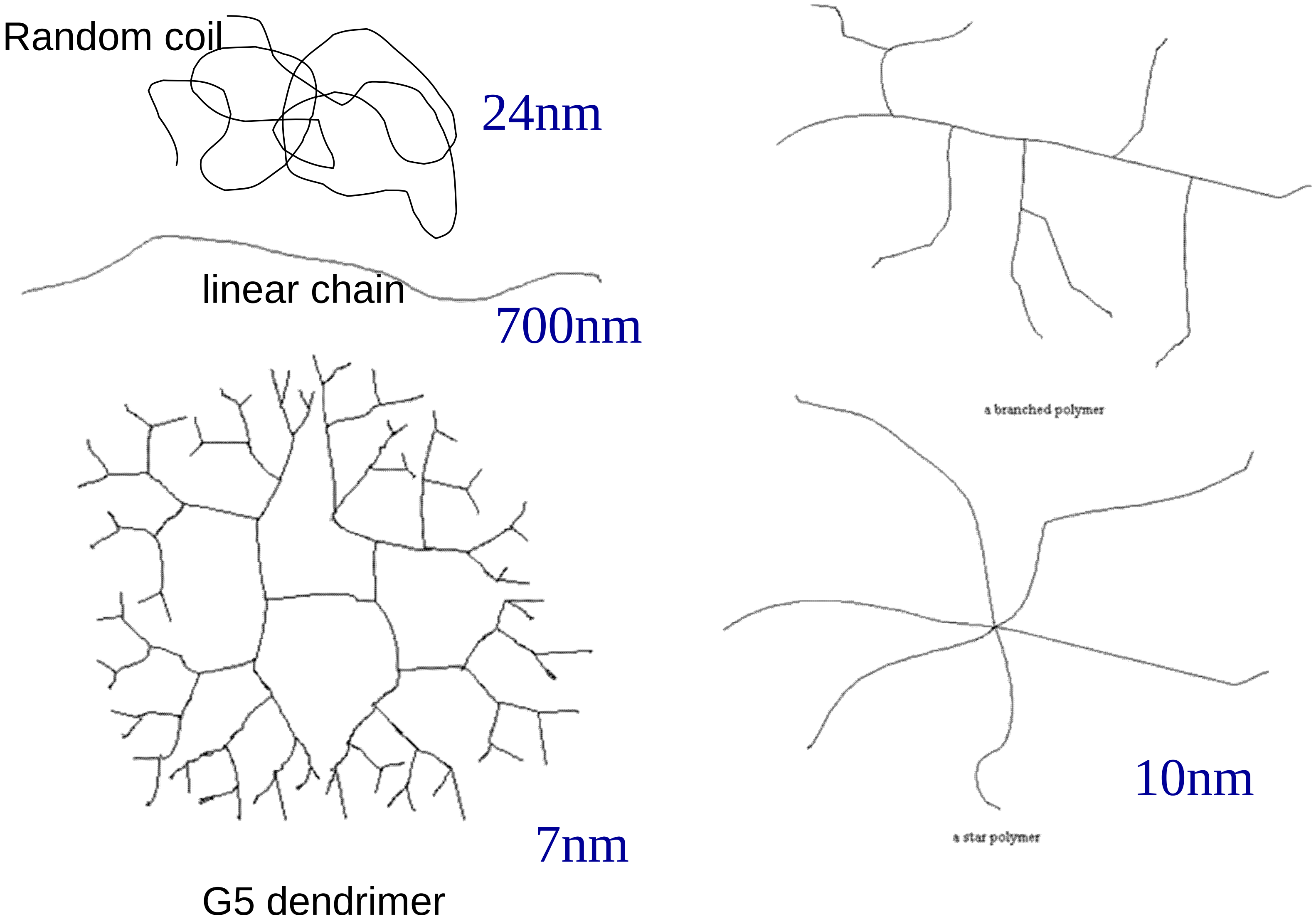
Advanced Polymer Laboratory
Nanostructured Polymer Research Center

Presented at the International Congress of Nanotechnology- October 31-November 3, 2005 San Francisco

Architecture - Size @ 100KD



Block copolymer architecture

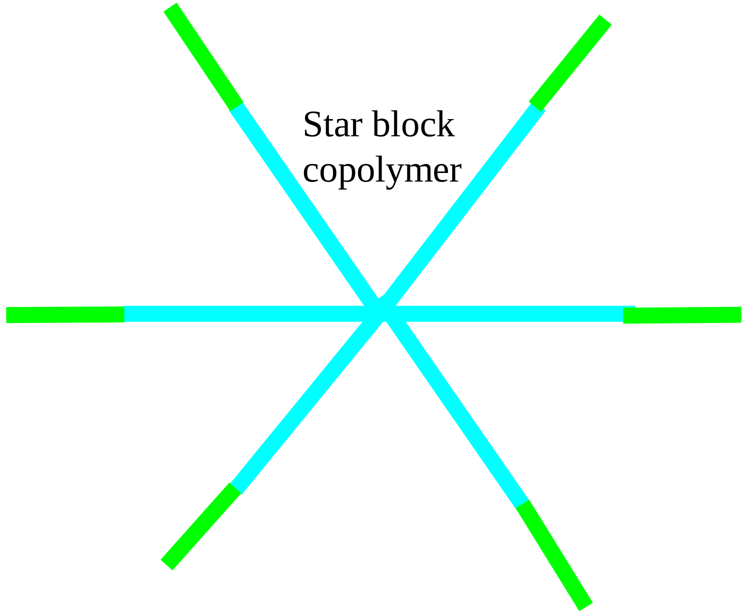
diblock-copolymers

Tri block-copolymers

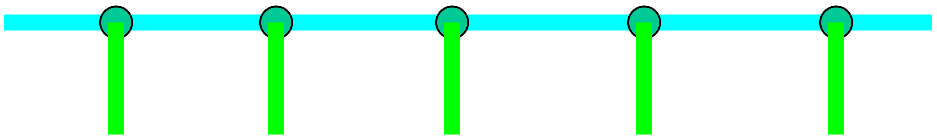
gradient-copolymers

Block-gradient -copolymers

Star block copolymer



Block pendant copolymer



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Why are polymer well suited for nanoscale manufacturing ?

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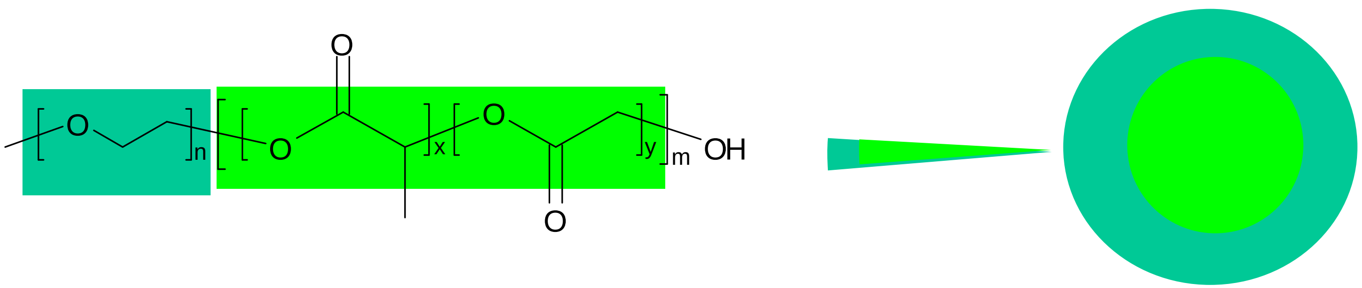
- Assume a block copolymer PEG-PGLA 55K-b-45K
  - Random coil size = Rg= $l(n_a)^{0.5}$ with l=0.2nm
  - Density of PGLA = 1.1 g/cm^3
- PGLA assembled in a 10nm "dry" core
- Number of chains/particle, n= $\pi D^3/6 * \rho$ /m.A
- N= 3.14*(10E-7) ^{3}/6*1.1/45000*6.02E23=8 chains
- Rg =$l(n_a)^{0.5}$ =0.2*((55000)/44) $^{0.5}$=0.2*$(1250)^{0.5}$ =7nm
- D=10+7*2=24nm



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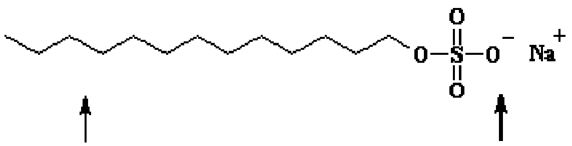
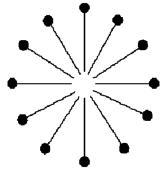
- **Polymeric Nanoparticles synthesis processes**
  - Mini-emulsion Polymerization
  - Self assembly
  - Directed assembly
- **Application to biotechnologies**
  - liposomes for transmembrane delivery
  - biosensors by molecularly imprinted polymers
  - Drug delivery

Emulsion Polymerization : soap opera

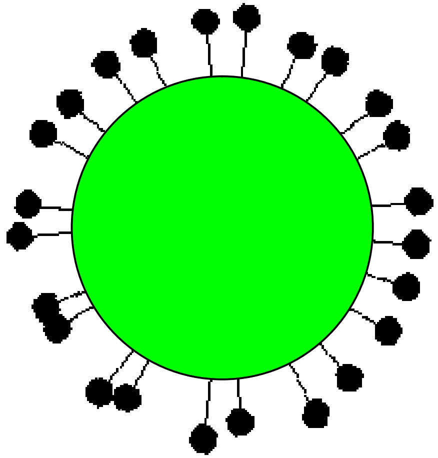


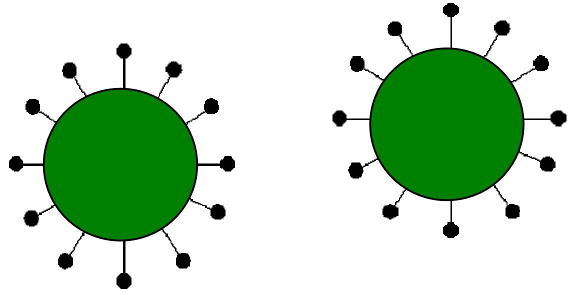
Sodium Lauryl Sulfate

Lazy chemist's representation of Sodium Lauryl Sulfate



Micelle:5nm





Stabilized Polymeric Particle: 50-500nm

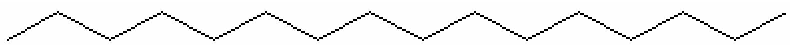
Stabilized Monomer droplet:5-50μm

Slide 6

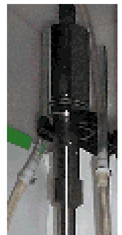
ygd1 Yvon Durant, 1/28/2002

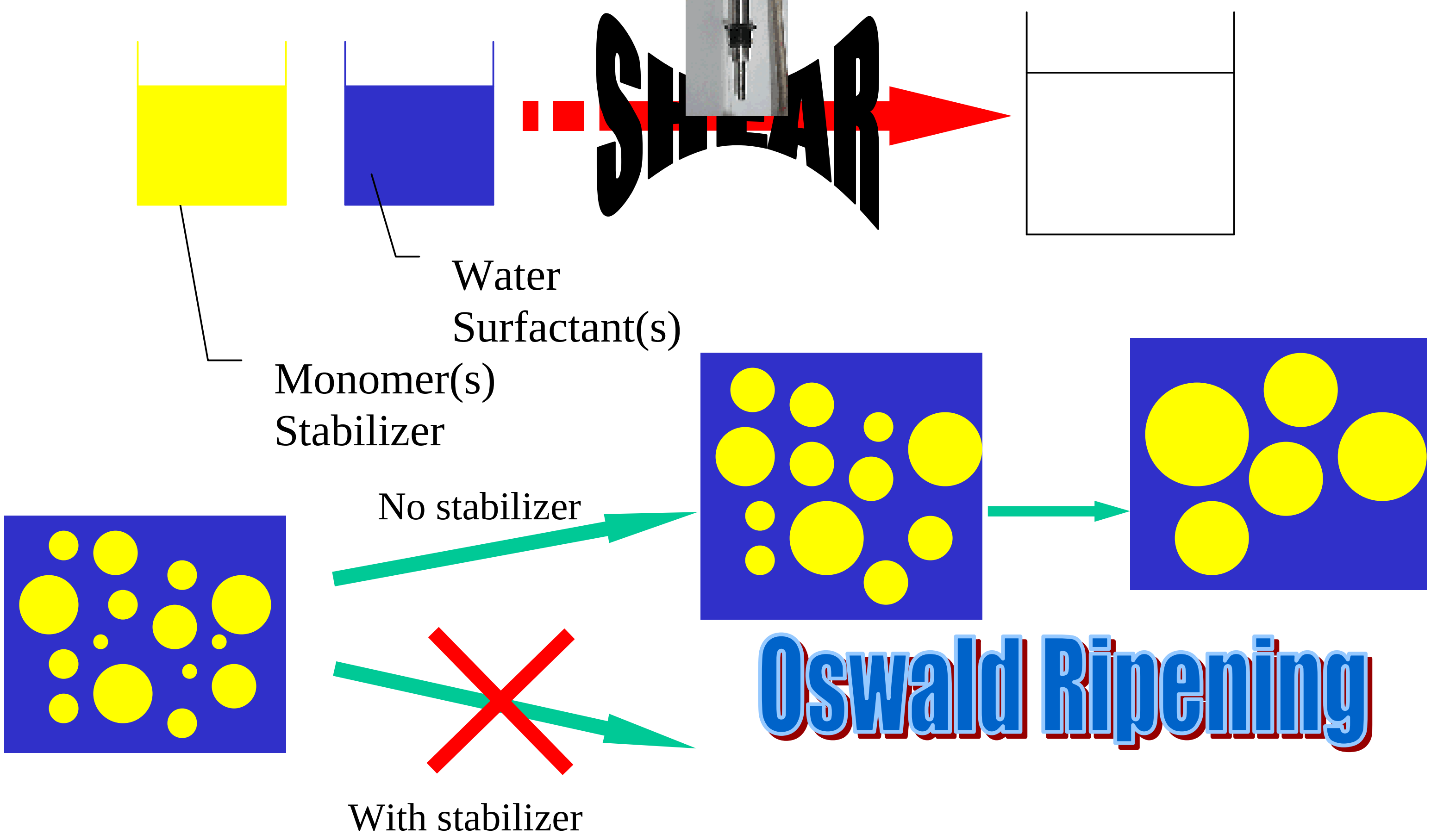
Miniemulsion Polymerization

- Create a meta-stable emulsion of the monomer(s).
- Use 2 key elements :
  - High shear source to break large droplets
    - Sonicator
    - Microfluidizer
    - Homogeneizer
  - Use a water insoluble molecule to stabilize the particle
    - Sometimes called cosurfactant (missleading)
    - Hexadecane, Eicosane, polymer, macromonomer, macroinitiator, CTA, ...



Miniemulsion stability





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Particle size control

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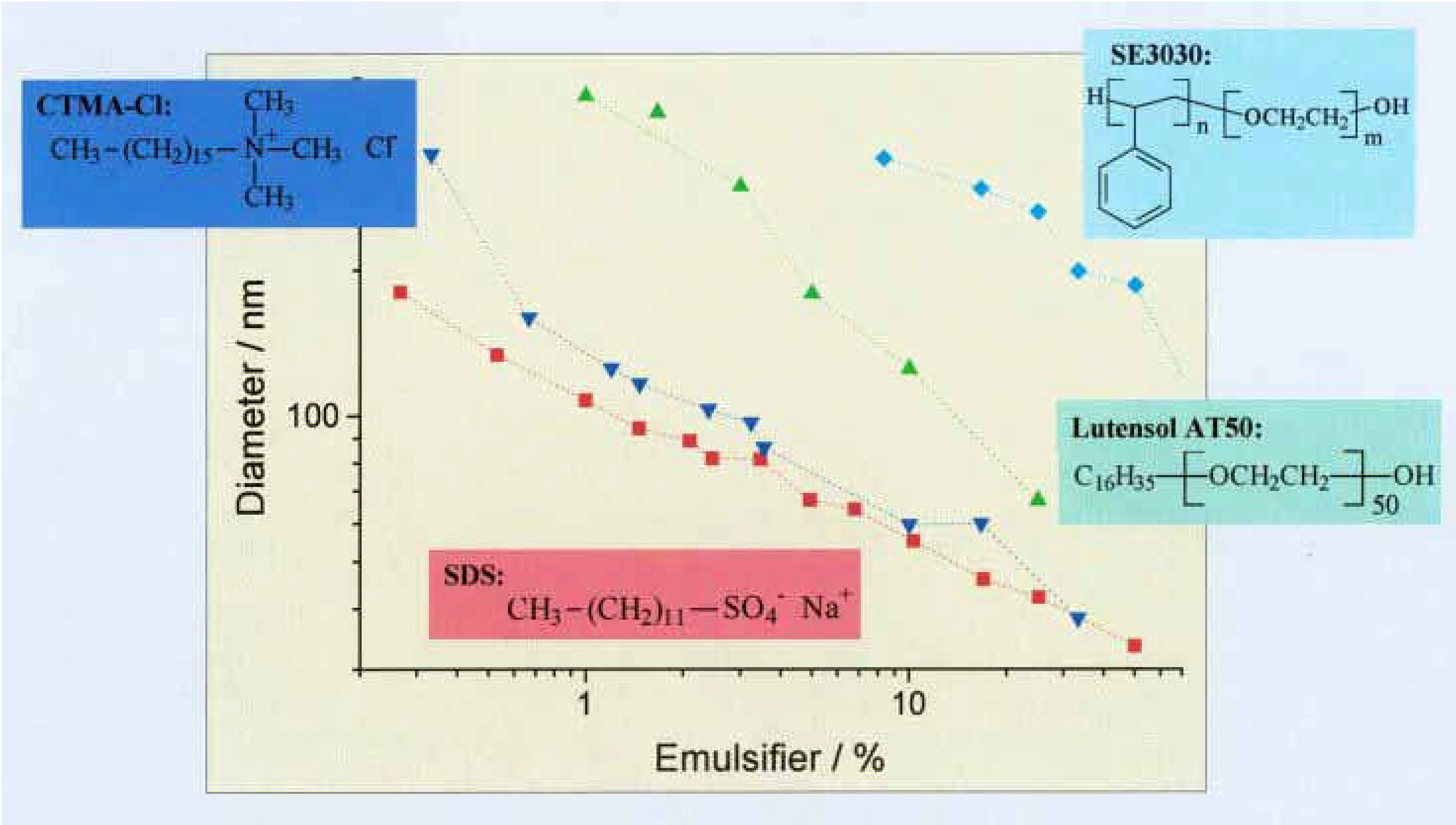

K. Landfester, N. Bechthold, F. Tiarks, and M. Antonietti, *Miniemulsion Polymerization with Cationic and Nonionic Surfactants: A Very Efficient Use of Surfactants for Heterophase Polymerization.* Macromolecules **1999**, *32*, 2679.

University of New Hampshire

Mini to micro emulsion

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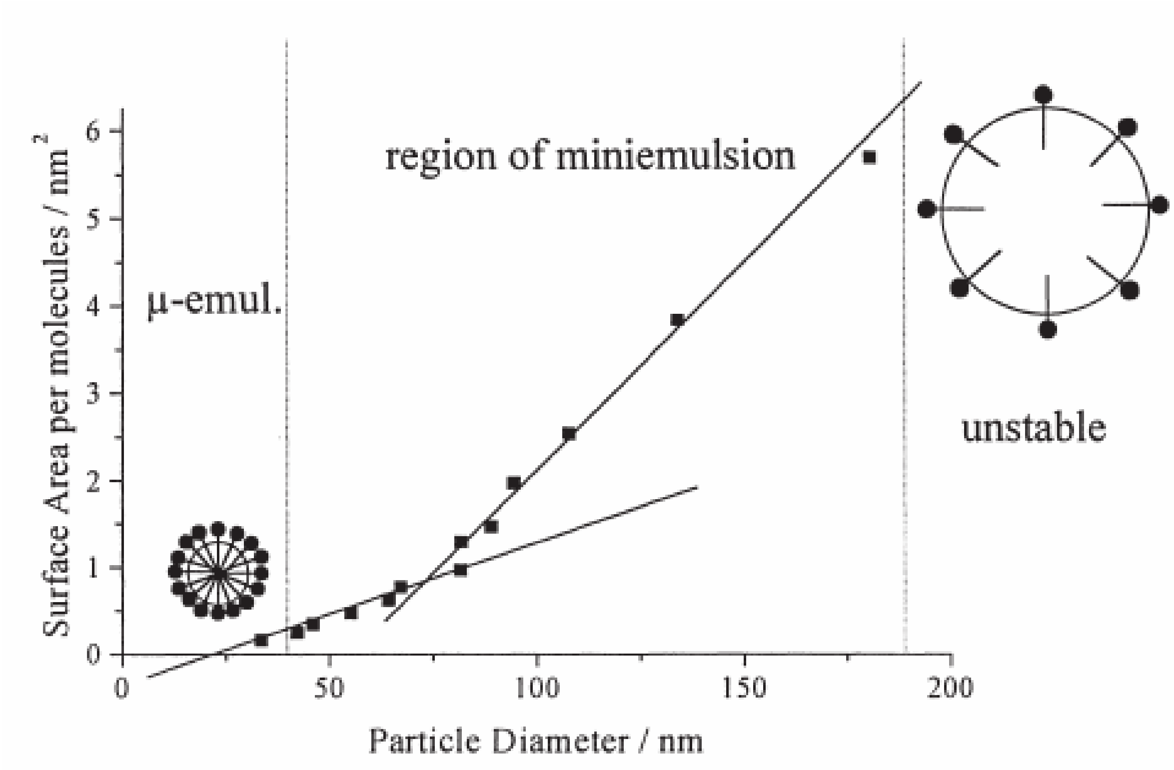

K. Landfester, *Recent Developments in Miniemulsions - Formation and Stability Mechanisms.* Macromol. Symp. **2000**, *150*, 171.

University of New Hampshire

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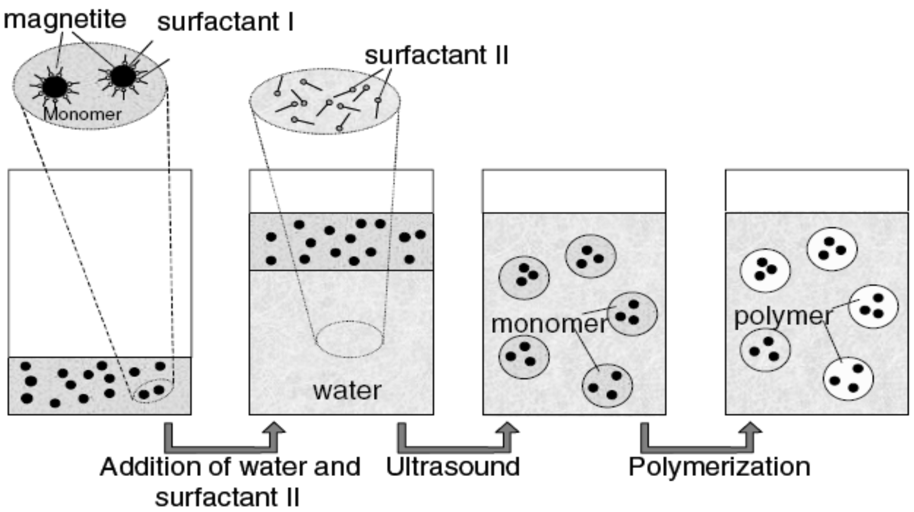
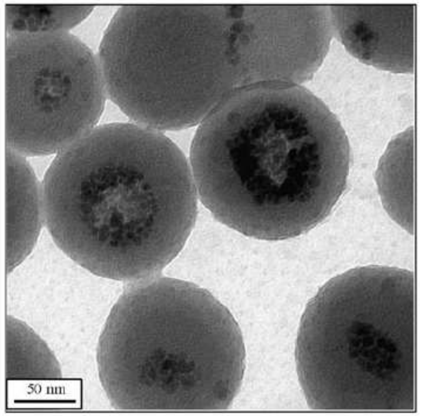
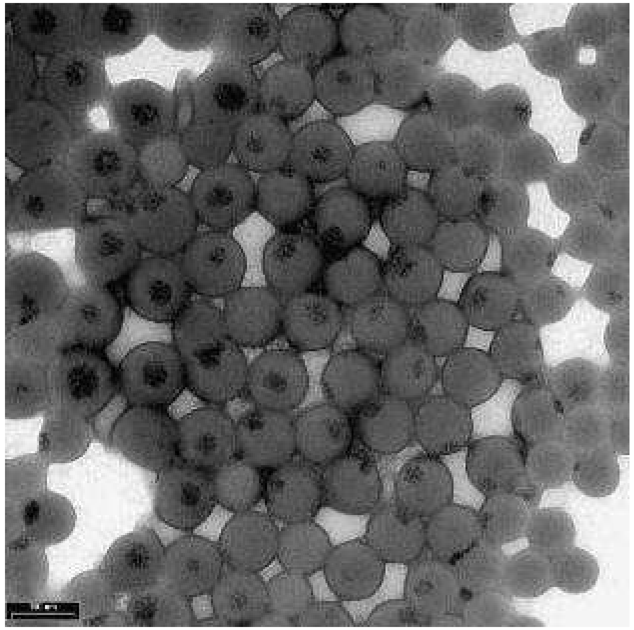
Encapsulation of magnetite in polymer particles by miniemulsion



J. Phys.: Condens. Matter **15** (2003) S1345–S1361





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Magnetite encapsulation

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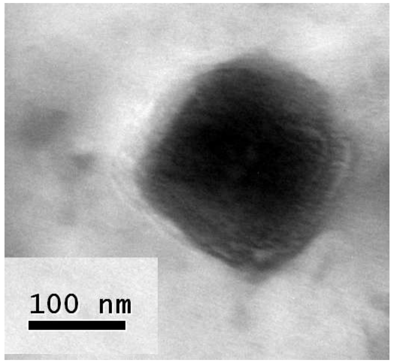
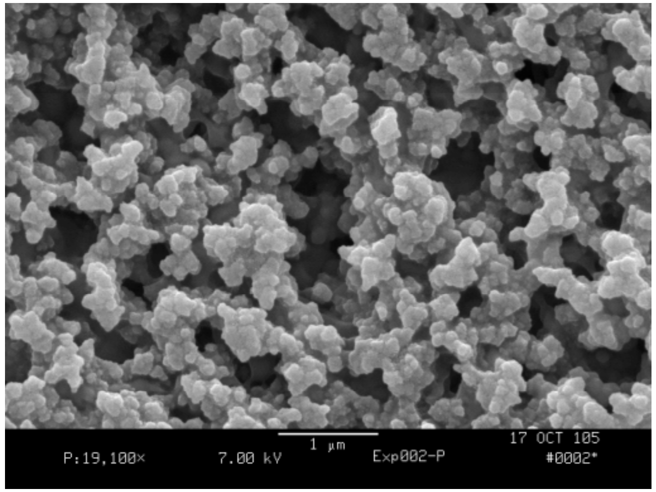
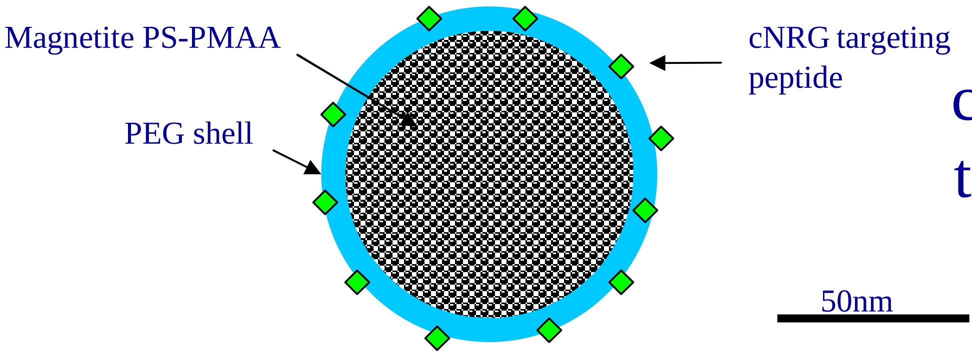


TEM

SEM





cNRG targets CD13 – tracer of engiogenesis

Magnetic nanoparticles functionalized with cNGR for atherosclerotic plaque diagnostic.

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  - Emulsion Polymerization
  - Mini-emulsion Polymerization
  - Self assembly
  - Directed assembly
- **Application to biotechnologies**
  - biosensors by molecularly imprinted polymers
  - liposomes for transmembrane delivery
  - Bypassing the BBB

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Molecularly Imprinted Polymers

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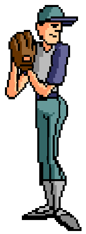

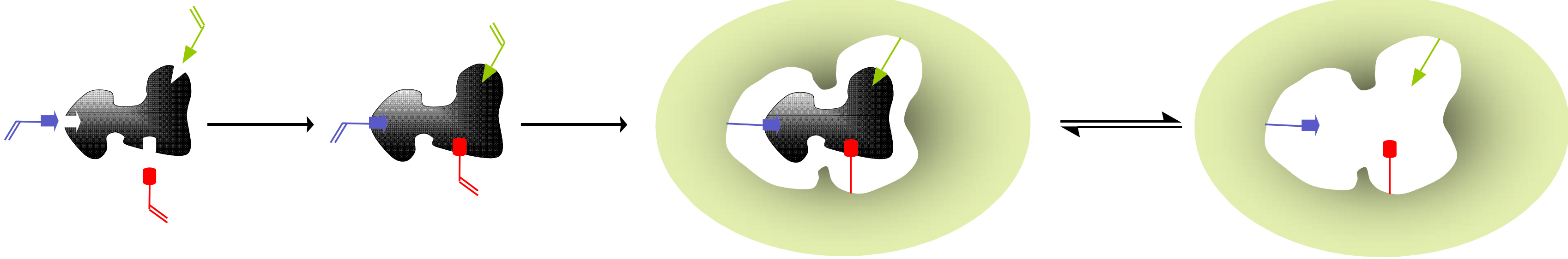

1. Selection of template molecule and functional monomers
2. Self-assembly of template molecule and functional monomers
3. Polymerization
4. Analyte Extraction

SINP : Surface Imprinted NanoParticle

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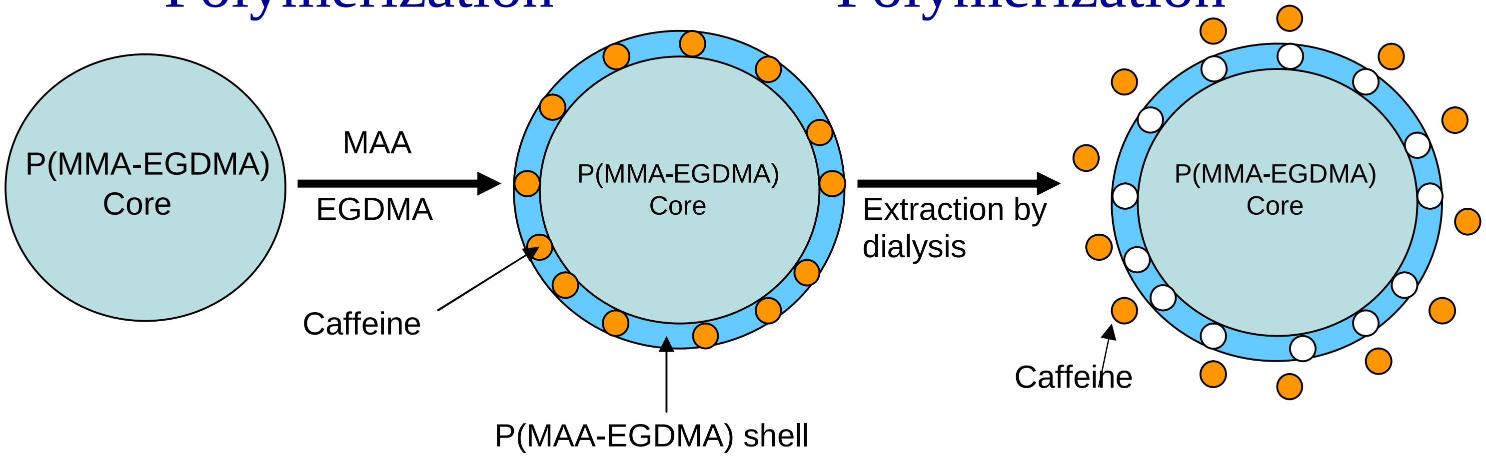
1st stage
Miniemulsion
Polymerization

2nd stage
Emulsion
Polymerization



MJB-20: miniemulsion seed

Organic phase = 23% : MMA 85.5%, EGDMA 9.5%, Hexadecane 5%,

Water phase = 77% : Water 99%, SDS 0.6%, KPS 0.025%, NP-50 0.39%

Prepare the two phases, mix them together, magnetically stir them for 15 minutes, then, sonicate the resulting emulsion for 2 minutes (90%, 9) in ice.

SCexp = 22.25%, Conversion = 98.96%,

Size = Malvern Nanosizer: Dz = 107.1 nm, Dv = 111.9 nm

MJB-21: 2nd stage imprinting

Water	57.74%
MJB20 (wet)	33.44%
NaHCO3	0.042%
KPS	0.047%
Caffeine	5.78%
EGDMA	2.63%
MAA	0.31%

Water, MJB-21, NaHCO3, were mixed and heated at 80C. When at temperature, add caffeine and start degassing. After 15 minutes, add KPS and start feeding with egdma+maa. Dilute with 250g of hot water (336%) while stirring.
SCexp = 2.635% (dilution) Conversion = 57.86%
Size = Malvern nanosizer Dz= 108.4 nm, Dv = 114.2nm
Brookhaven 90+: Dz = 104.9 nm, Effective Dv = 105.2 nm

SEM+DLS of SNIP

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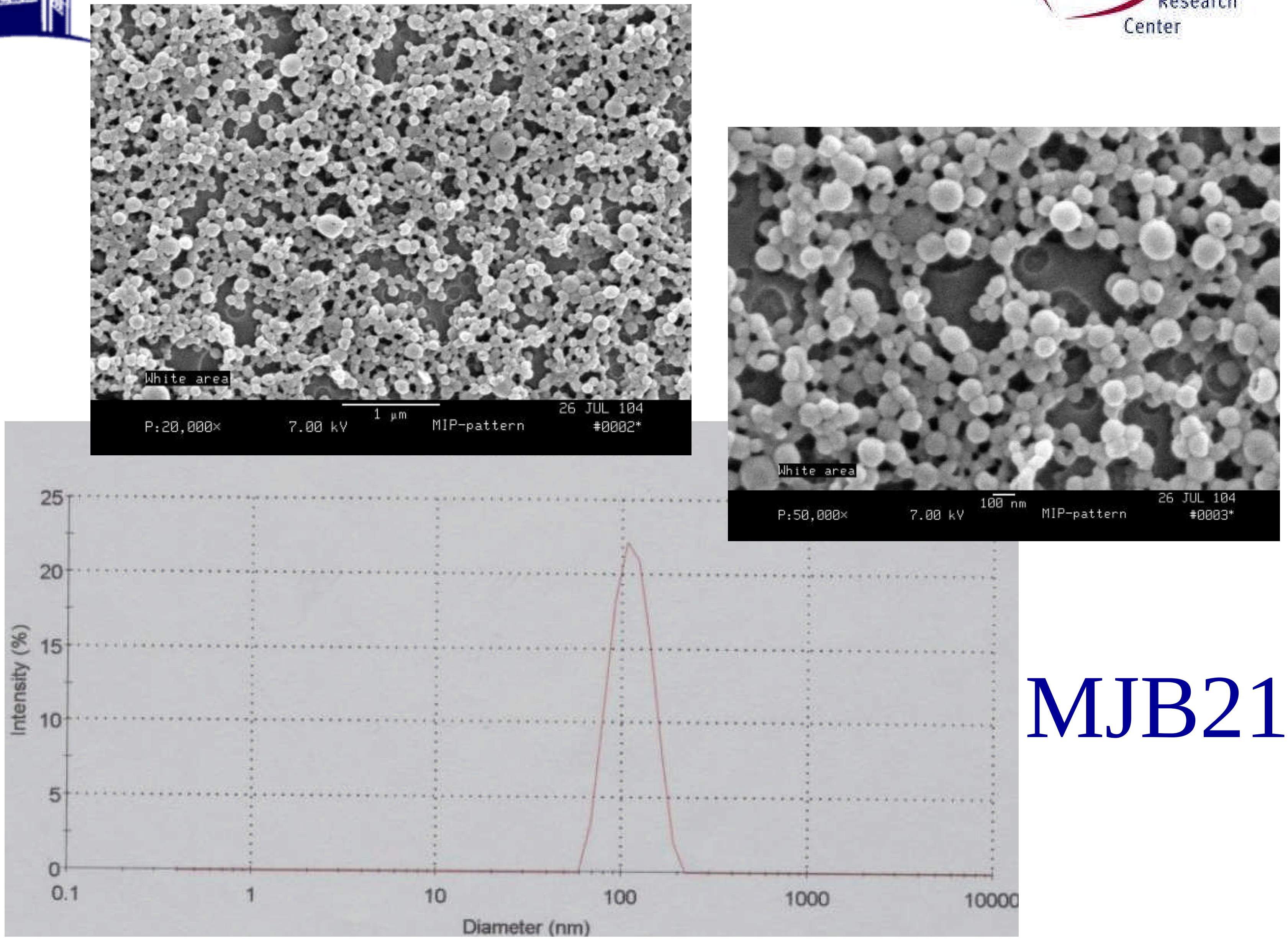
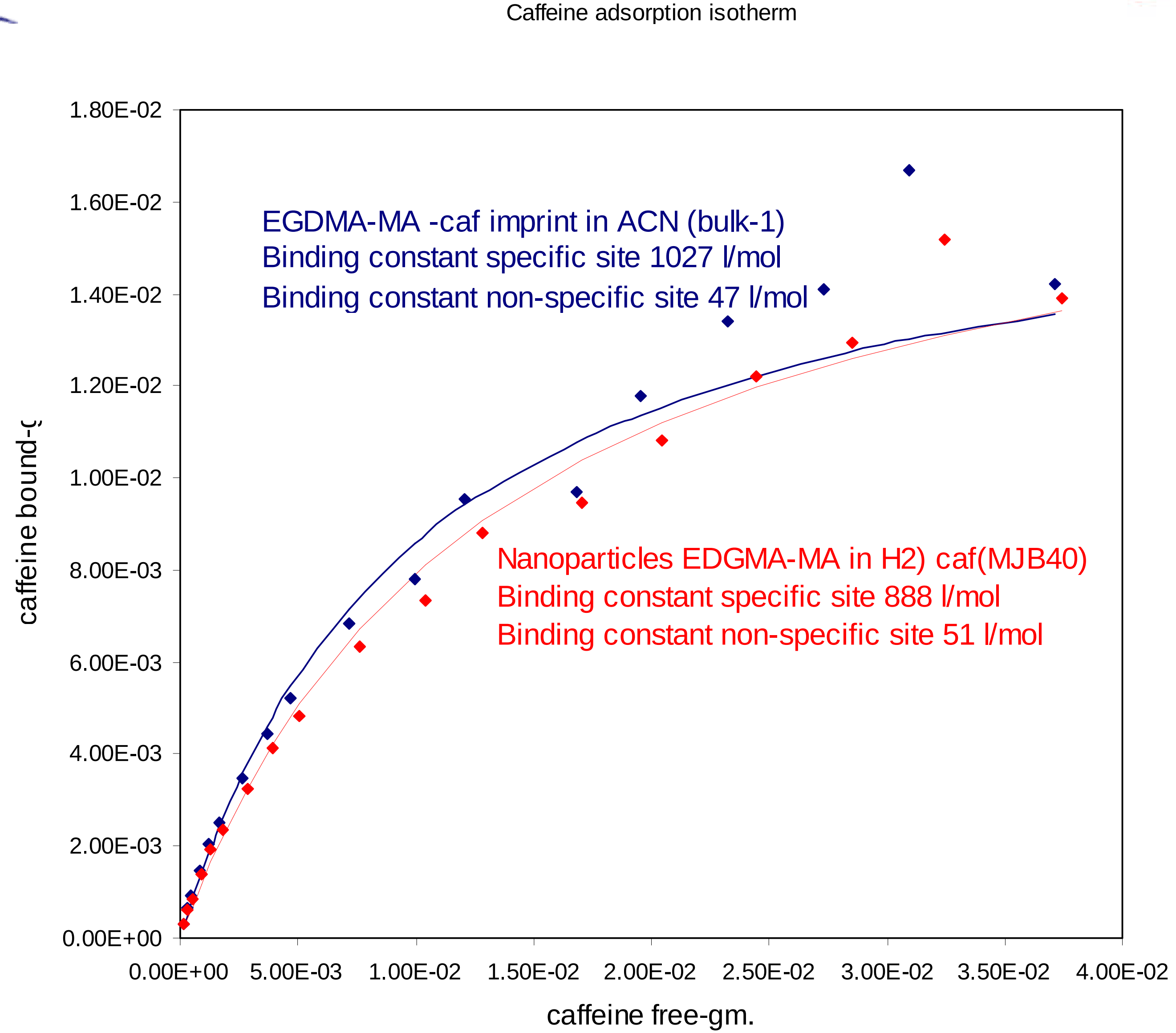


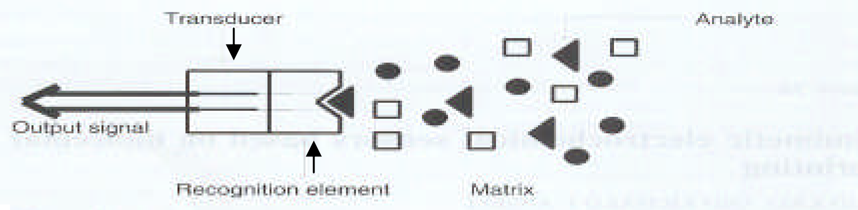
MJB21

Adsorption studies by HPLC



Biomimetic electrochemical sensors based on molecular imprinting

- A chemical sensor selectively recognizes a target analyte molecule in a complex matrix and gives an output signal which correlates with the concentration of the analyte.



The principle of a chemical sensor.

The transducer: When the analyte interacts with the recognition element of a sensor, there is a change in one or more physicochemical parameters associated with the interaction. Transducer convert these parameters into an electrical output signal than can be amplified, processed and displayed in a suitable form.

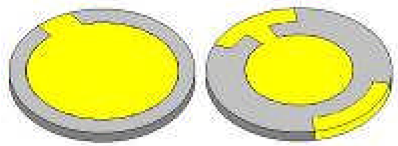
⇒ Molecular imprinting use as sensing materials

Advantage: cheap, stable and robust under a wide range of conditions including pH, humidity and temperature

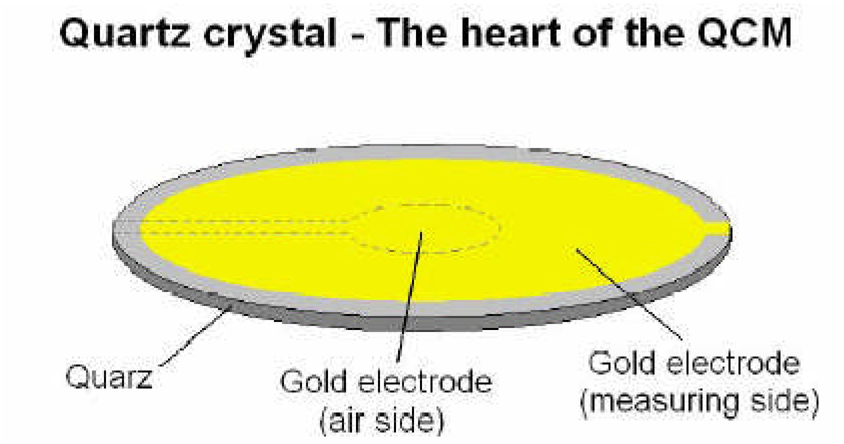
Problem: Signal transduction is so low that it seem to be environmental artifacts. Due to the insulating nature of the polymer constituting the MIP

Biomimetic electrochemical sensors based on molecular imprinting / Chap.18 MIP – D. Kriz, R. J. Ansell- Vol 23 -Elsevier

QCM



- A QCM consists of a thin quartz disc sandwiched between a pair of electrodes. Due to the piezoelectric properties of quartz, it is possible to excite the crystal to oscillation by applying an AC voltage across its electrodes.

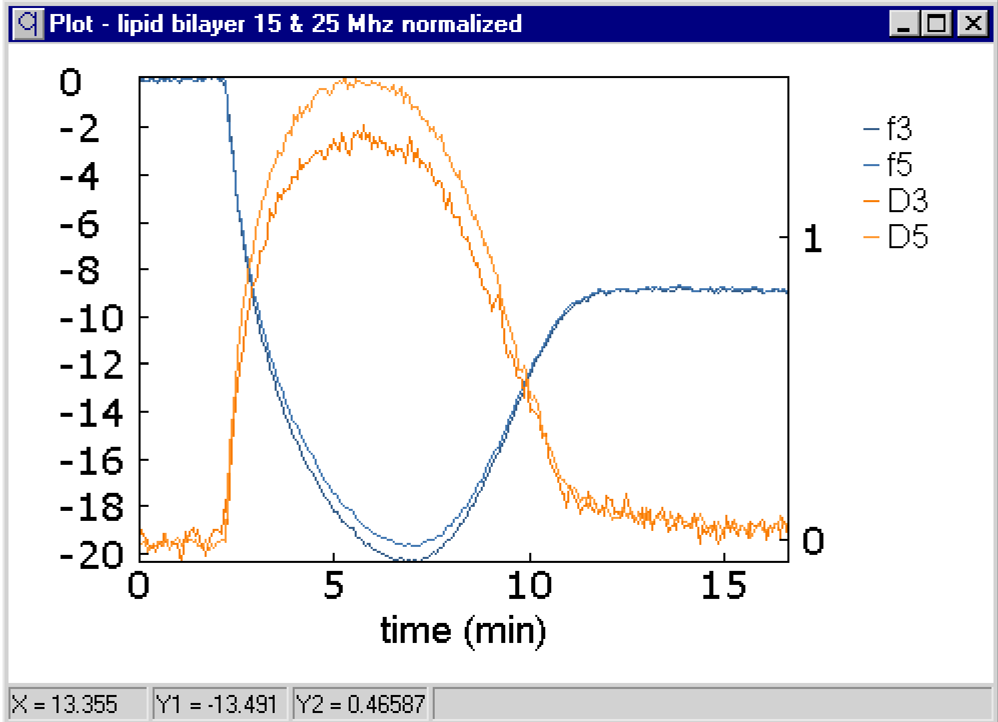


$\Delta f = -f_u^{2/3} \left[(\rho_L \eta_L) / (\pi \times (\rho_q \mu_q)\right]^{1/2}$, where

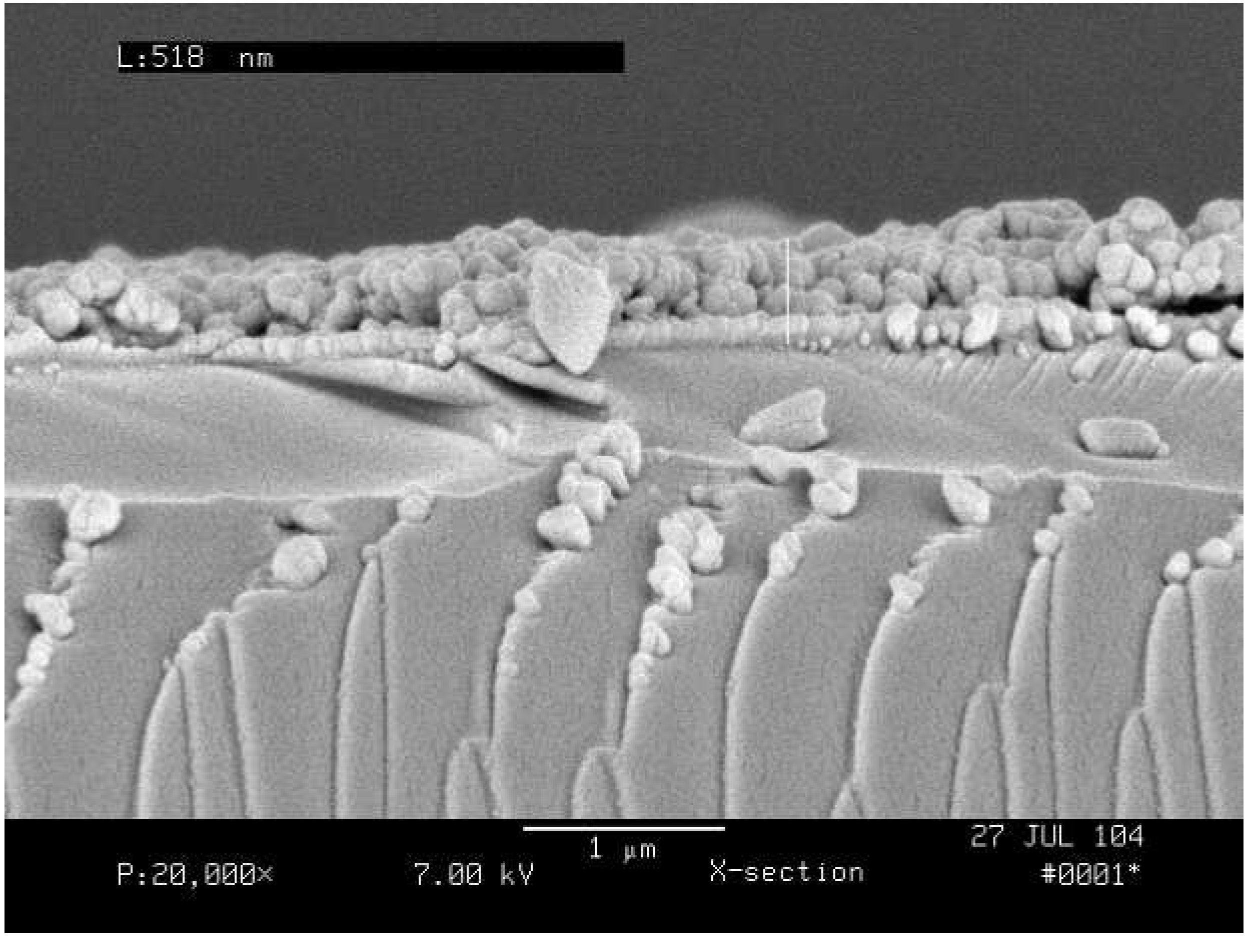
Δf = measured frequency shift,
f_u = resonant frequency of the unloaded crystal,
ρ_L = density of liquid in contact with the crystal,
η_L = viscosity of liquid in contact with the crystal,
ρ_q = density of quartz, 2.648 g/cm^3,
μ_q = shear modulus of quartz, 2.947×10^{11} g/cm$\times$s^2.

Q-Sense D300


qsense


Plot - lipid bilayer 15 & 25 Mhz normalized
0
-2
-4
-6
-8
-10
-12
-14
-16
-18
-20
0
5
10
15
time (min)
1
0
f3
f5
D3
D5
X = 13.355
Y1 = -13.491
Y2 = 0.46587

Coated QCM sensor
Fracture SEM



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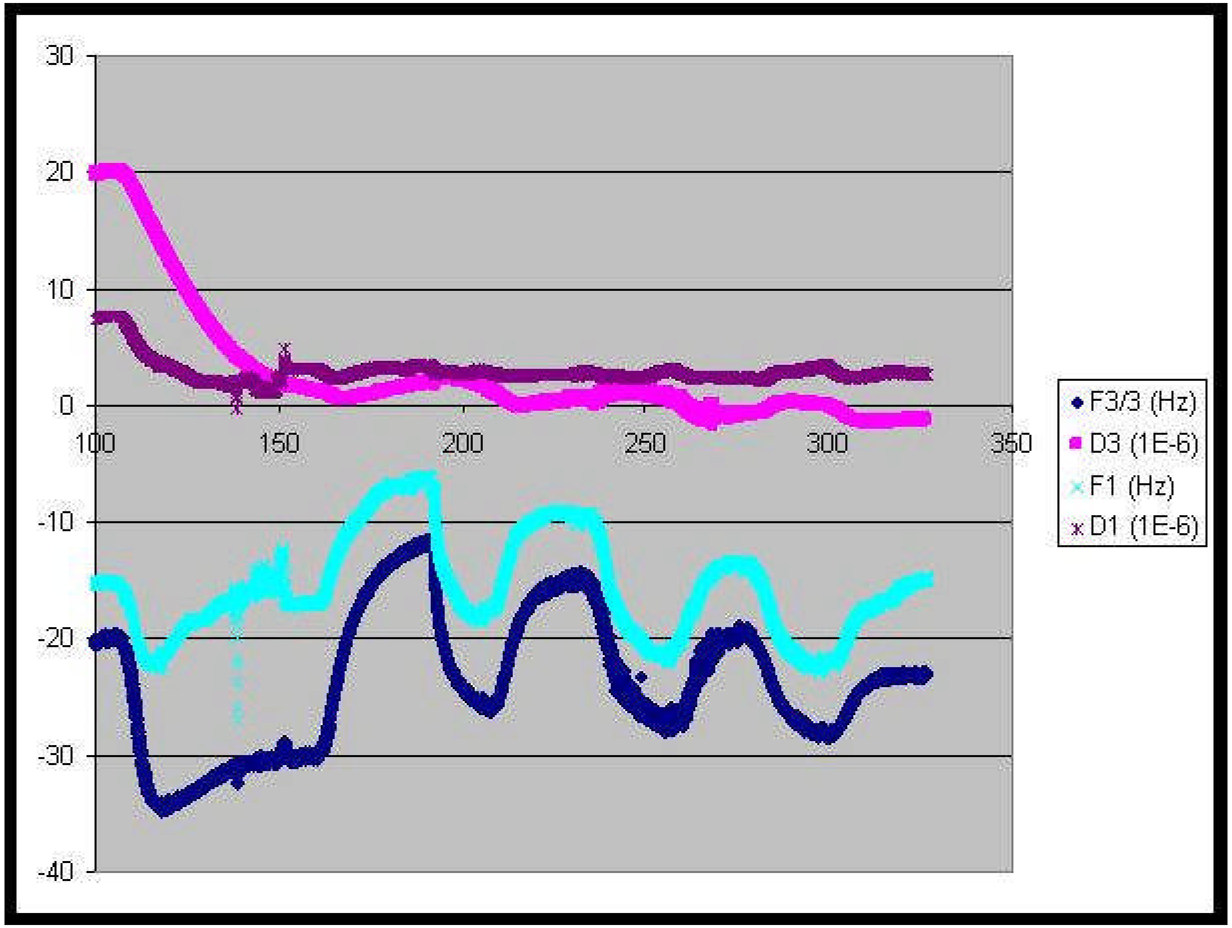
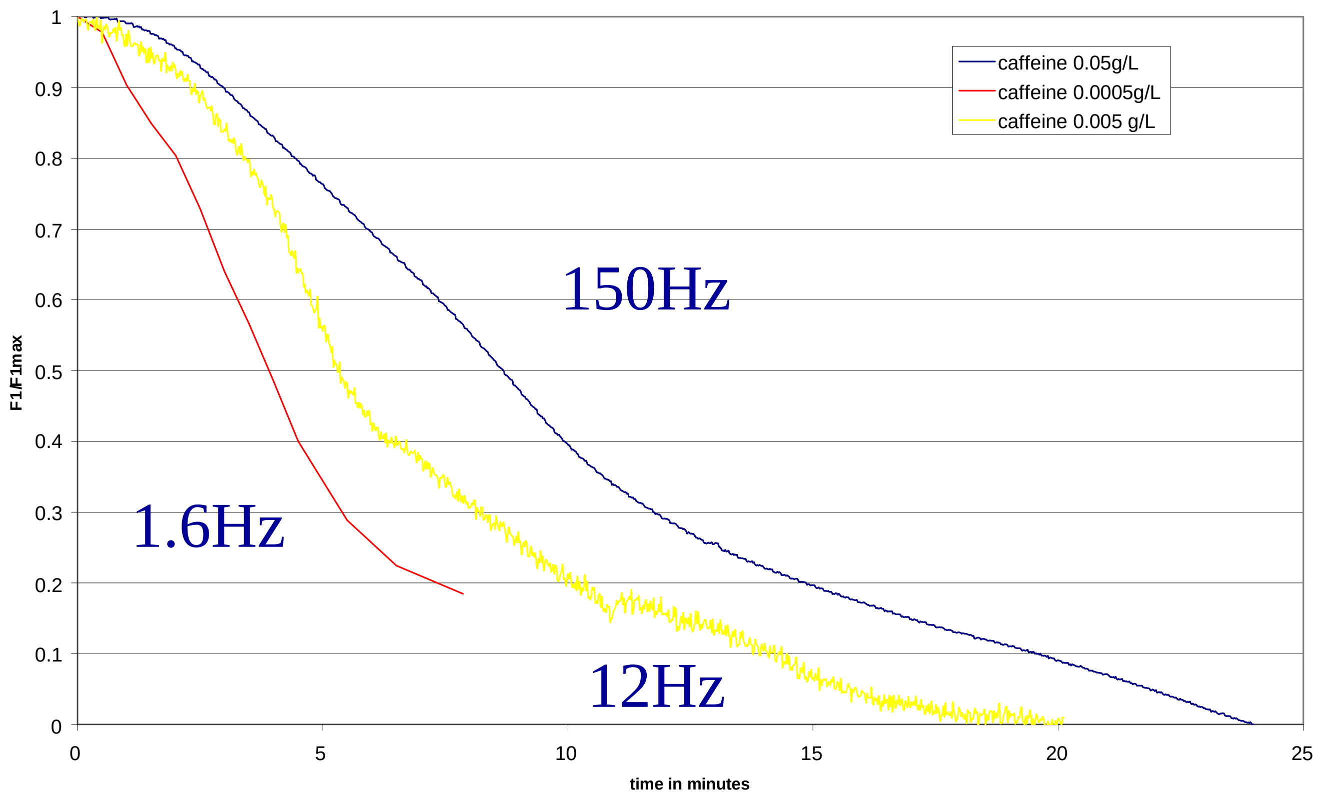
Raw data



QCM results

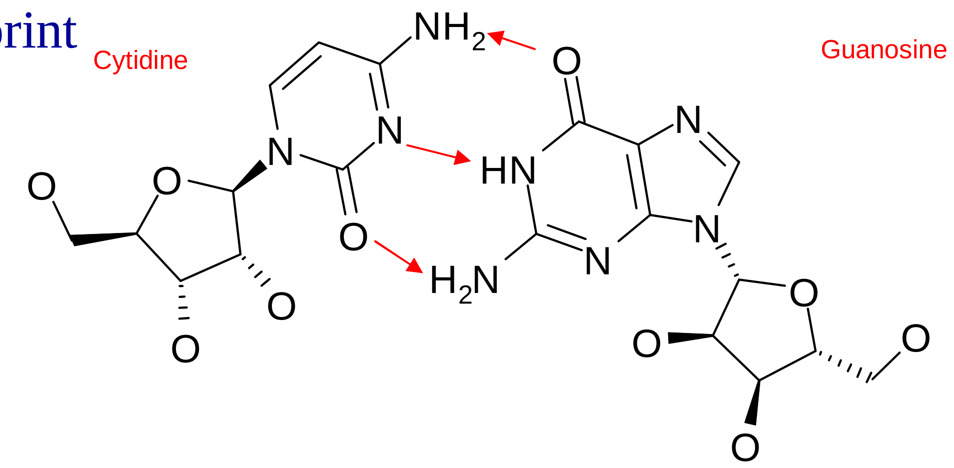
Adsorption of caffeine at different caffeine solution concentrations



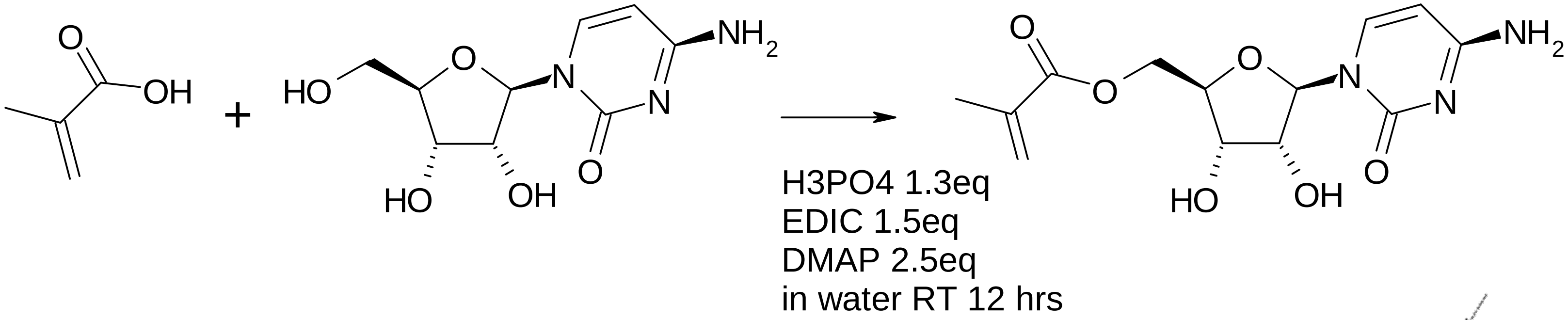
With the Langmuir equation the quantity adsorbed can be calculated for the caffeine MIP at a concentration of 0.0005g/L. This value is found to be equal to 7.3×10-6g of caffeine per gram of MIP. The mass of MIP on the crystal is equal to 4×10-5g. With these two values, the minimum amount detected in this experiment was equal to 0.3nanogram.

Guanosine Recognition

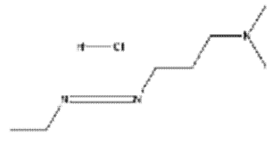
- Perfect complement to imprint guanosine is cytidine

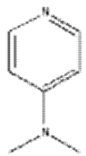


- Modified cytidine monomer



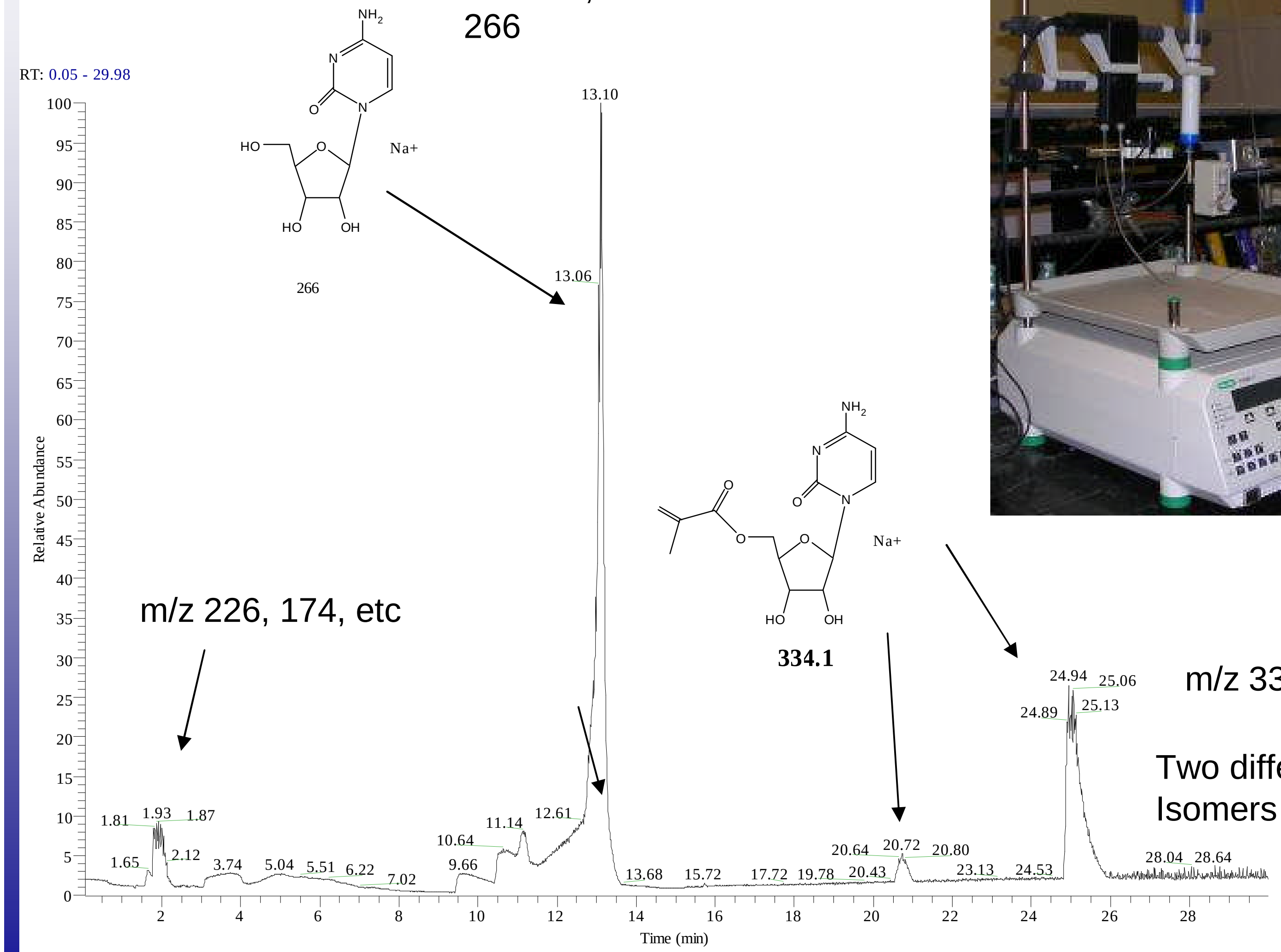
EDCI: 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
DAMP: 4-dimethylaminopyridine





LC/MS Base peak chromatogram

m/z 112, 266



m/z 226, 174, etc

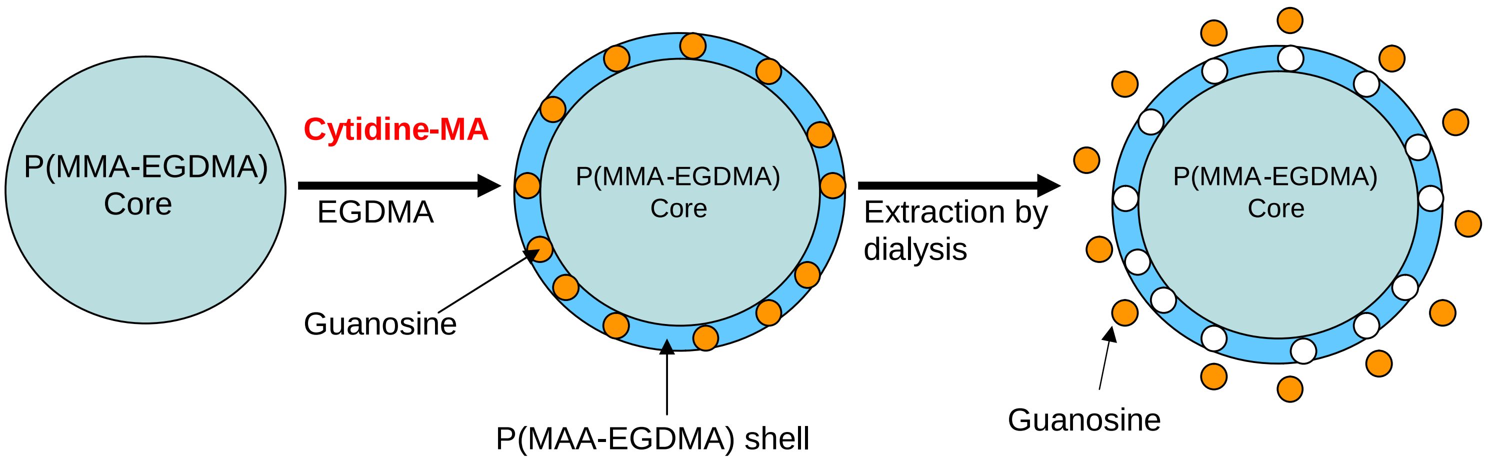
m/z 334

Two different
Isomers apparently

SINP : Guanosine detection

1st stage
Precipitation Polymerization

2nd stage
Emulsion Polymerization



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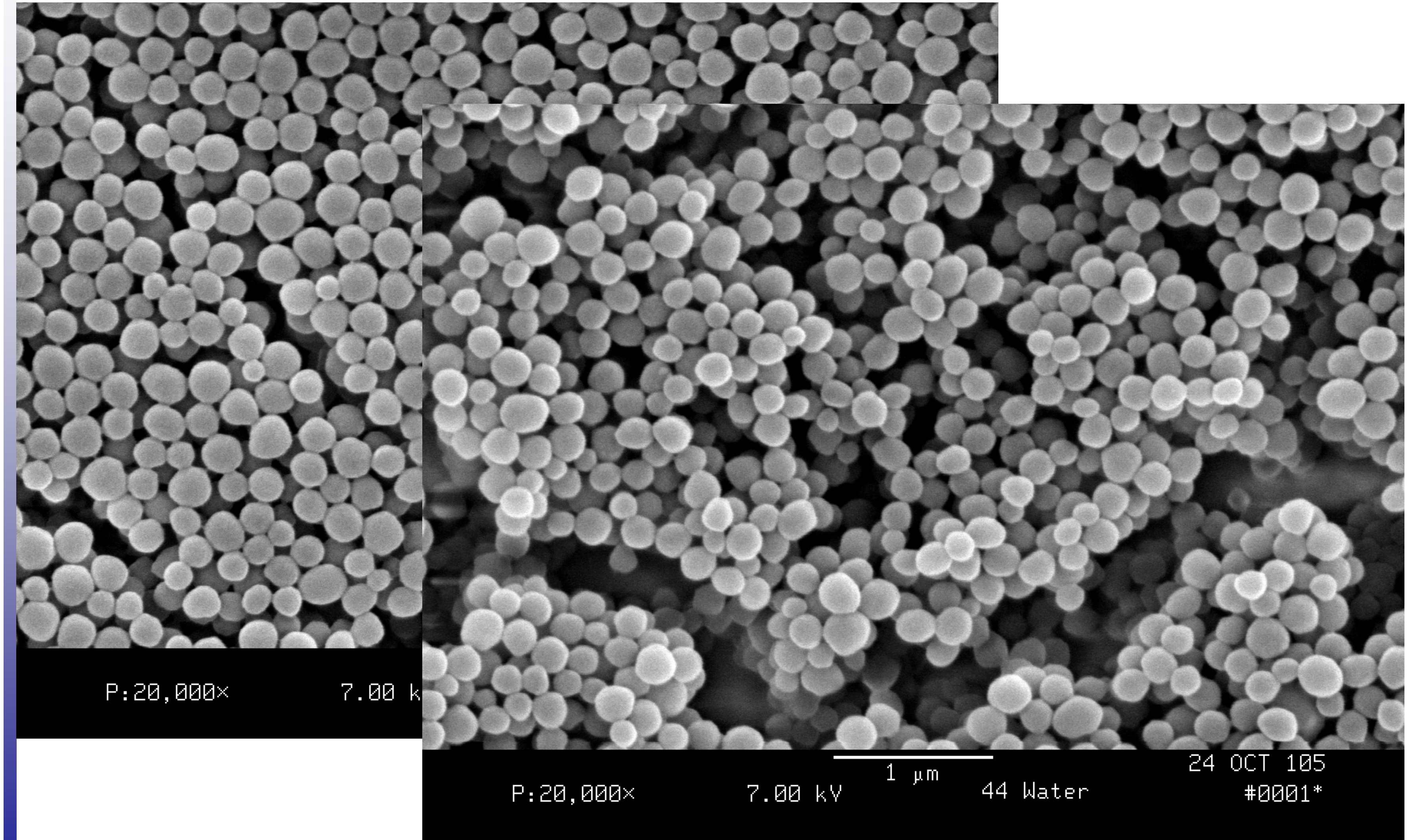
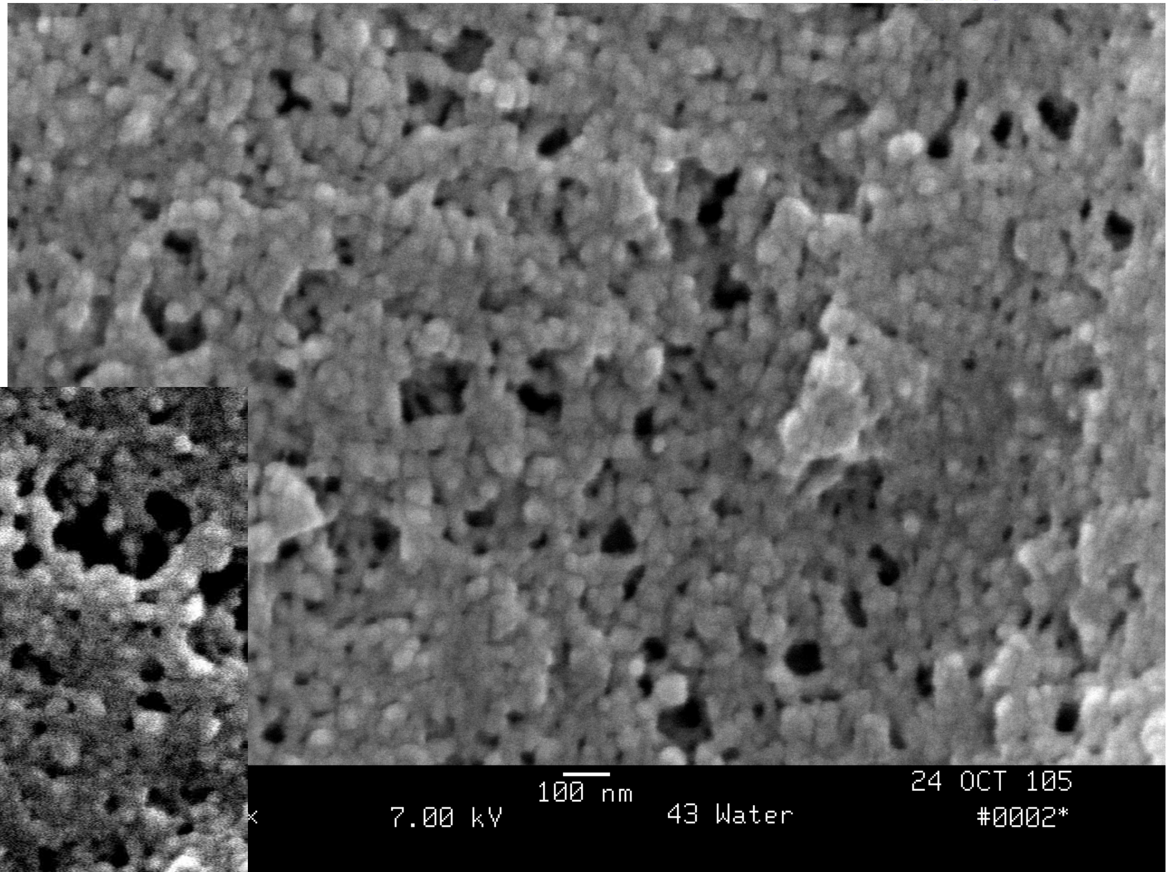
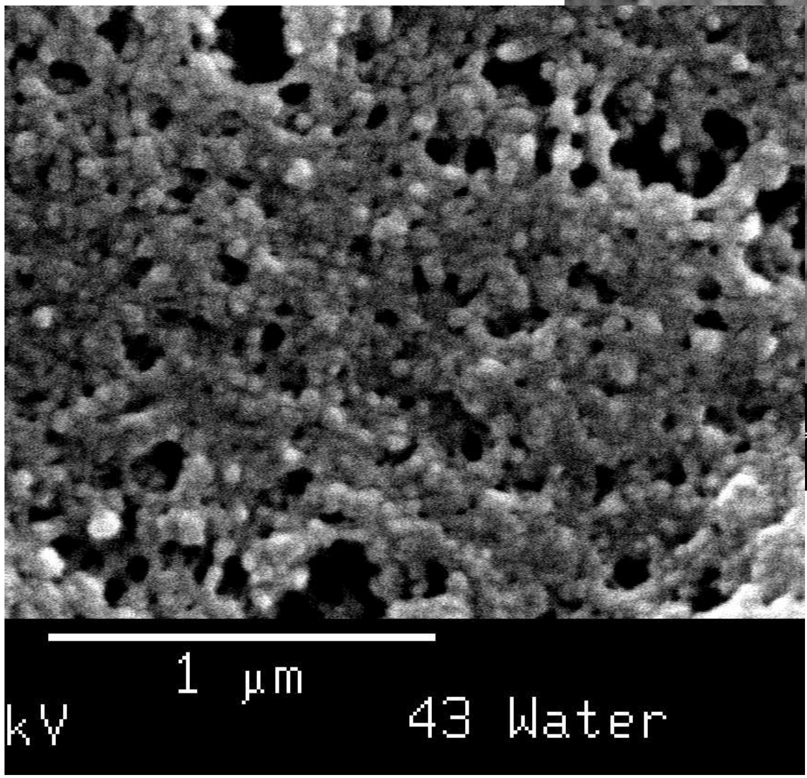
Precipitation Polymerization in ACN



Precipitation polymerization

- Smaller…
- 20nm
- Higher sensitivity





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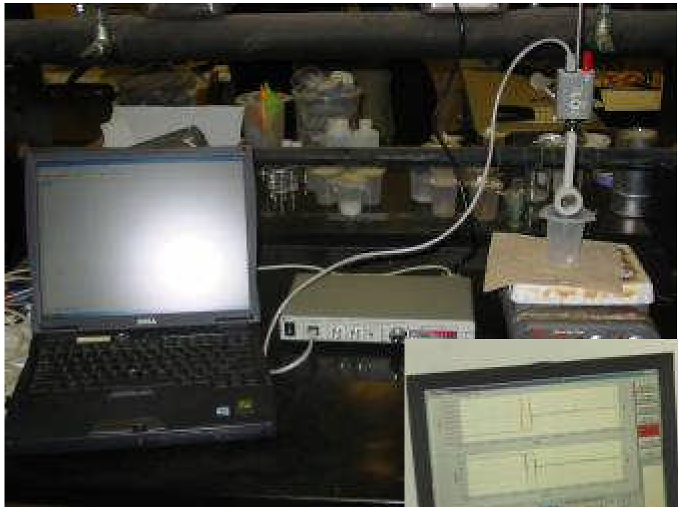
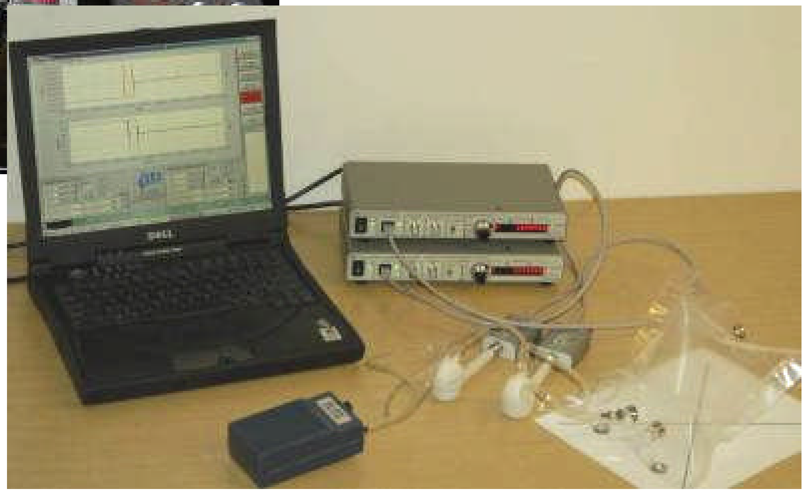
Low cost QCM





QCM200

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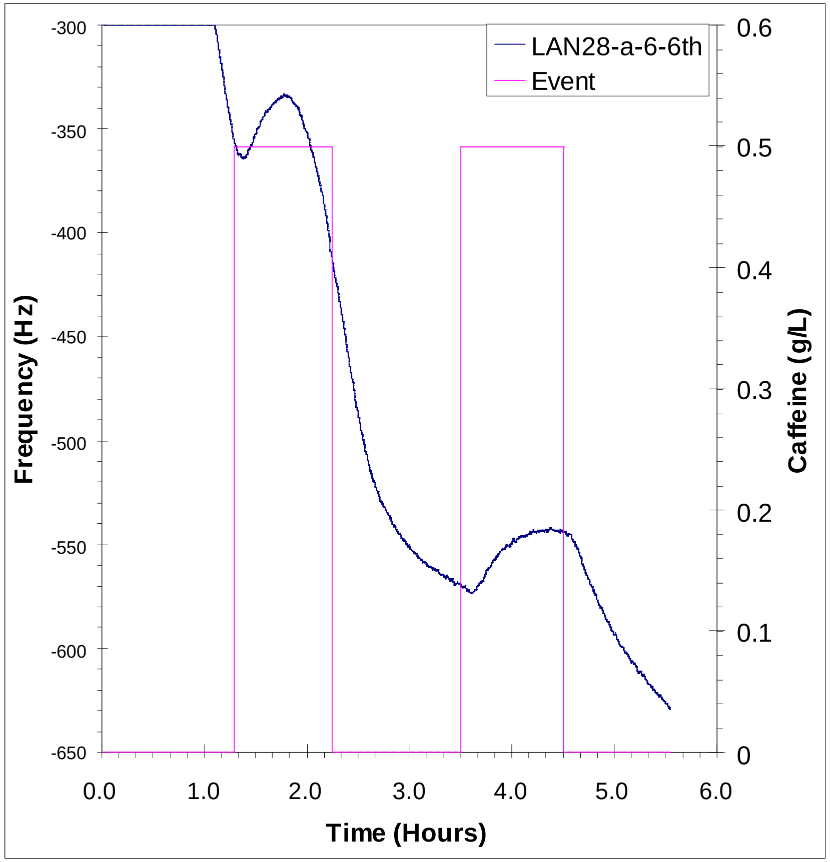

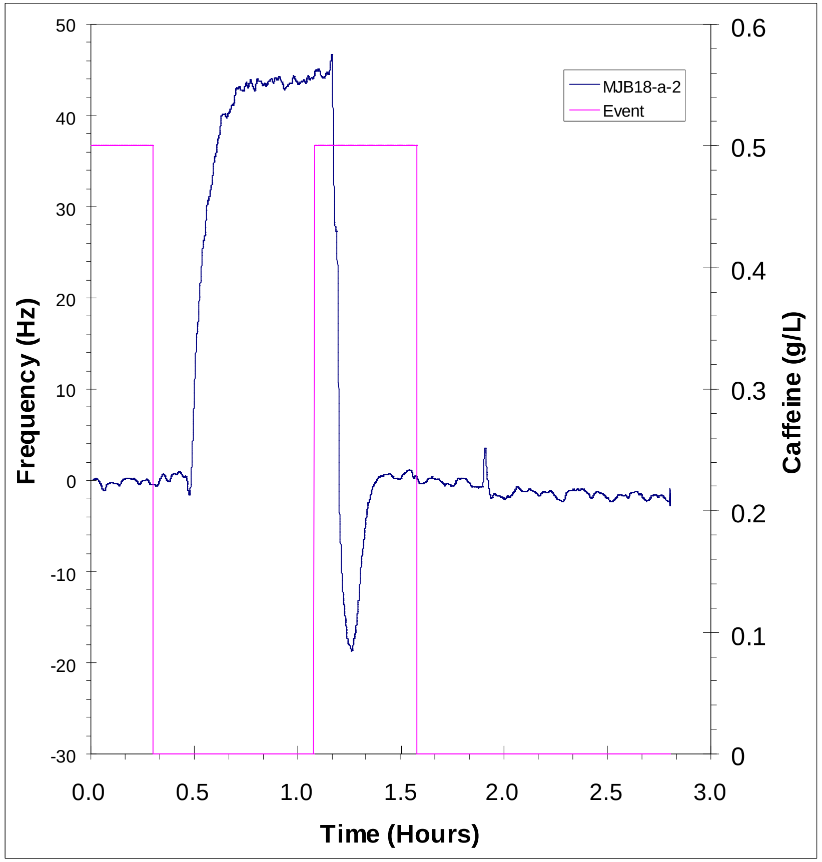

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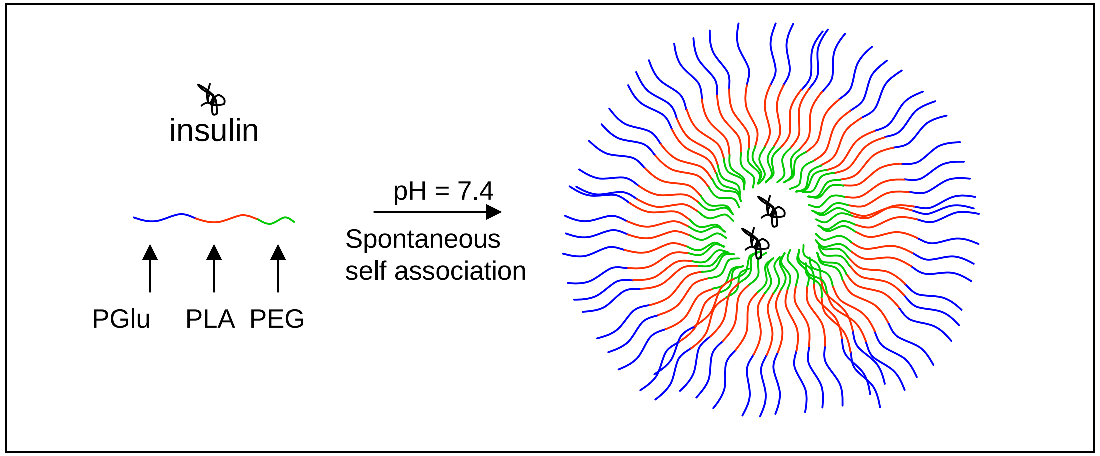
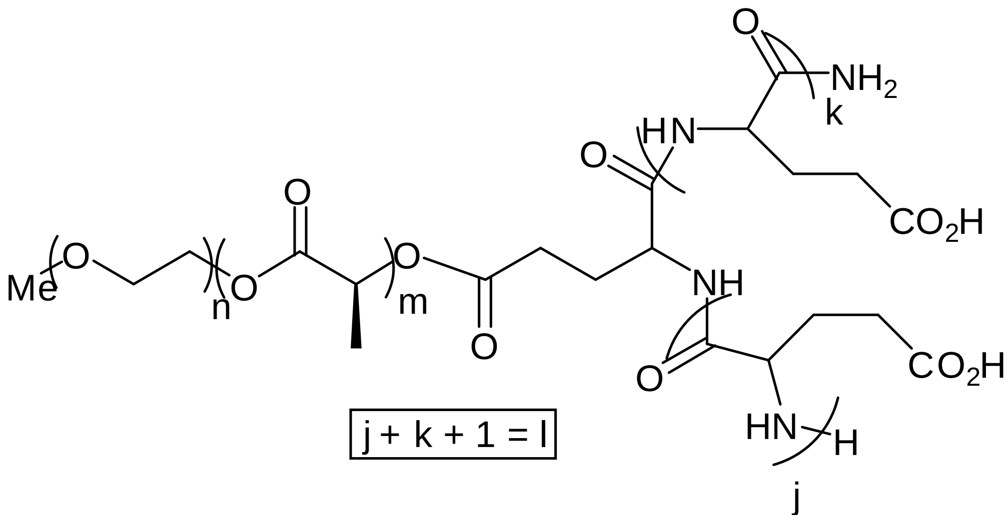
  - Drug delivery

Self assembly (Claverie)





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Parental delivery of insulin

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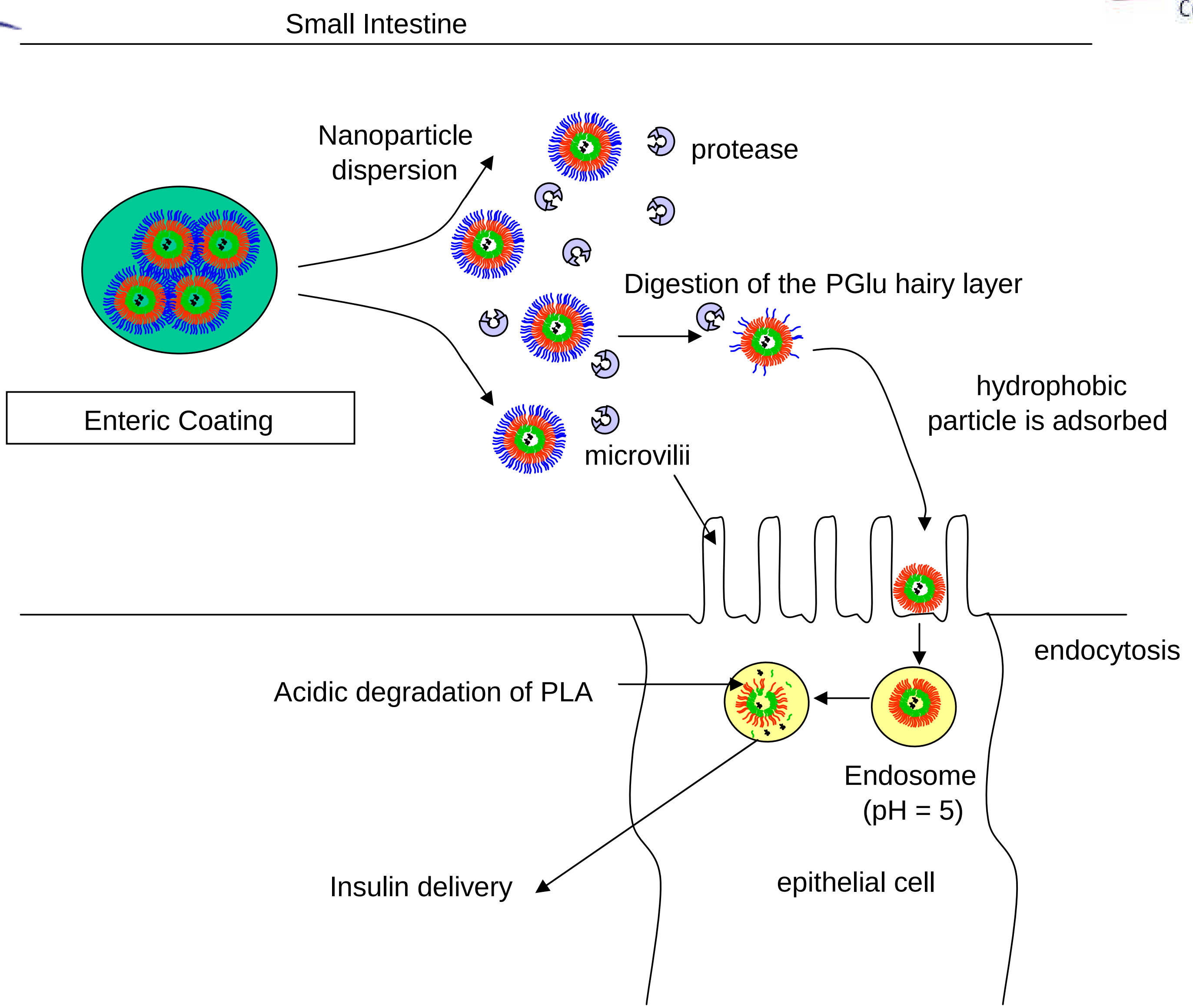
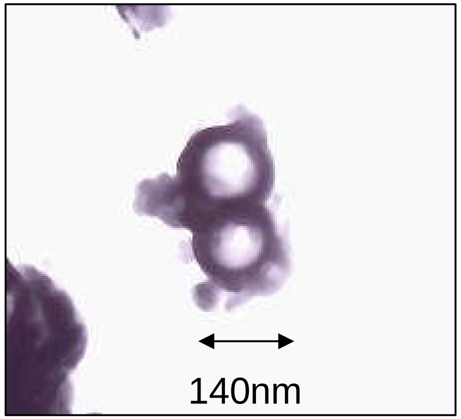


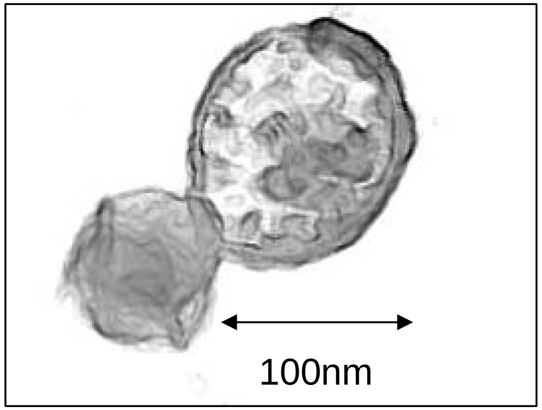
Properties of the vesicles

- White / translucent liquid (nanosize)
- Does not contain any solid in suspension
- Has the viscosity of water

Electronic Microscopy

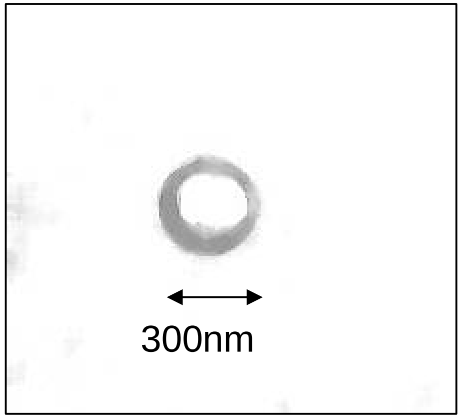


FMC 150

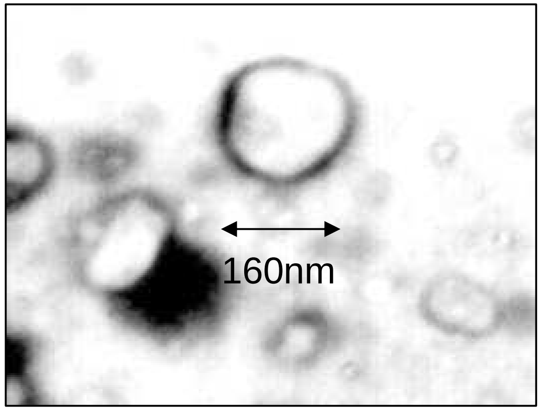


FMC 66

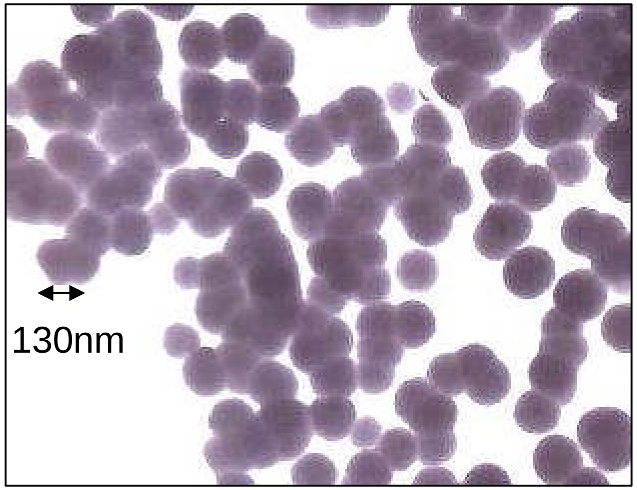
Branched Triblock



FMC 179



FMC 146



Linear Triblock

- **Polymeric Nanoparticles synthesis processes**
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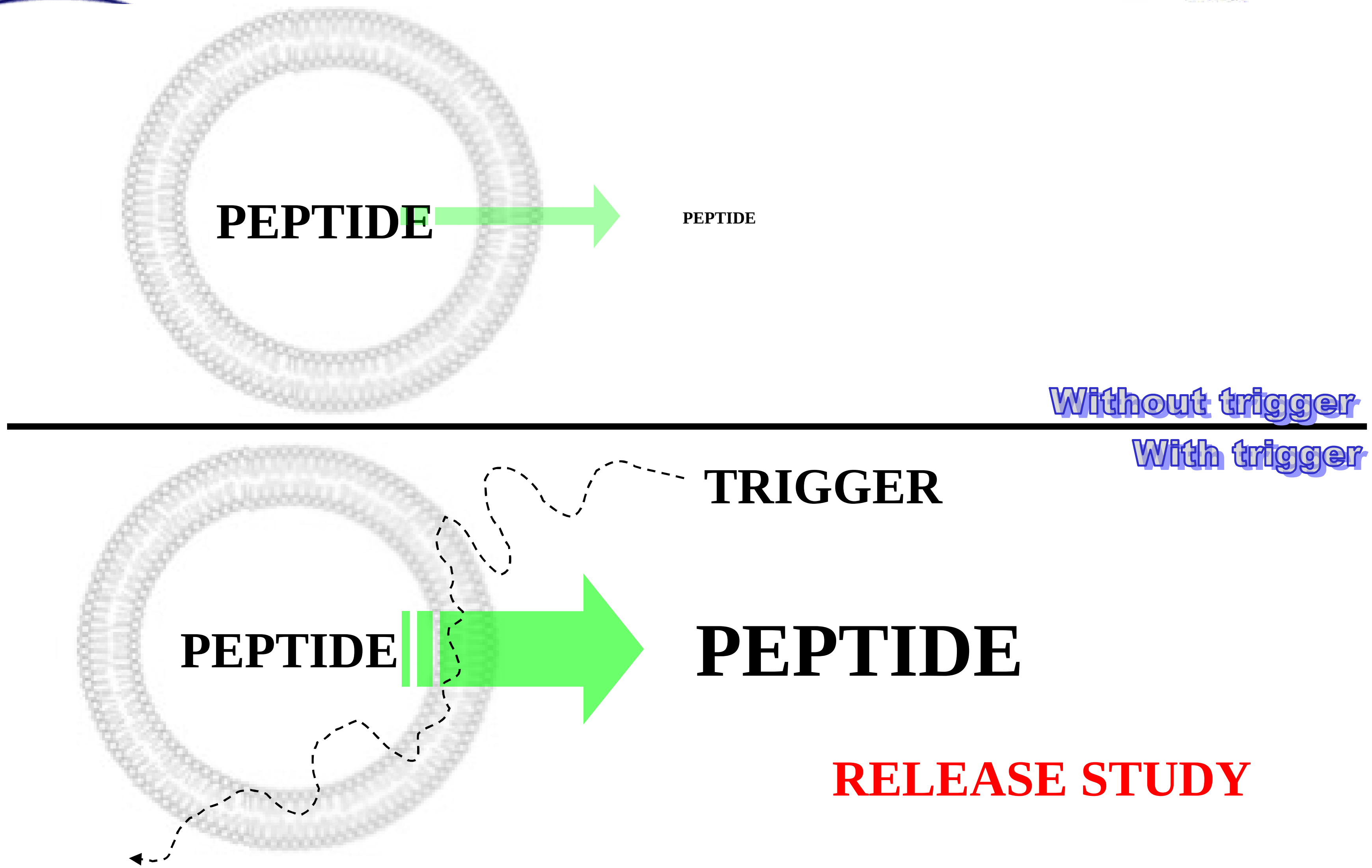
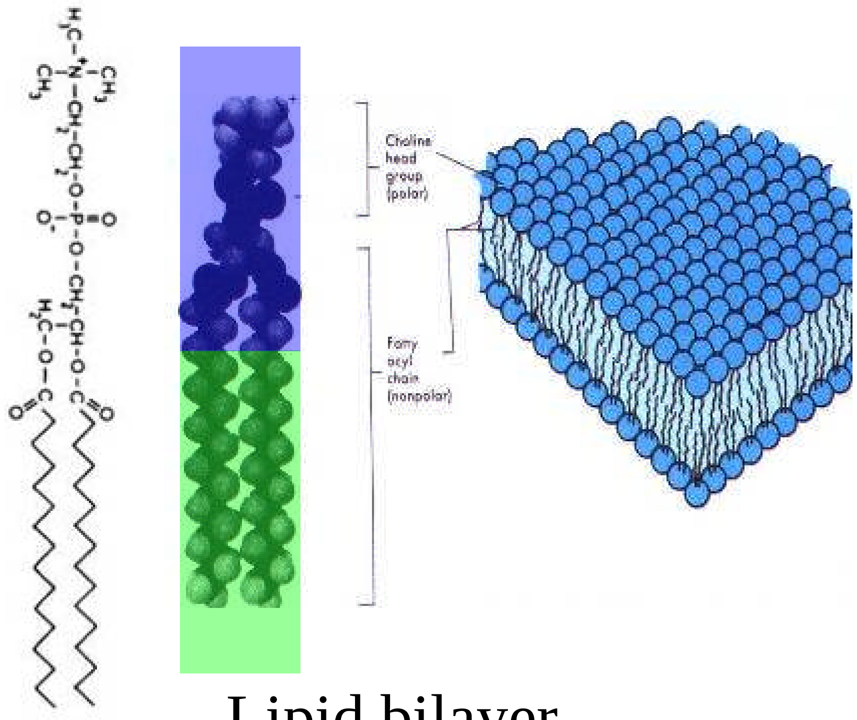
  - Self assembly
  - Directed assembly
- **Application to biotechnologies**
  - biosensors by molecularly imprinted polymers
  - liposomes for transmembrane delivery
  - Drug delivery

Trigger strategy (in vitro)

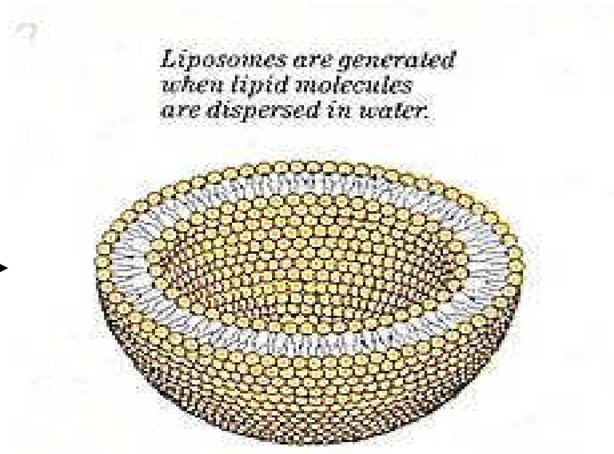


RELEASE STUDY

Liposomes



Lipid bilayer



Liposome

http://www.avantilipids.com/PreparationOfLiposomes.html

Self assembly of Liposome

Multi Lamellar Vesicles

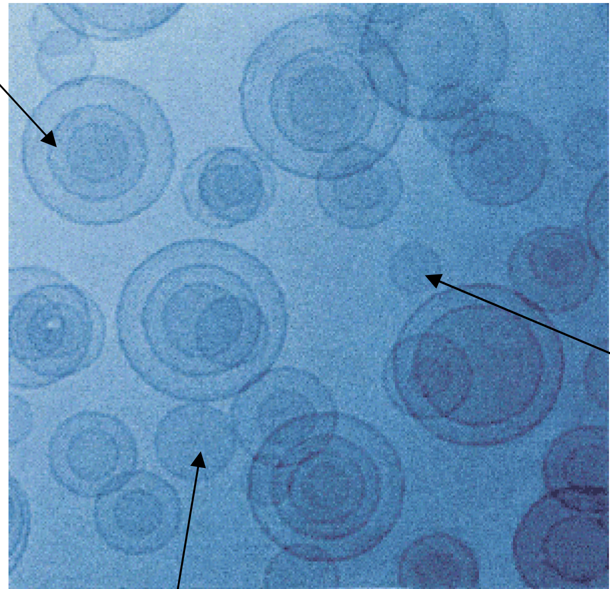


Photo courtesy of FEI Company Japan Ltd.)

Small Unilamellar Vesicles

Large Unilamellar Vesicles : LUV

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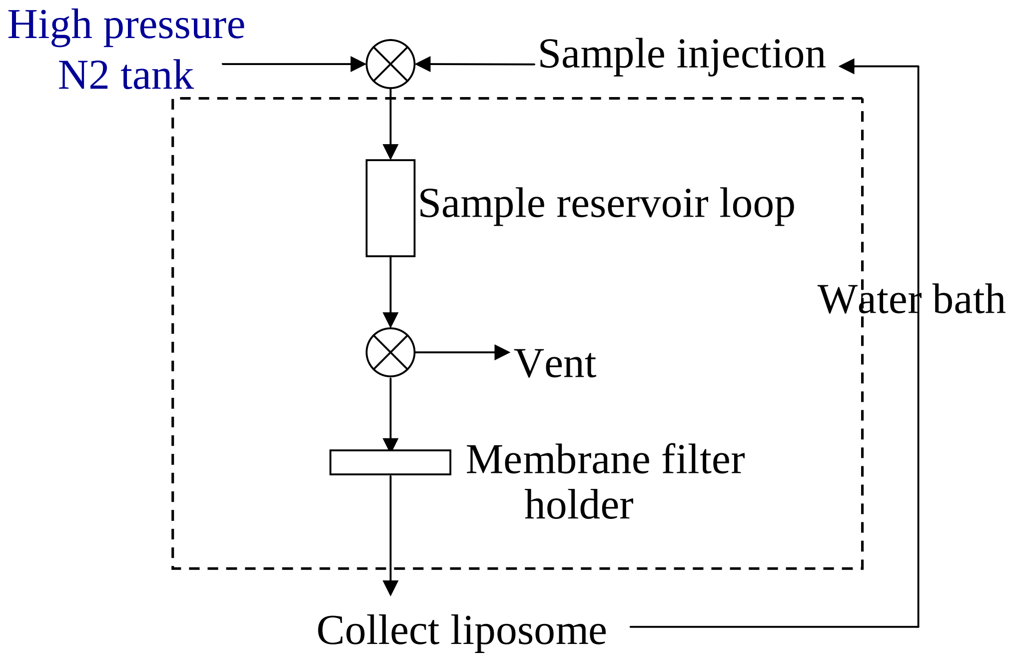
Directed assembly : extrusion

- Operates above Tc
- Membrane pore size control vesicle size
- Multiple extrusion (typically 5 passes)
- Good reproducibility
- Can operate at up to 10 bar (typically 4)
- “Wide” range of LUV



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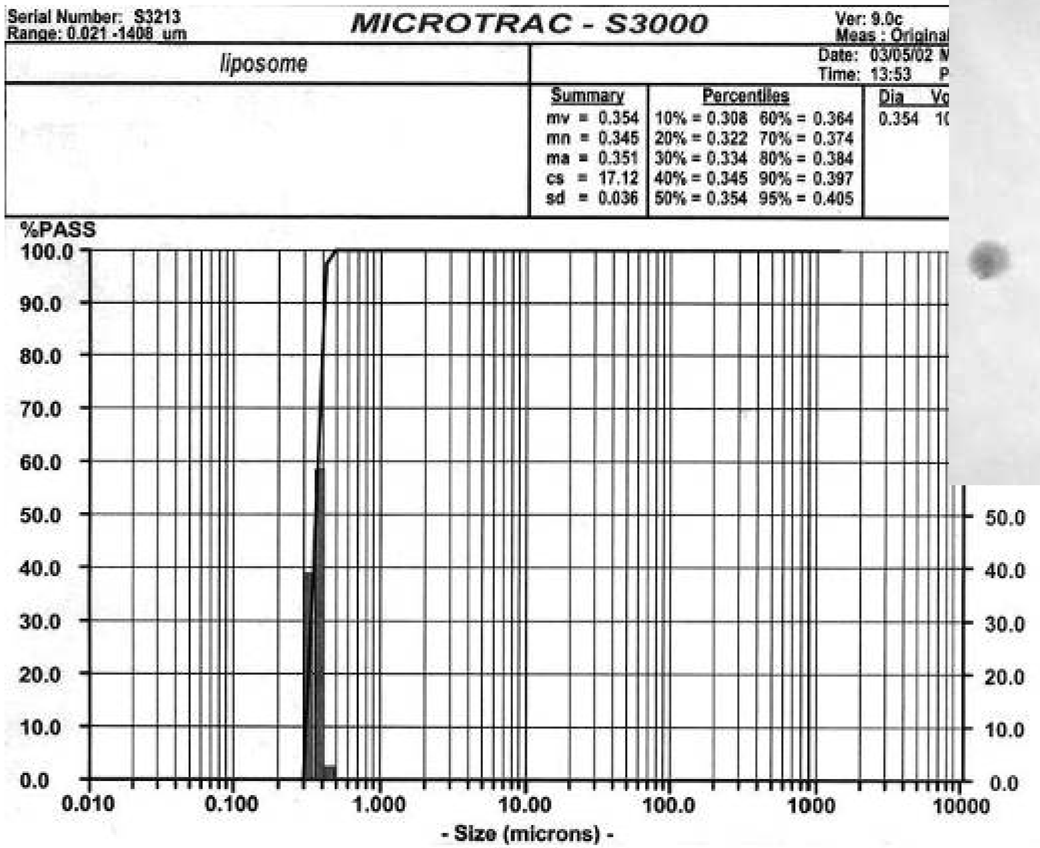
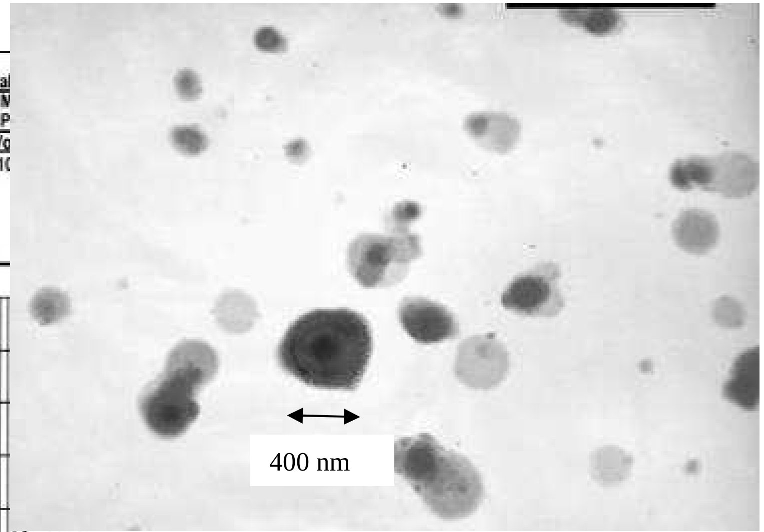
DPPC liposome size distribution after extrusion through a 400 nm polycarbonate membrane filter.

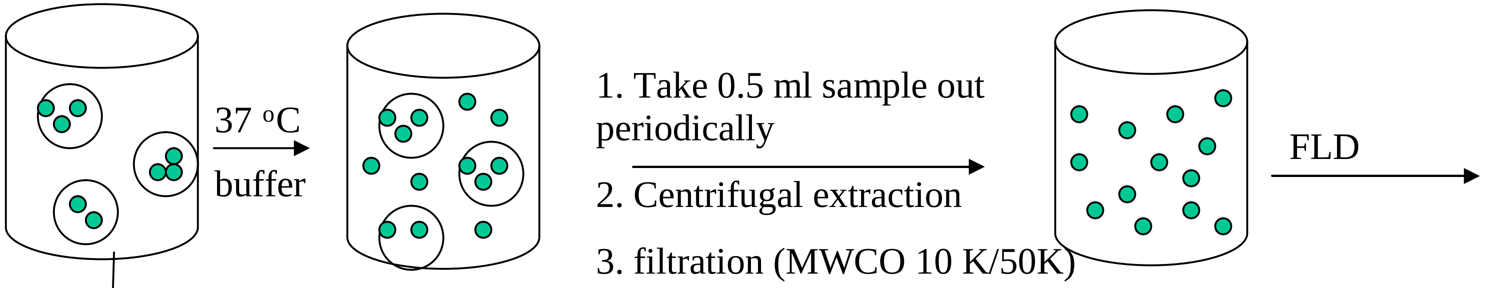


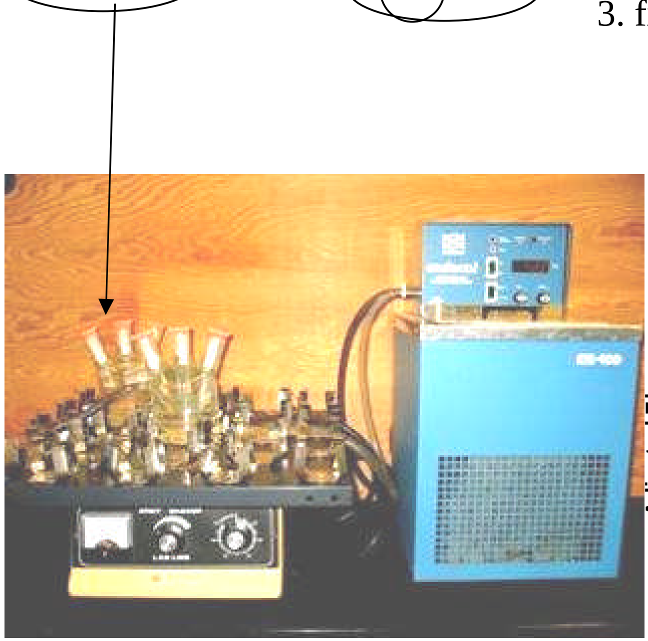


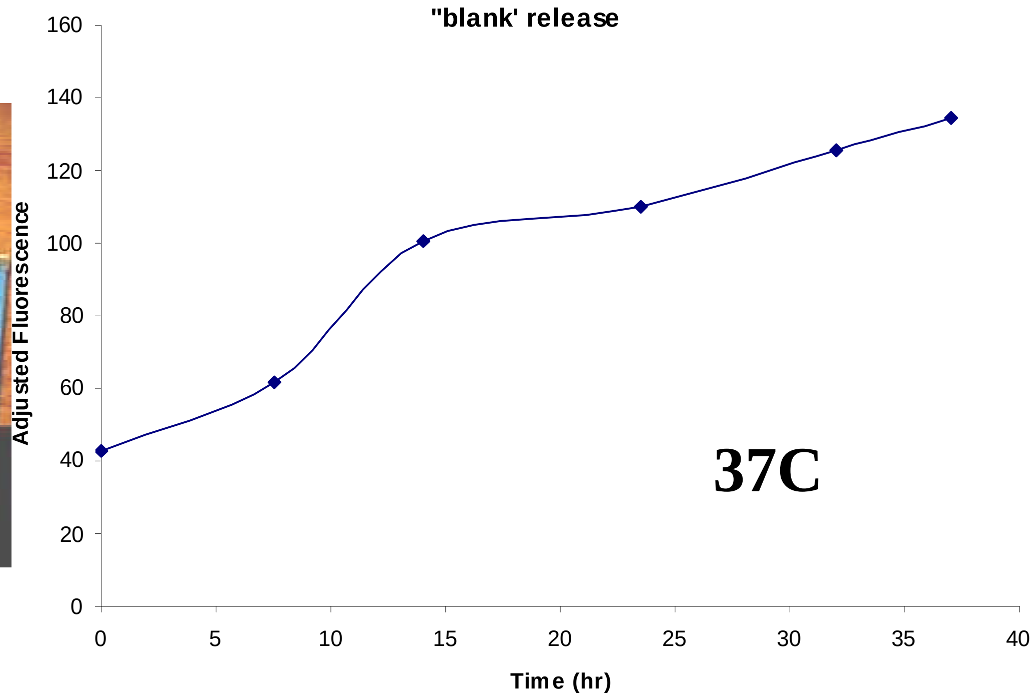
Negatively-stained TEM

Can be VERY monodispersed

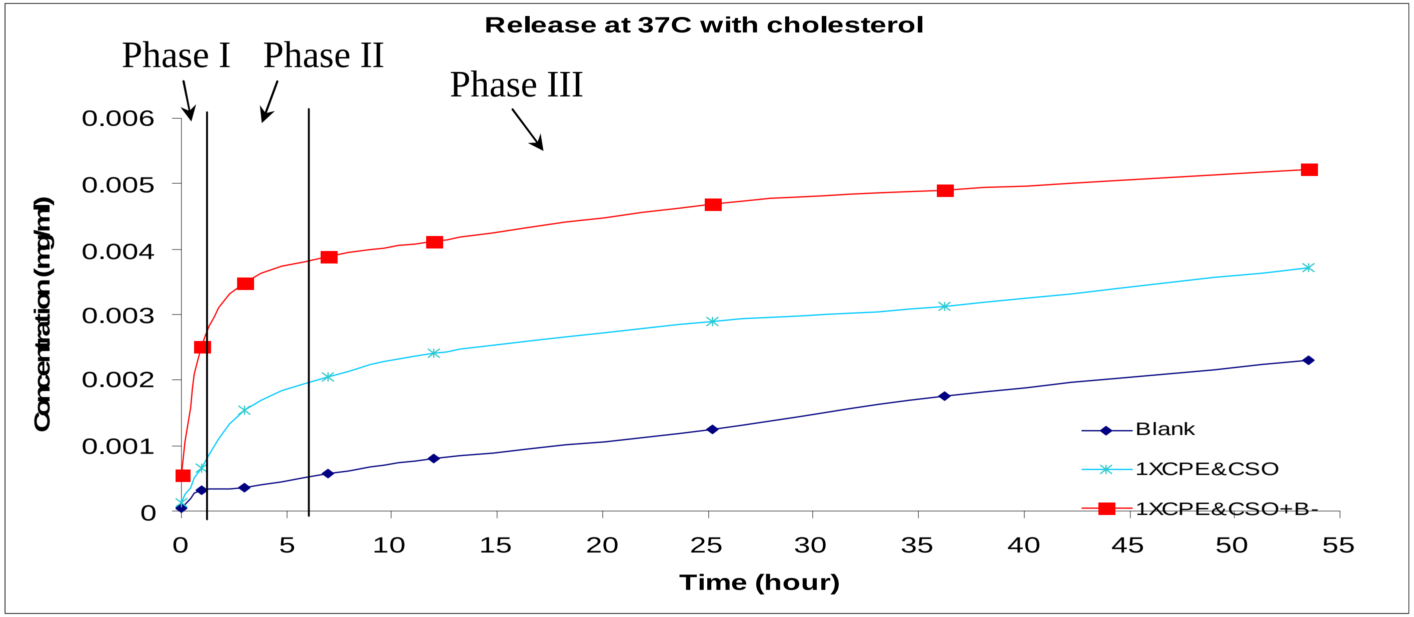
Release study strategy

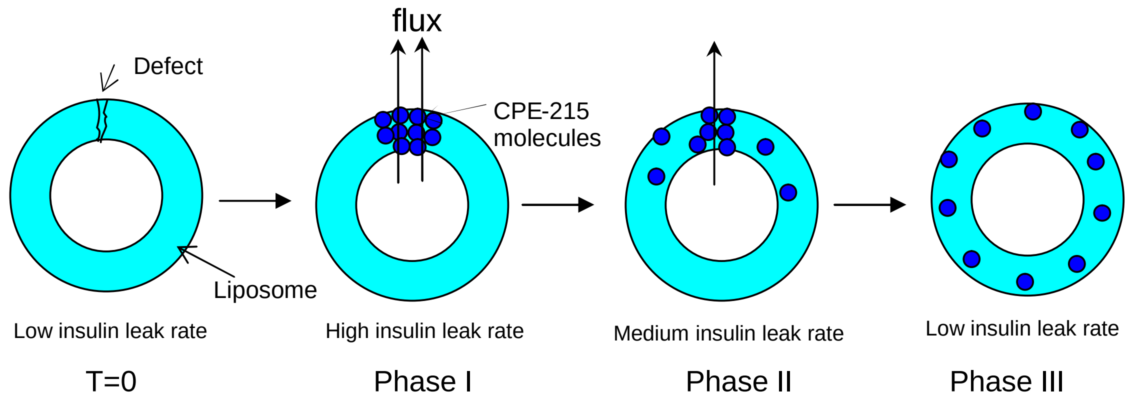






Transmembrane transport mechanism of insulin with excipient triggering





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Microemulsion

<u>Recipe MJB-10: microemulsion (seed)</u>

Water	82.84%
NaHCO3	0.043%
Na2O5S2	0.011%
SDS	8.27%
KPS	0.17%
Styrene	8.67%

Water, Salts, SDS, stirred, degassed. Add 20% of styrene. Heat. When at 80C, add KPS. Let react for 20 minutes. Start feeding with styrene, over 2 hours. 30 minutes of Post polymerization.

SCexp = 15.1% Conversion = 77.47%

Size = CHDF:

Dv = 35.5 nm, Dn = 33.2 nm

Nanotrac:

Dv = 36.8 nm, Dn = 25.13 nm