Engineering Pharmaceutical Nanoparticles

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The University of Kansas

The University of KANSAS

Acknowledgements

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Undergraduates:

Casey Morris, Tina Coop, Ryan Ellis

Funding:

NIH, American Heart Association, Cystic Fibrosis Foundation, PhRMA Foundation, Juvenile Diabetes Research Foundation, HBC, KMCRI

Special thanks:

The Microscopy Lab at KU, Prof. Russ Middaugh and lab members.

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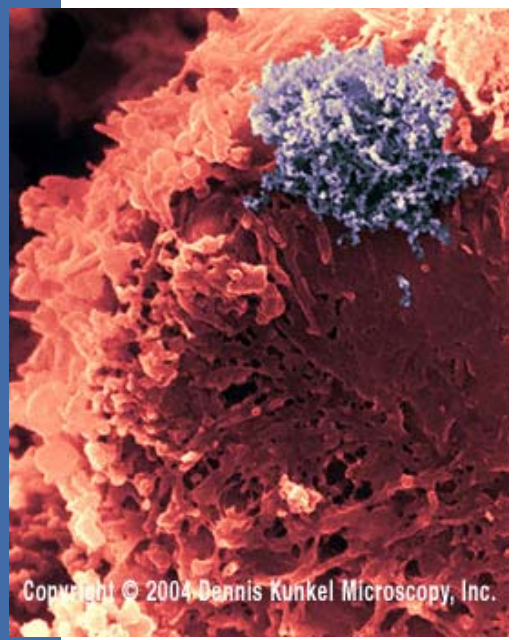
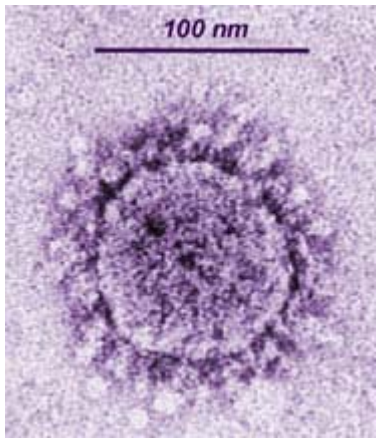
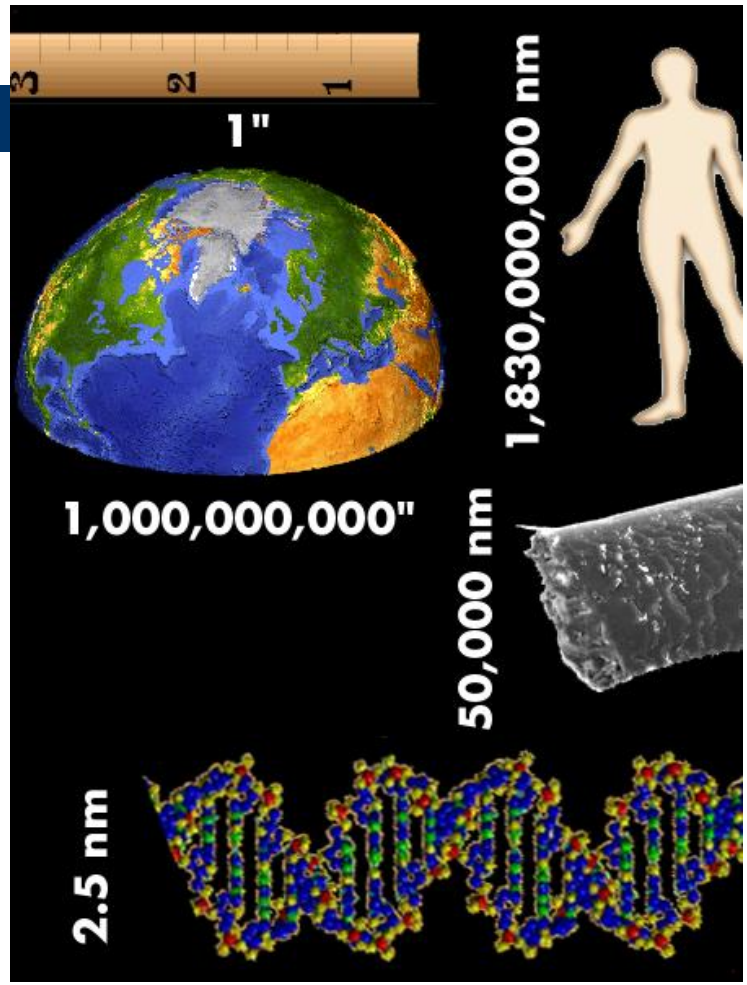
Nano perspective….



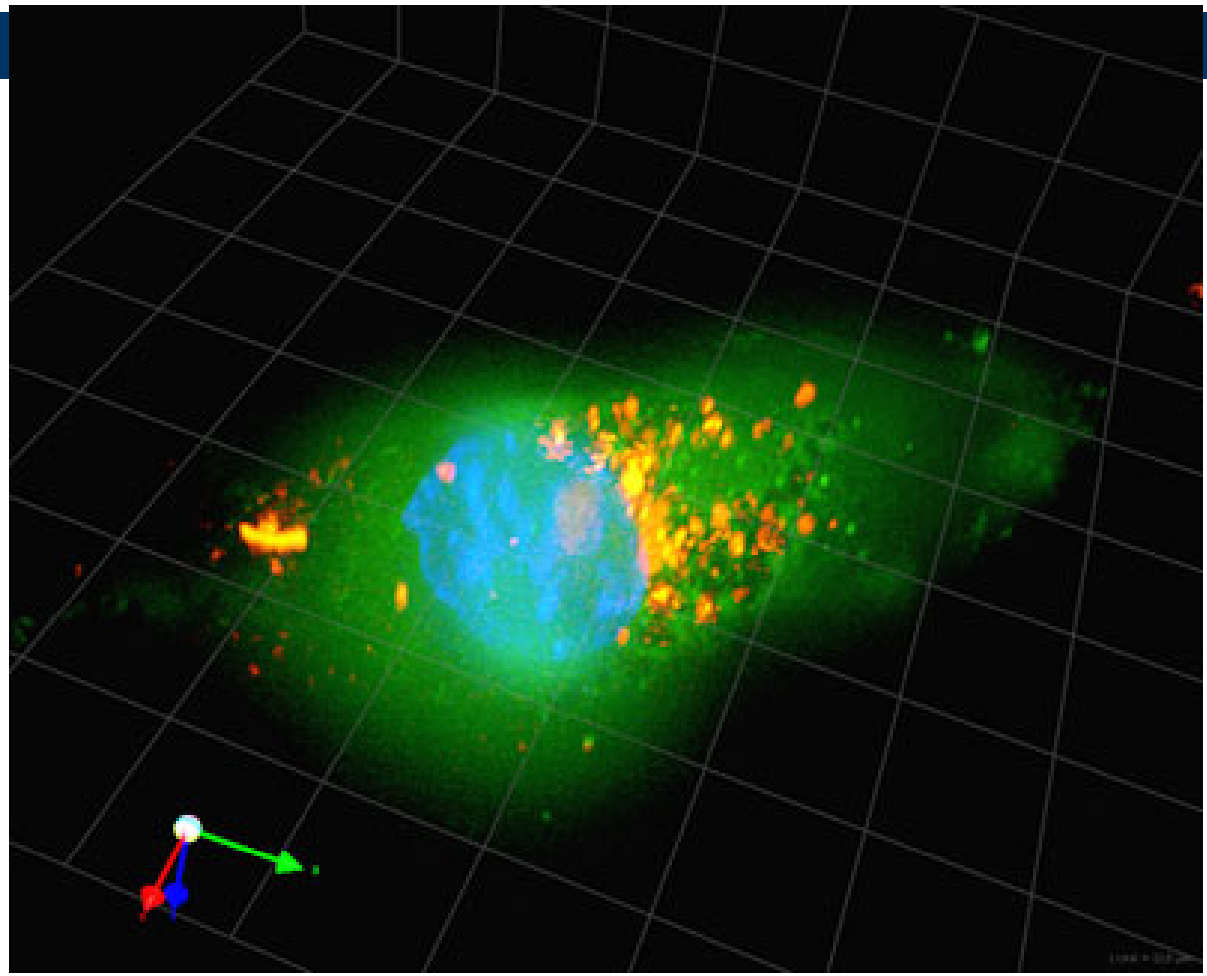
human T-lymphotropic virus



SARS virus



Nanometer size particles are small enough to enter cells.



Particle size >200 nm enables intracellular delivery.

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Particle engineering is critical for pharmaceutical applications.

- Control particle…
  - Size and distribution
  - Morphology
  - Surface roughness
    - Dispersibility
  - Surface chemistry
    - Passivate
    - Activate
  - Consistency/quality control
  - Product lifecycle management

- Dissolution rate
  - Control size
- Pulmonary delivery
  - ~3 microns
- Nasal delivery
  - ~5-20 microns
- Embolism
  - ~10-20 microns
- Avoid RES
  - >150 nm
- Target “leaky” vessels
  - <250 nm
- Endocytosis
  - ~200 nm

Retsch

Current practices are process intensive and harsh for fragile API.

Shear
Heat
Contact Materials

→

Polymorphism
Loss of activity
Contamination

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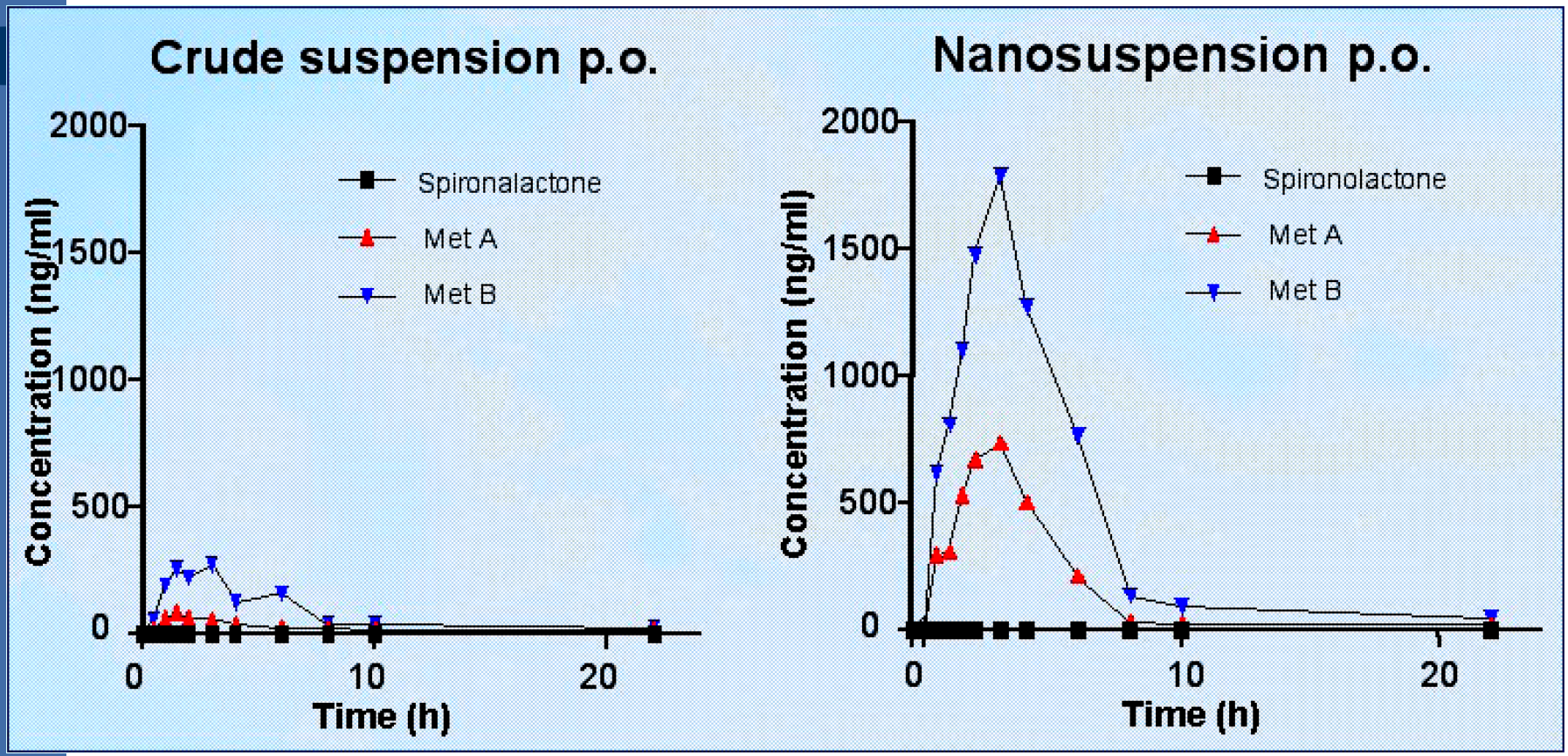
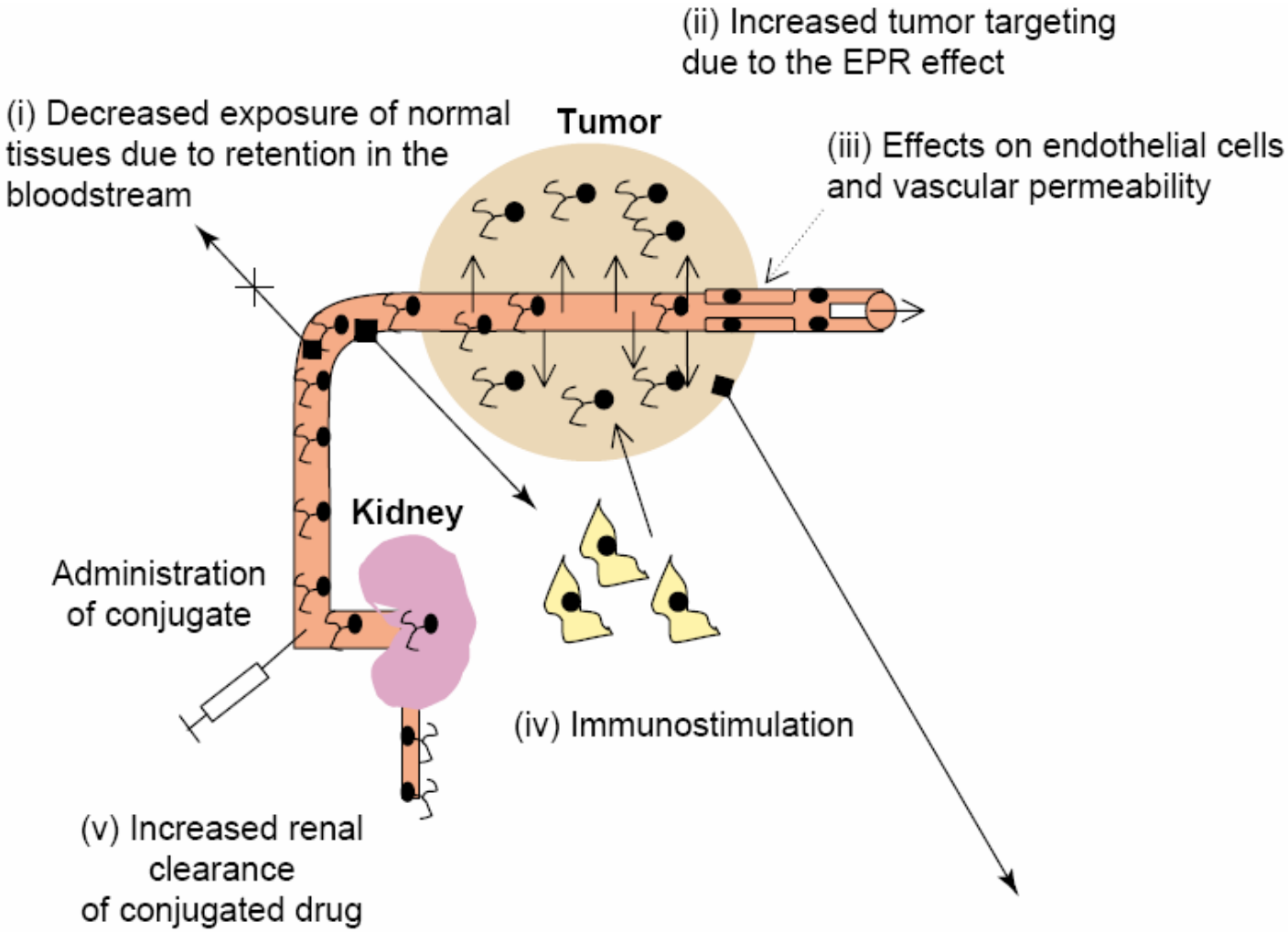
For example, the bioavailability of poorly soluble drugs can be enhanced.



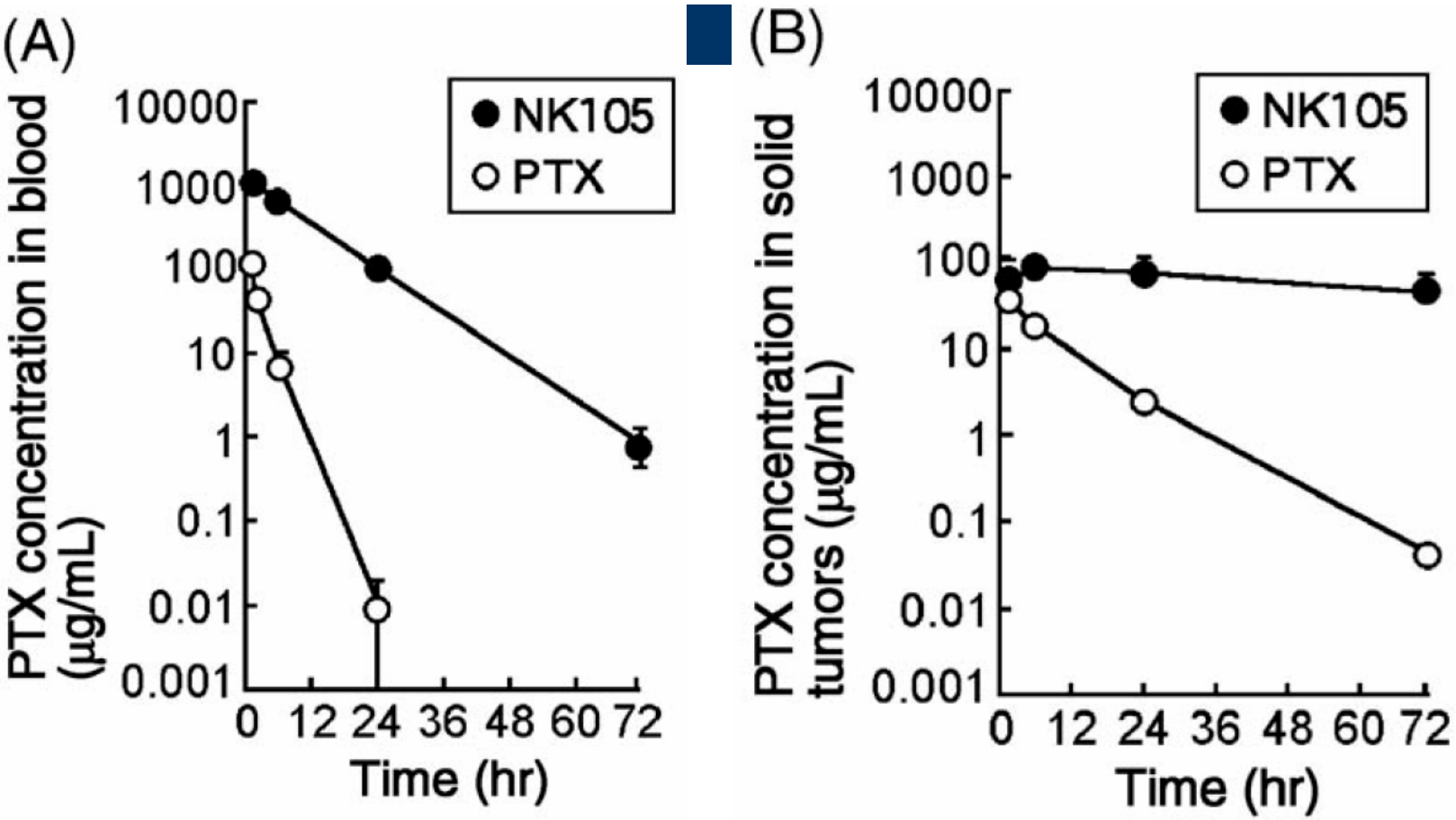
Spironalactone is a synthetic 17-lactone steroid. Nanoparticle suspensions of this drug dramatically enhance the drug dissolution.

SkyePharma

For example, tumor accumulation via "enhanced permeability and retention."



For example, tumor accumulation via "enhanced permeability and retention."



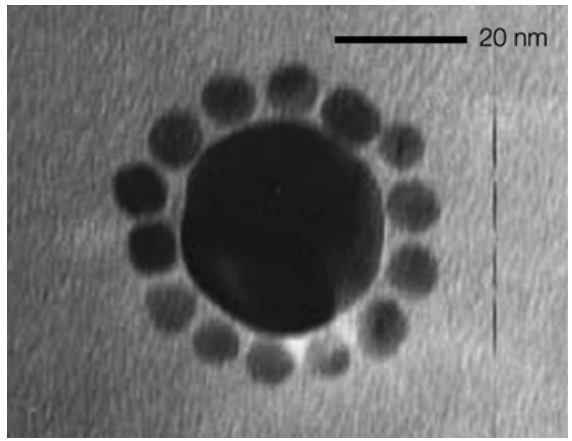
Nishiyama (2006)

Question:
How do you create particles?

A multitude of methods may be used to engineer particles.

- Top Down
  - Milling/grinding

- Bottom up
  - Crystallization
  - Spray drying
  - Ionic complexation
  - Self assembly



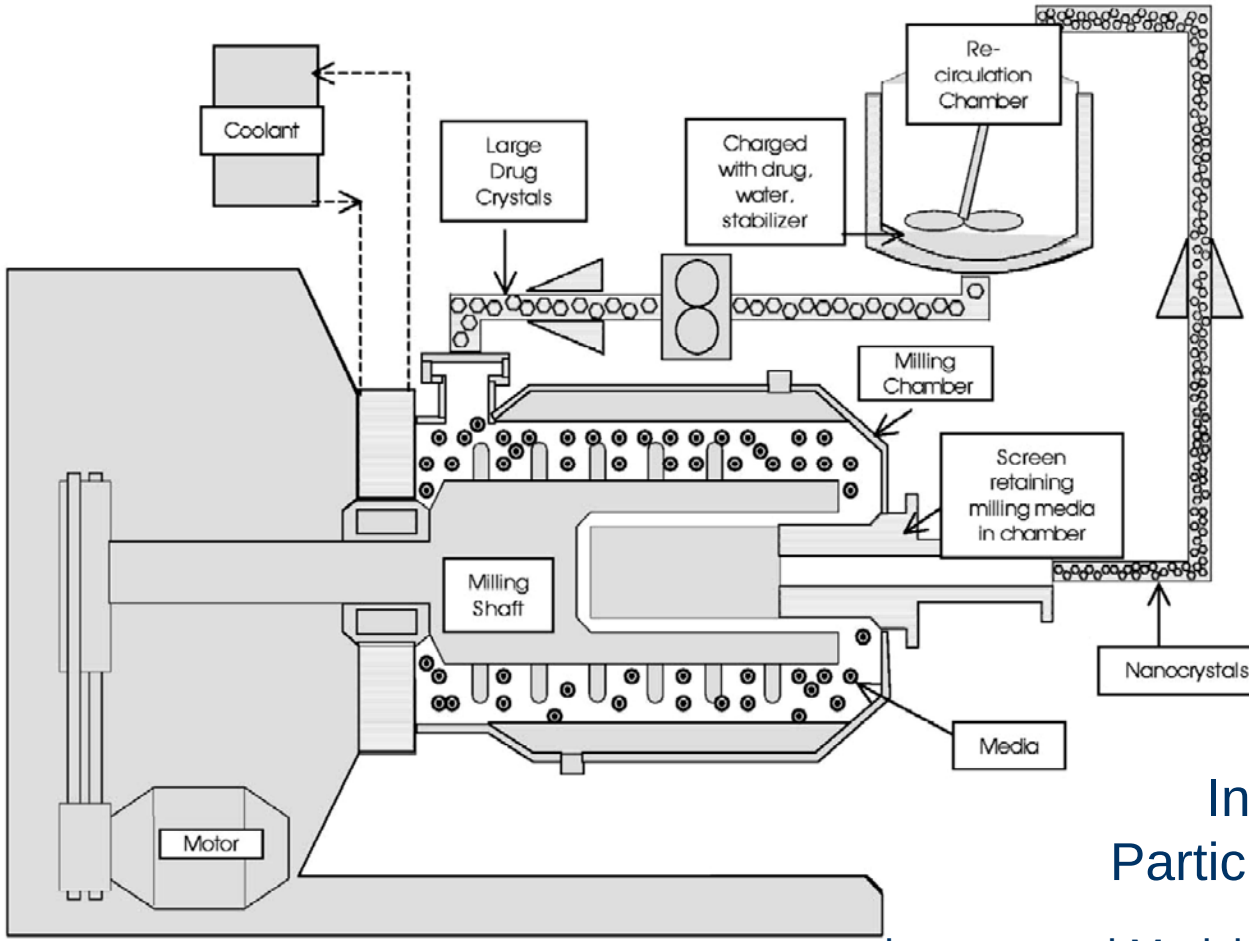
www.buchi.com and LaVan, *et al.* (2002)

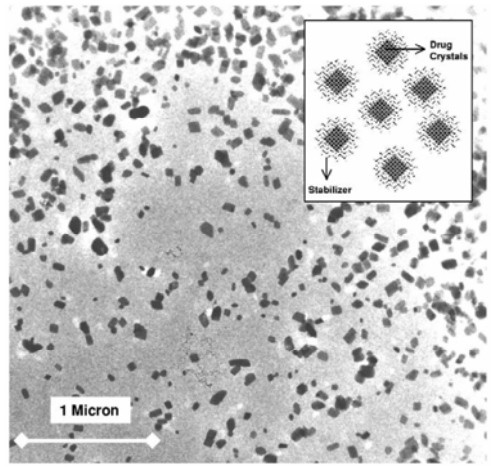
Outline of particle engineering technologies covered.

- Milling/Spraying Technology
- Crystallization Technology
- Supercritical Fluids
- Polymer Nanoparticles
- Molecular Technology/Polyplexes
- Block copolymers – micelles
- Liposomes/Polymersomes
- Polymer/Drug conjugates
- Berkland Lab

Milling/Spraying Technology

Wet milling provides a method to reduce the particle size of poorly soluble API.



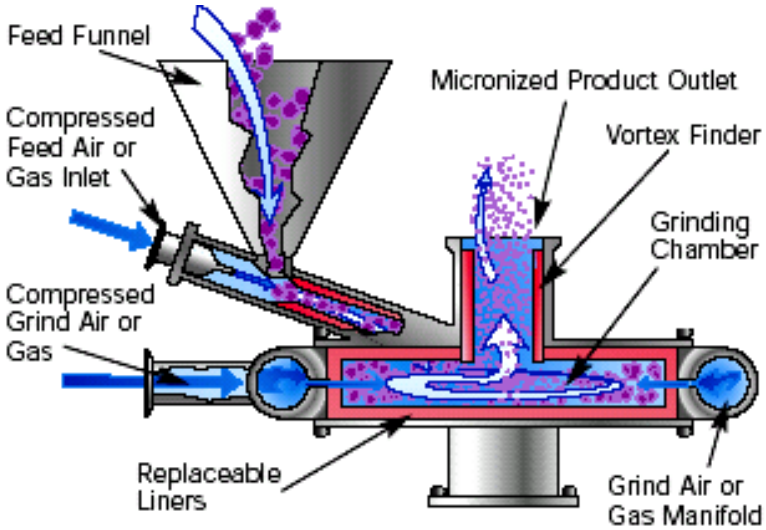


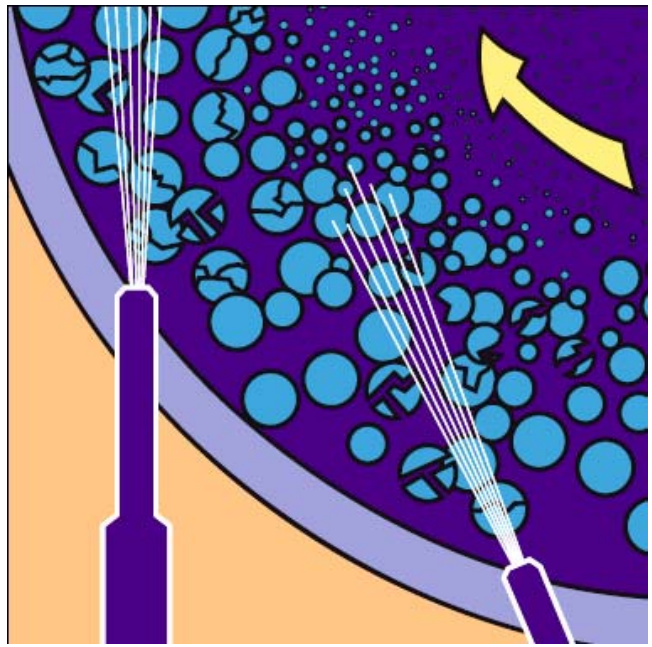
Intensive process
Particle recovery required

www.elan.com and Merisko-Liversidge, *et al.* (2003)

Air jet milling reduces particle size to 1-30 microns or smaller.

- Air jet milling
  - Particle-particle collisions
  - No heat or moving parts





>30 kg/hr production
Low power consumption

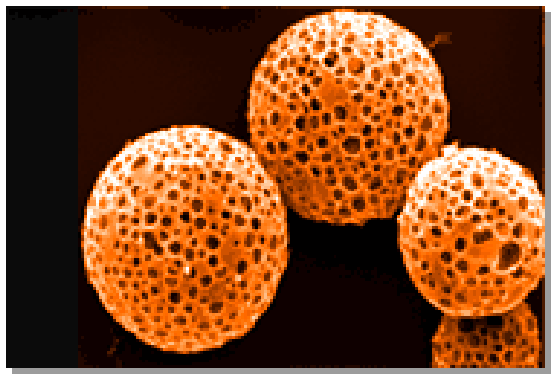
www.microntech.com and www.sturtevantinc.com

Spray drying can be utilized for engineering particle size/morphology.

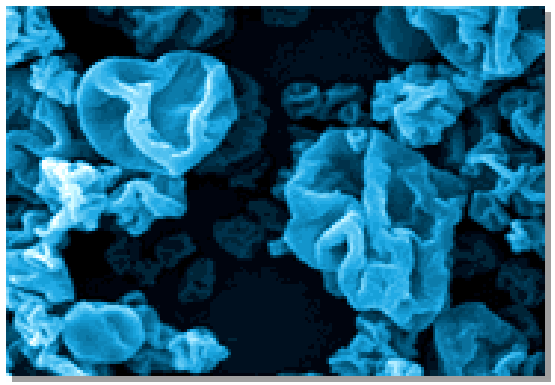
- Size control
  - Somewhat
- Density control
- Dispersibility
- Stability

Particle diameter ~3 microns leads to deep lung deposition

$$d_{aero} = d_p (\rho/\rho_{ref})^{0.5}$$



Small molecule API

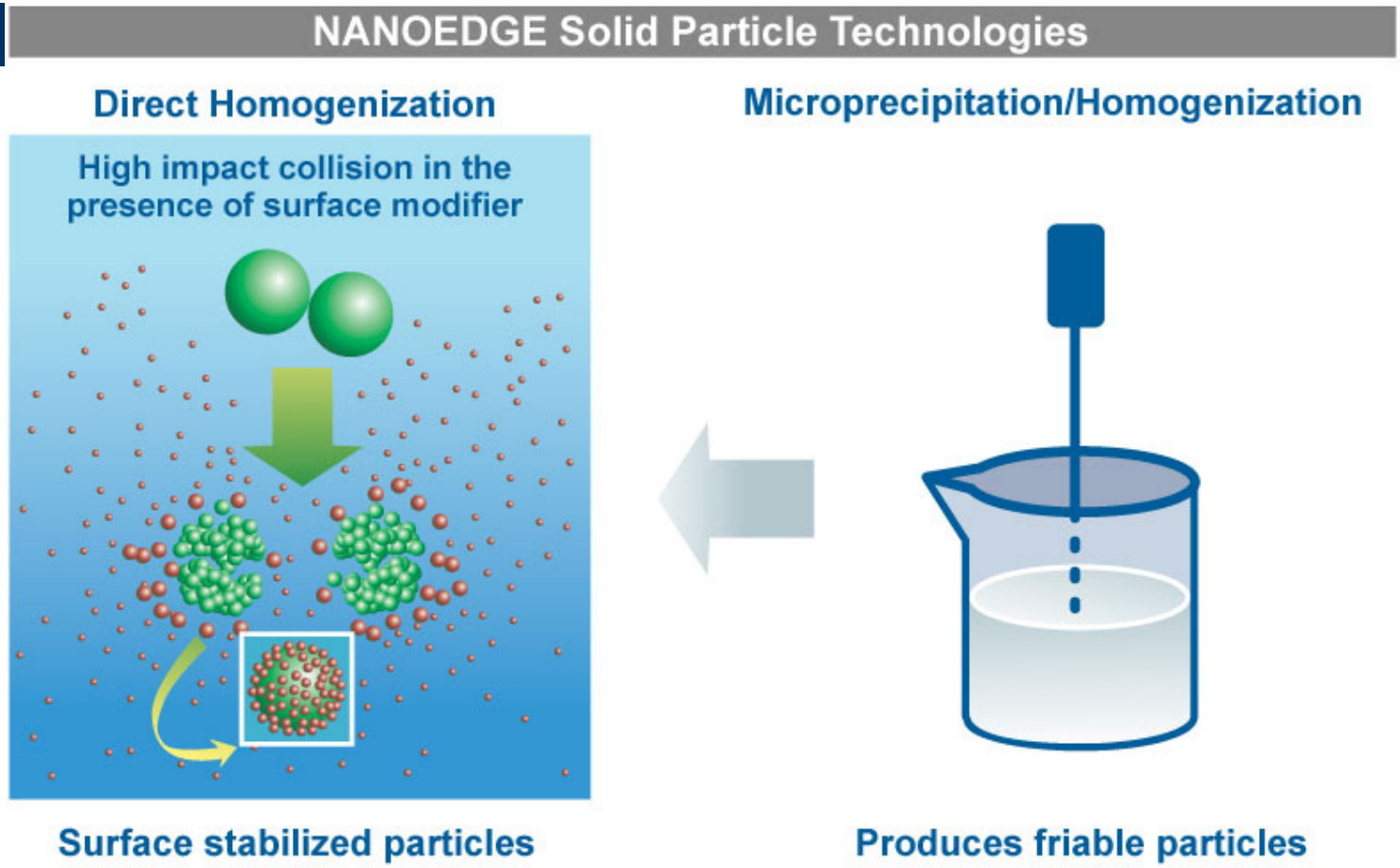


Large molecule API

Crystallization Technology

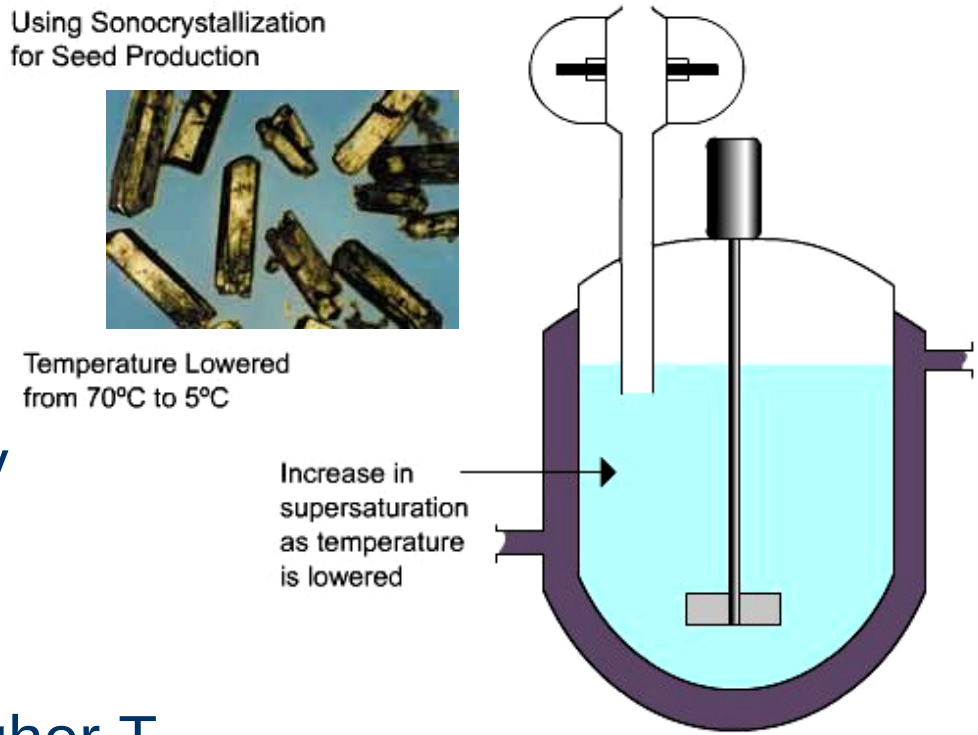
For background see Rabinow (2004)

Crystals can be designed to be small and friable for nanosizing.



C^3 technology uses ultrasound to control crystal formation.

- Control nucleation
- Enhance yield
- Reduce agglomeration
- Fewer imperfections
- Increase reproducibility
- Eliminate seeding?
- No sonicator contact
- Crystal formation at higher T

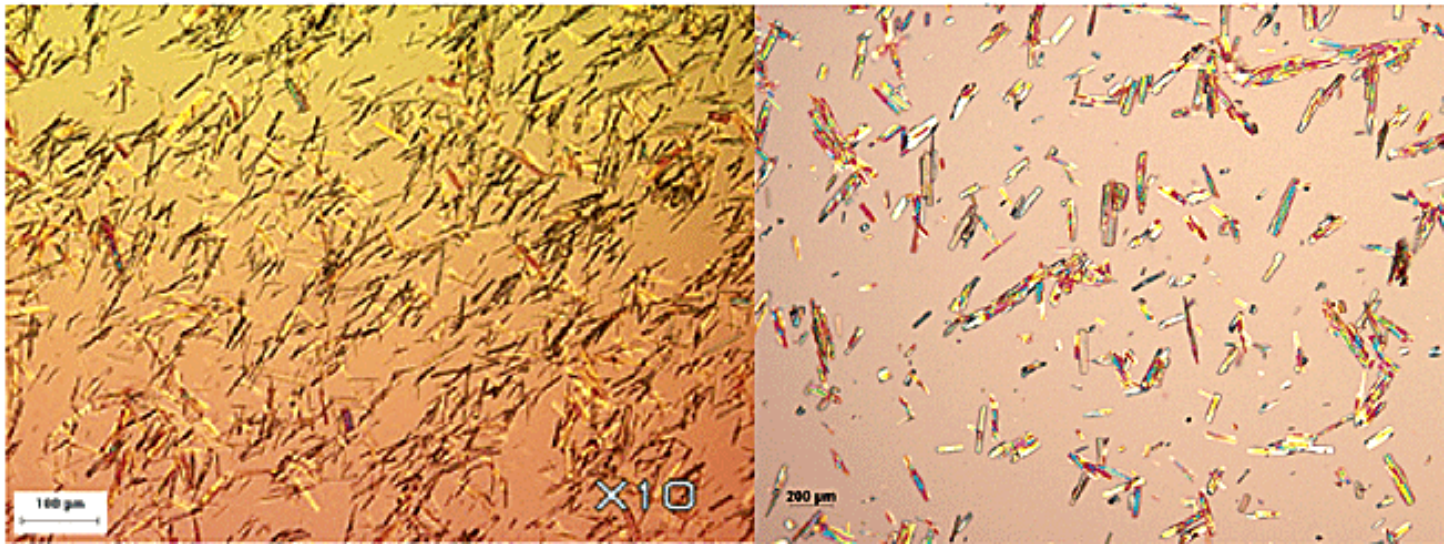


GlaxoSmithKline

Nucleation induced by sonication increases crystal homogeneity and yield.

Sonocrystallised

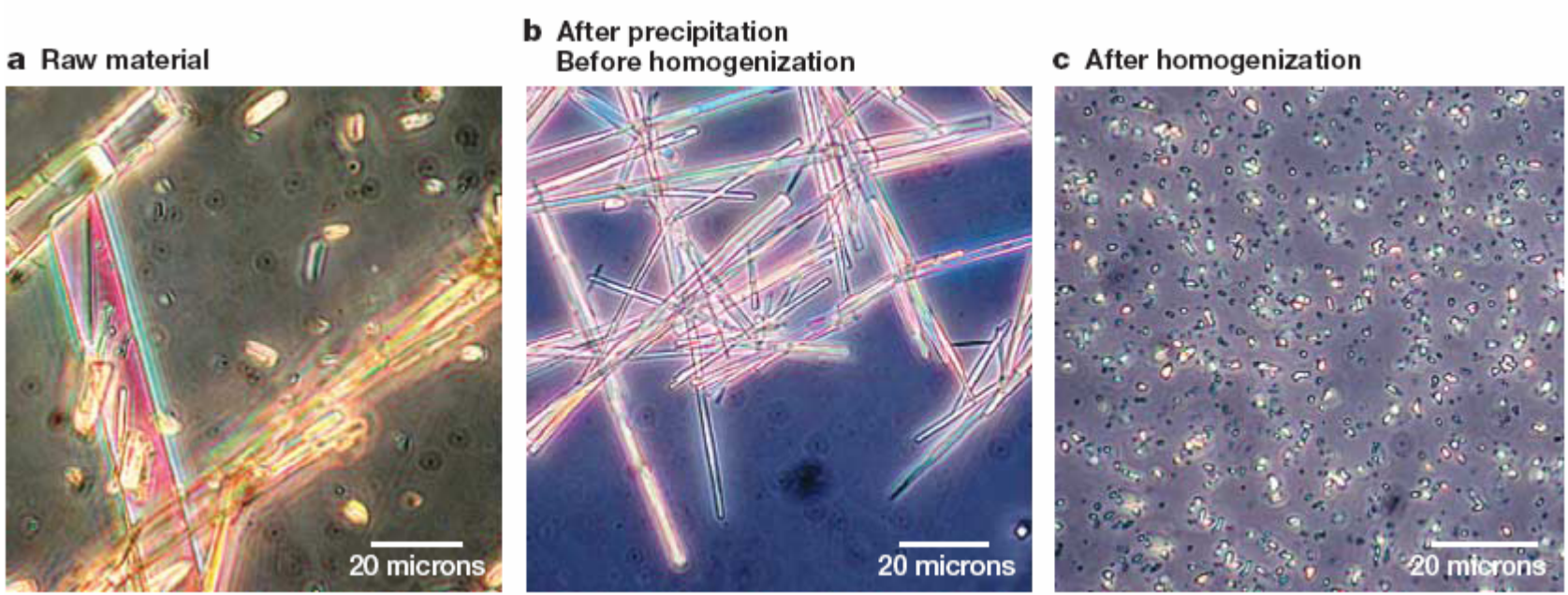
Batch crystallised



Sonication techniques applied to emulsion crystalization processes provide control over nucleation, crystal size, and quality.

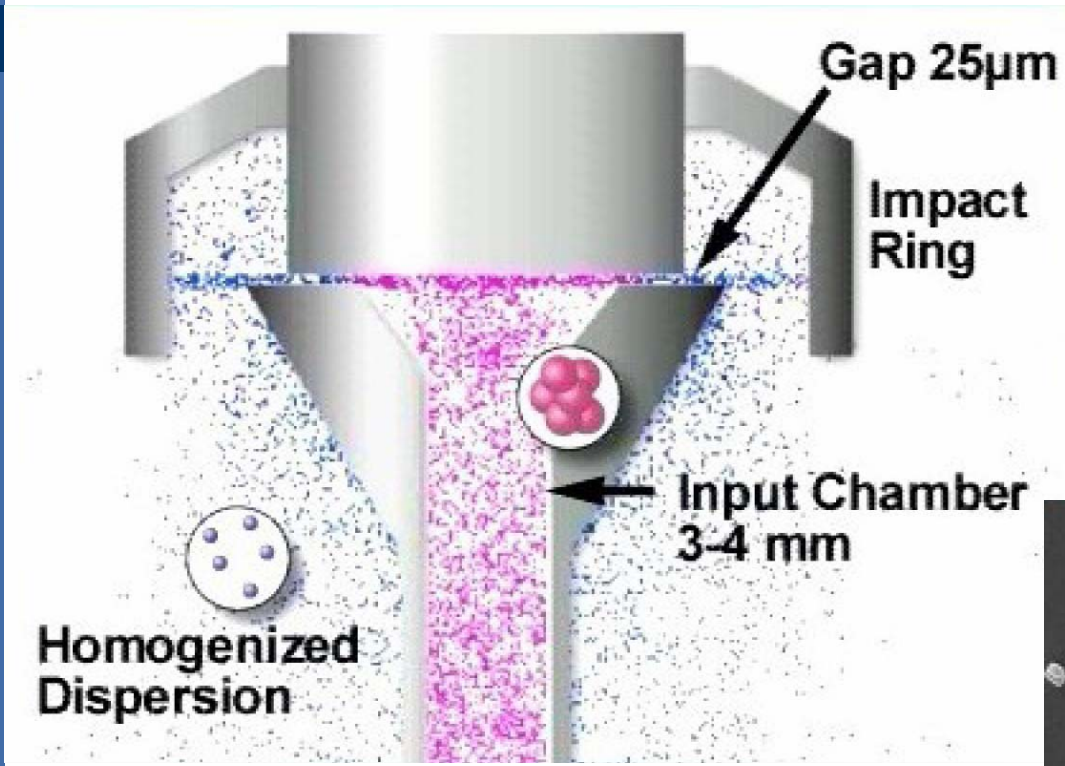
www.glaxosmithkline.com,

Post-crystallization processing decreases particle size (submicron).



Rabinow (2004)

High pressure homogenization is another technique.



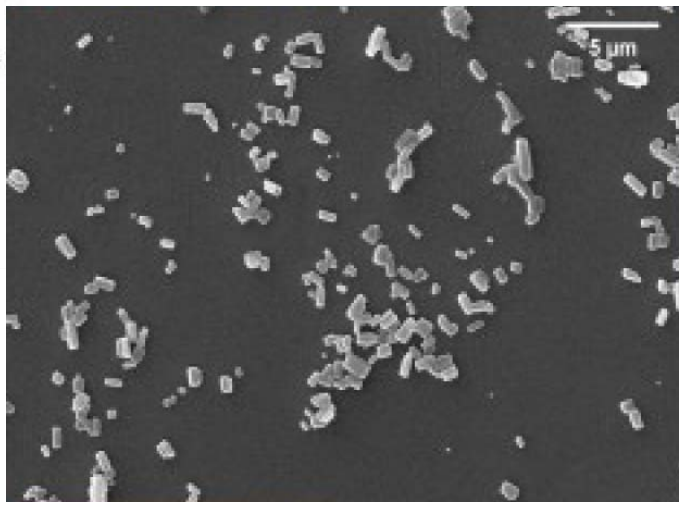


Table 2 | **Solid-particulate-nanosuspension-based formulations in development and in the market**

Drug	Indication	Drug delivery company	Pharma company	Route	Status
Paclitaxel	Anticancer	American BioScience	American Pharmaceutical Partners	Intravenous	Phase III
Undisclosed multiple	Anti-infective	Baxter NANOEDGE	Undisclosed	Oral/ intravenous	Preclinical to Phase II
Undisclosed	Anticancer	Baxter NANOEDGE	Undisclosed	Intravenous/ oral	Preclinical to Phase I
Rapamune	Immuno-suppressant	Elan Nanosystems	Wyeth	Oral	Marketed
Emend	Anti-emetic	Elan Nanosystems	Merck	Oral	Marketed
Cytokine inhibitor	Crohn's disease	Elan Nanosystems	Cytokine PharmaSciences	Oral	Phase II
Diagnostic Agent	Imaging agent	Elan Nanosystems	Photogen	Intravenous	Phase I/II
Thymectacin	Anticancer	Elan Nanosystems	NewBiotics./Ilex Oncology	Intravenous	Phase I/II
Fenofibrate	Lipid lowering	SkyePharma	Undisclosed	Oral	Phase I
Busulfan	Anticancer	SkyePharma	Supergen	Intrathecal	Phase I
Budesonide	Asthma	Elan Nanosystems	Sheffield Pharmaceuticals	Pulmonary	Phase I
Silver	Eczema, atopic dermatitis	NUCRYST	Self-developed	Topical	Phase I
Calcium phosphate	Mucosal vaccine adjuvant for herpes	BioSante	Self-developed	Oral	Phase I
Insulin	Diabetes	BioSante	Self-developed	Oral	Phase I

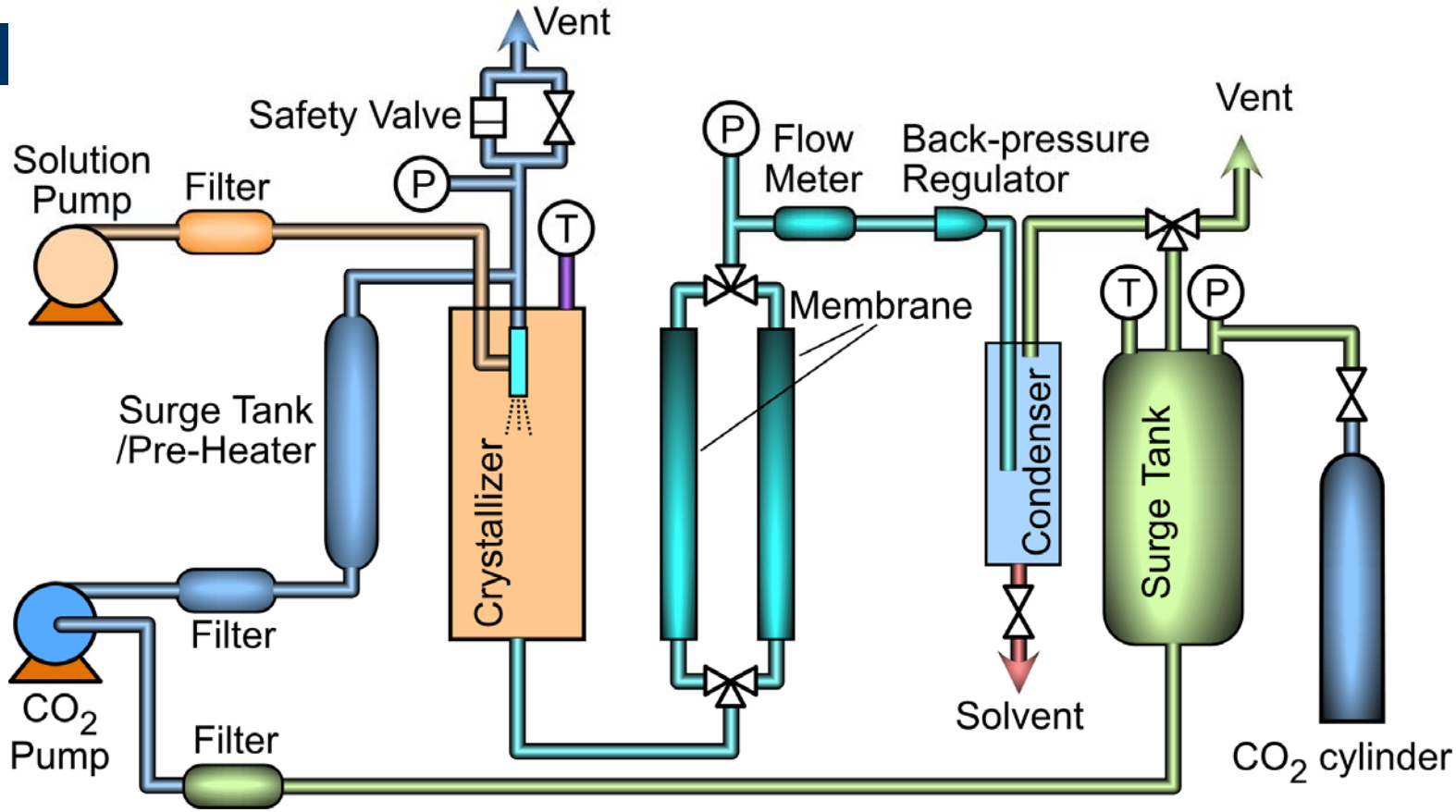
Rabinow (2004)

Supercritical Fluids (SCF)

For a review, see Yeo, *et al.* (2005)

CritiTech

Ultrasonic nozzle, SCF anti-solvent (SAS) process offers decent scalability.



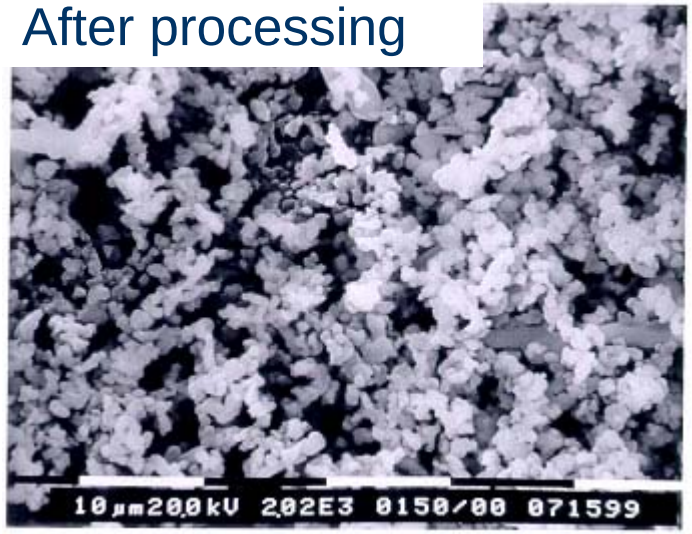
Maximum Capacity: 0.5 kg/8 h

CritiTech

SCFs provide high diffusion coefficients for rapid/complete solvent extraction.

Before processing

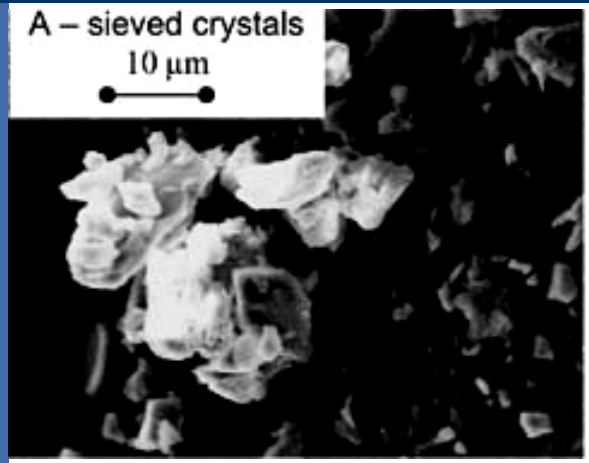
After processing

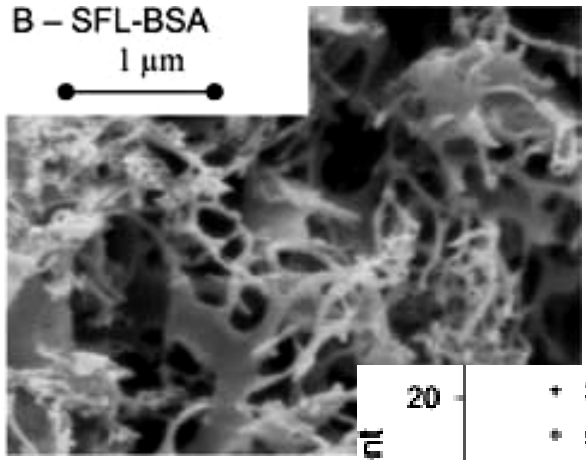


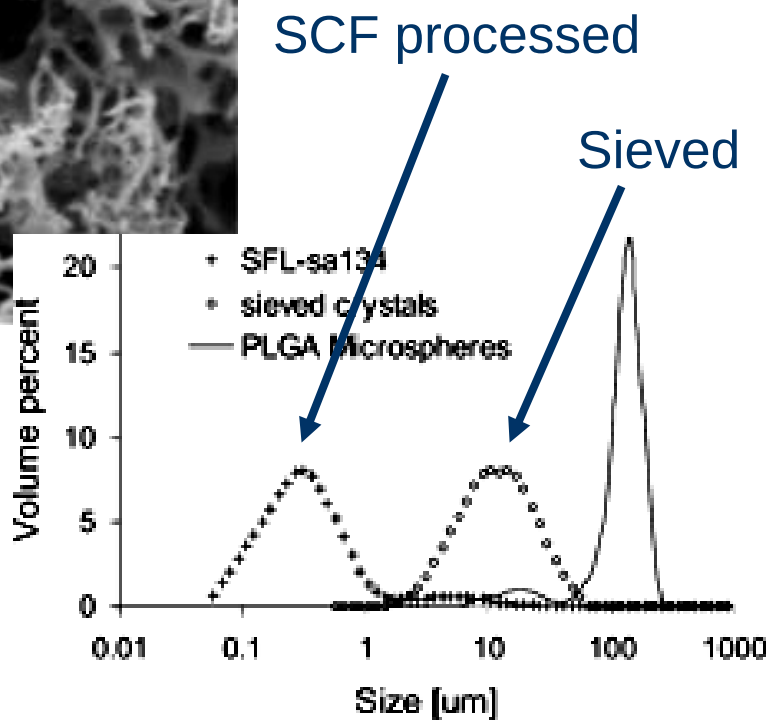
- Reduced particle size by SAS
- Narrow size distributions
- Small molecules, proteins and peptides
- Minimal residual solvent

- Expensive?

Spray-freezing into SCF provides a novel means to create protein particles.







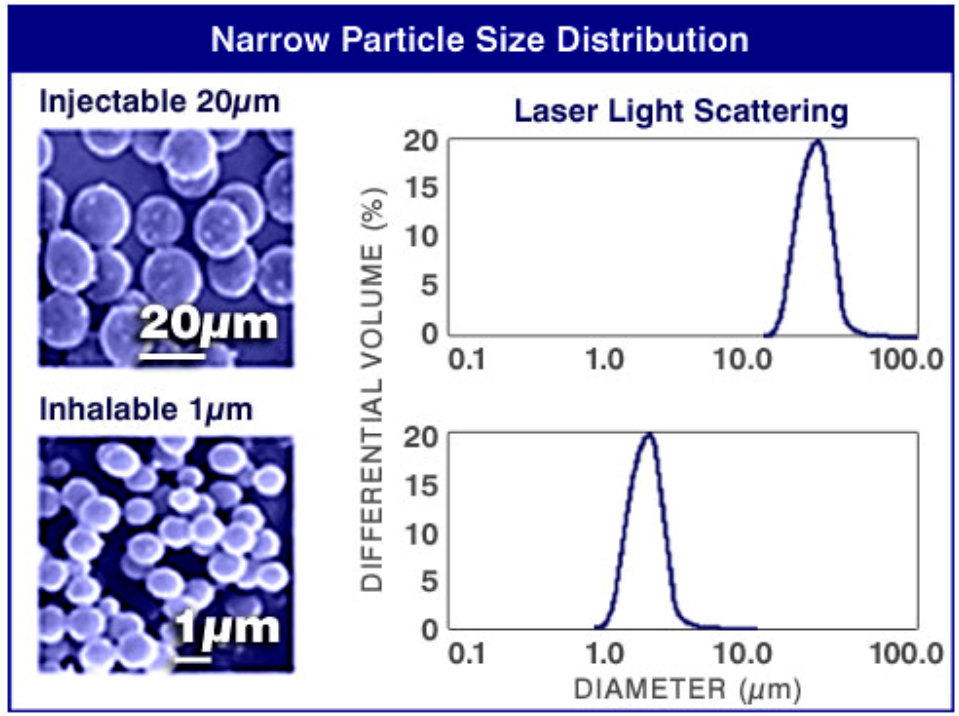
Spray-freezing into SCF produces micron or sub-micron protein particles. Particles can also be homogenized in SCF (cryo-milled) to further reduce particle size.

Williams, *et al.* (2004), Leach, *et al.* (2005) and www.alkermes.com

Polymer Nanoparticles

Baxter's PROMAXX microspheres have decent size control for API delivery.

- Sustained or immediate release
- Sized for delivery
  - Deep lung
- Fabrication
  - Aqueous
  - Polyethylene glycol
  - Insulin crystals
  - Lower T
- Stable (dry)

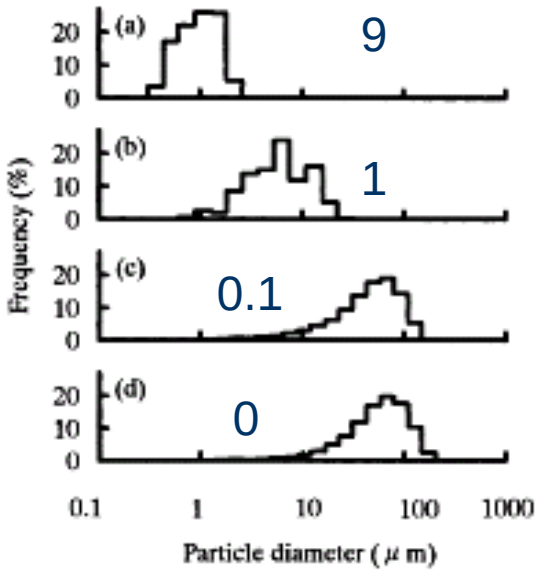


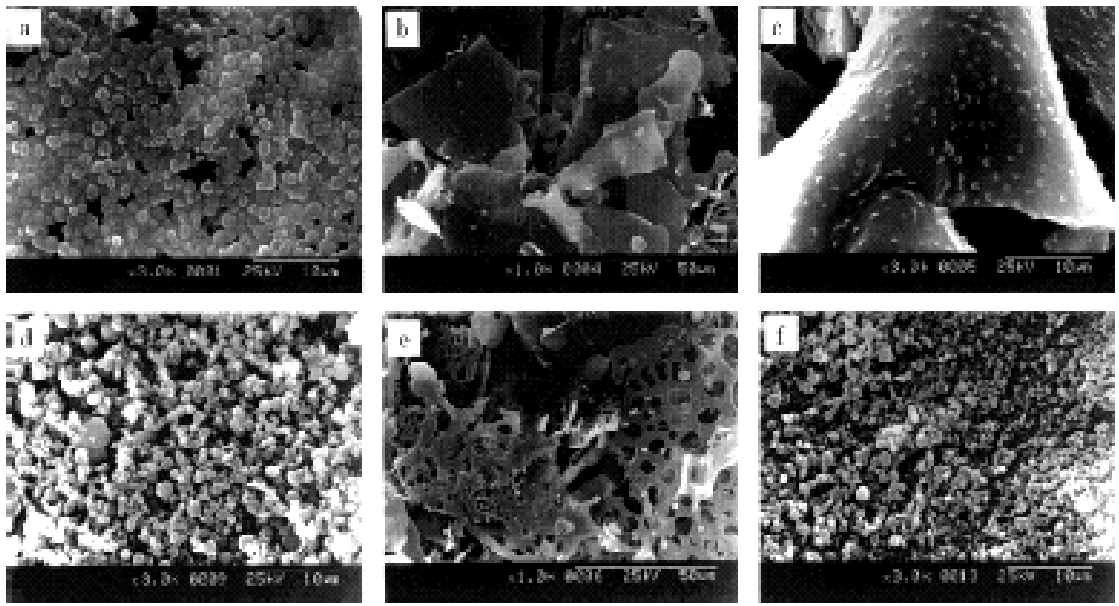
www.baxter.com

Pure protein particles produced by freeze-drying with PEG, removing PEG.

- Freeze-drying albumin with PEG → particles.

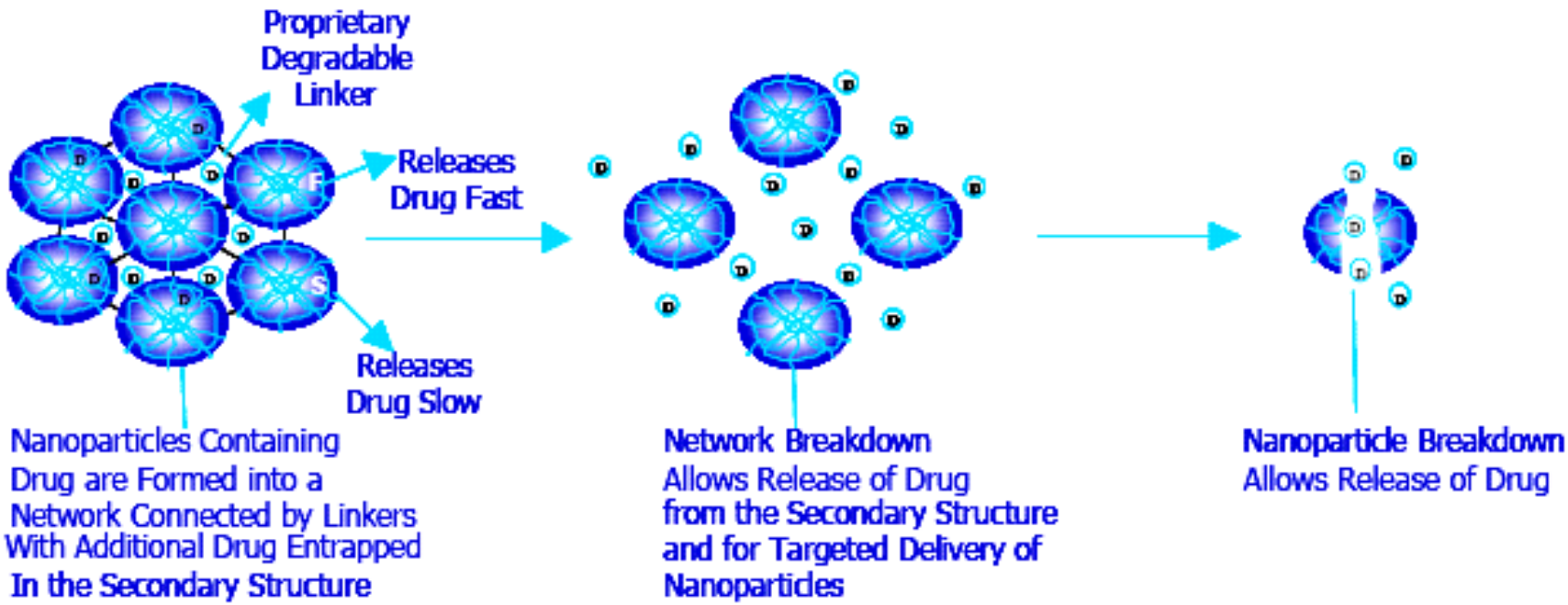
PEG:Albumin 1 0.1 9





Morita, *et al.* (2000)

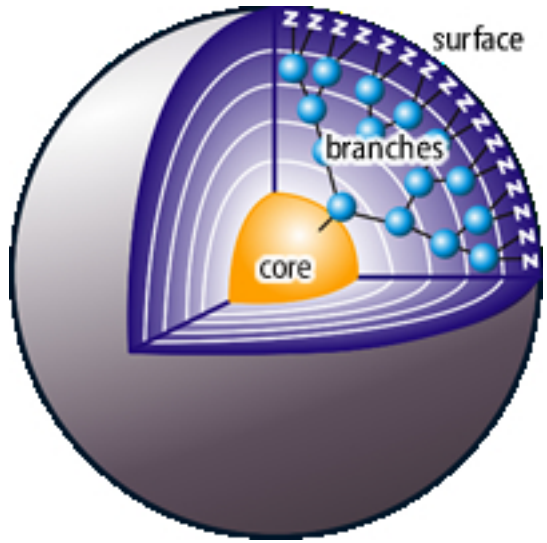
Bulk "gel" materials can be made from crosslinked nanoparticles (oral delivery).



Molecular Technology/ Polyplexes

Dendrimers are gaining interest for delivering drugs and sensing.

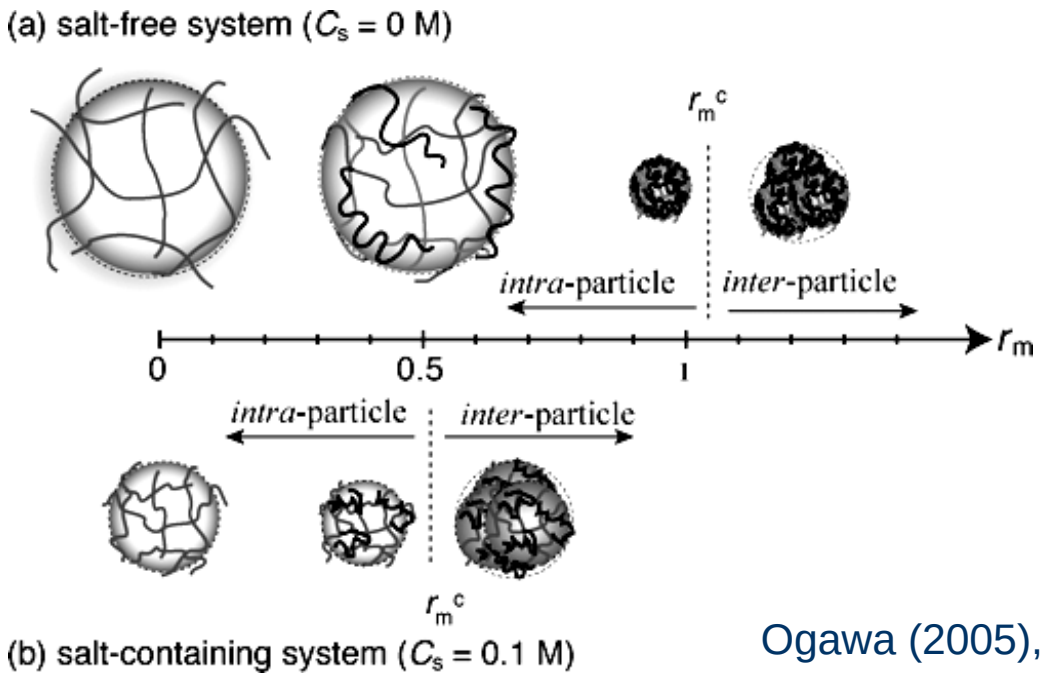
- Easy formulation?
- Size control (Mw)
- Complex or intercolate drugs
  - Low drug loading
- Marketed products



Product	Purpose	Company
VivaGel™	Prevention of HIV	Starpharma
Stratus® CS	Cardiac marker diagnostic	Dade Behring
SuperFect®	Gene transfection	Qiagen
Alert Ticket™	Anthrax detection	U.S. Army Research Laboratory

Polyelectrolyte complexes can be formulated to contain API.

- Long used for gene delivery, PEI-DNA
- Recently, for enhanced transport across BBB



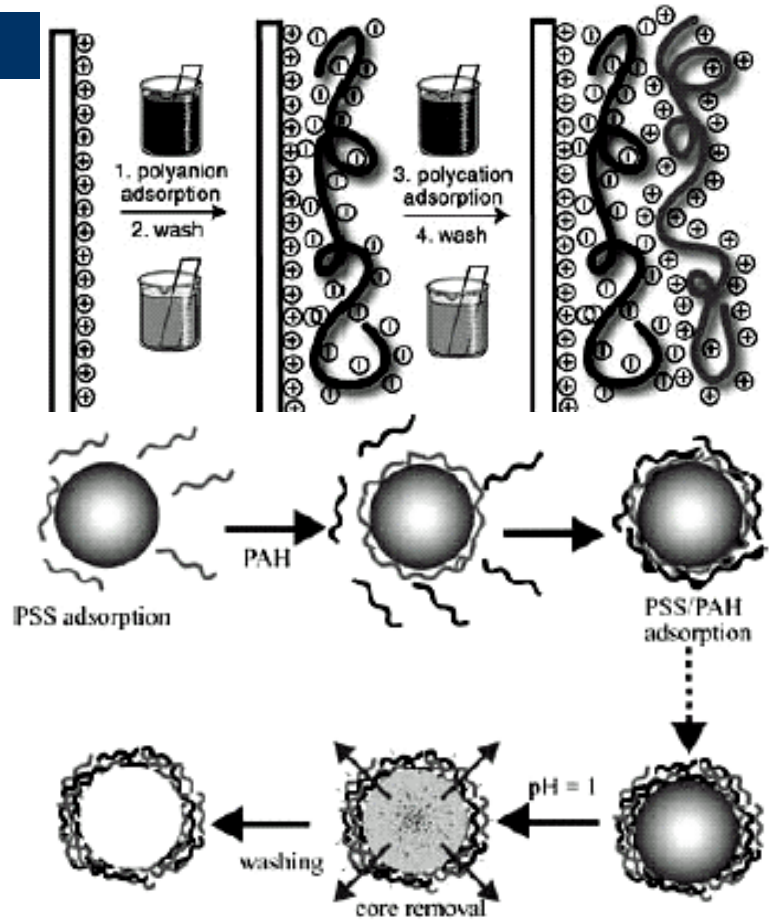
Drug is usually mixed with polymer 1 (binding) and polymer 2 (opposite charge) is dripped in with mixing to form nanogels via ionic complexation.

Ogawa (2005), Li (2004) and Vinogradov (2004)

Capsulution

LBL-Technology® can form capsules by using uniform particle templates.

- Ordered shells
- Dense shells
- 4-24+ layers (8-50 nm)
- API encapsulation
  - Entrapped in layer
  - Partitioned into core
- Surface active

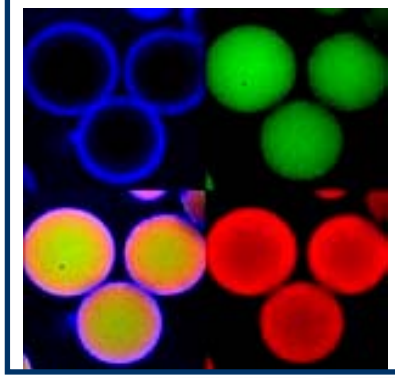


Ger. Offen. (2004) and Peyratout (2004)

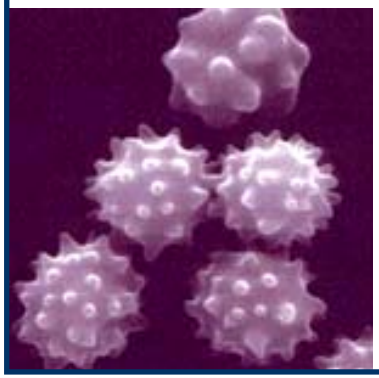
Capsulution

LBL controls drug locale, particle morphology or coating of drug crystals.

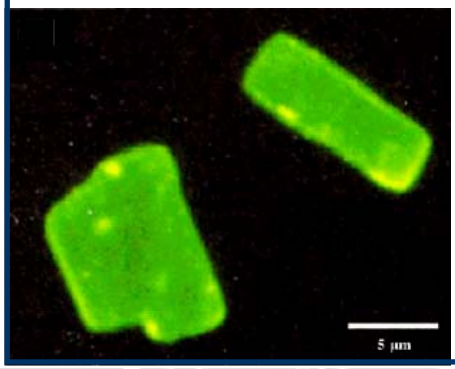
Drug localized to specific shell layer or core.

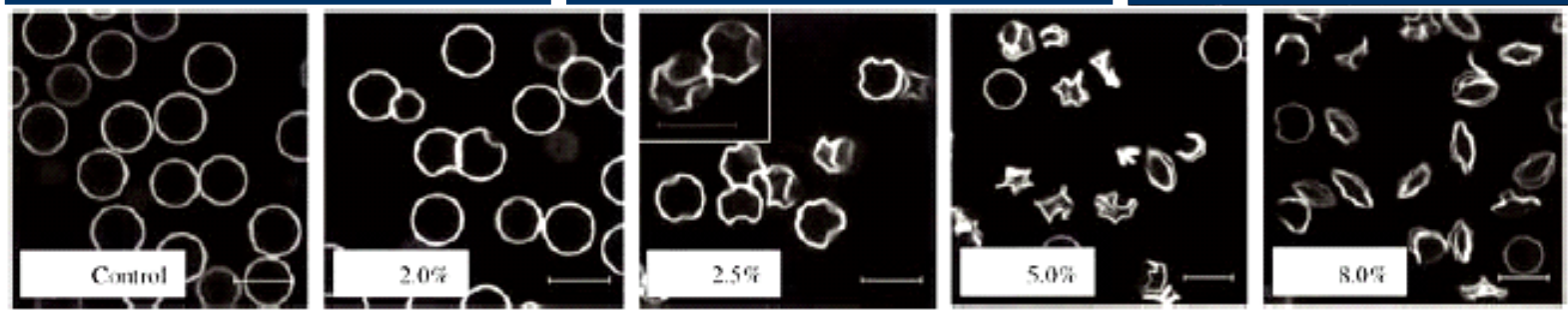


Morphology control may reduce aggregation



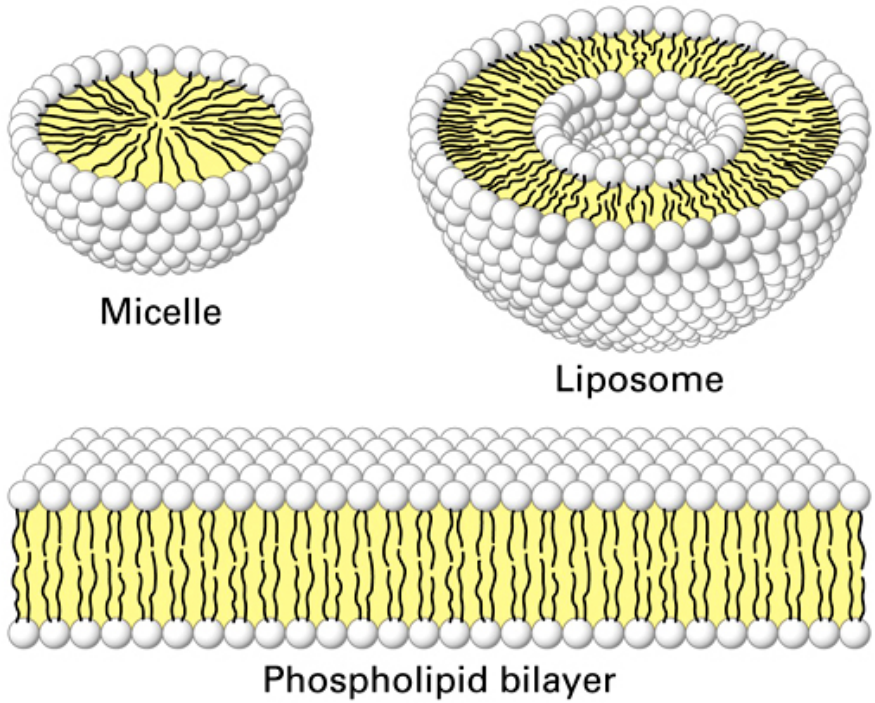
API crystals can be selectively coated



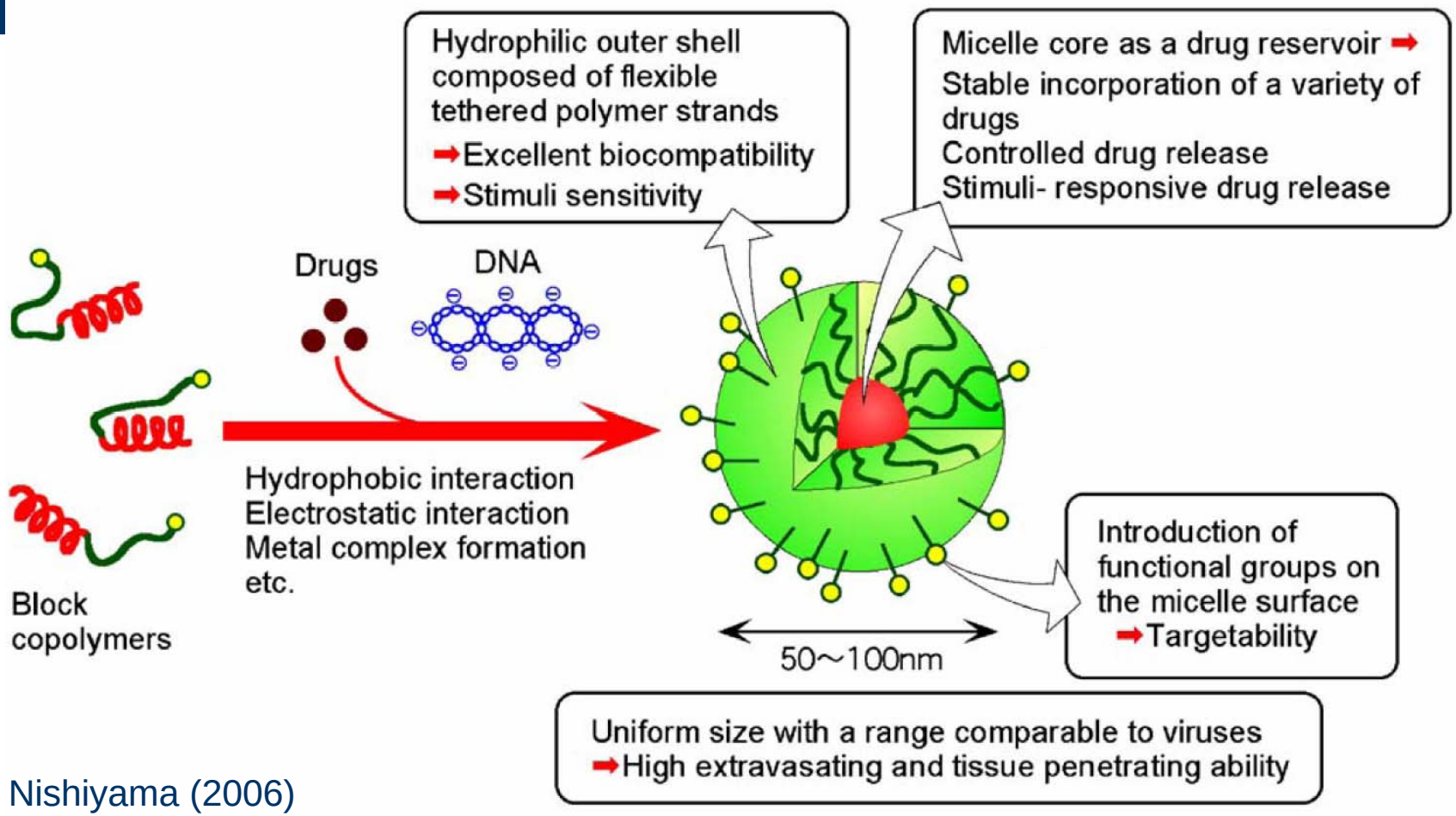


Ger. Offen. (2004) and Peyratout (2004)

Block copolymers - micelles

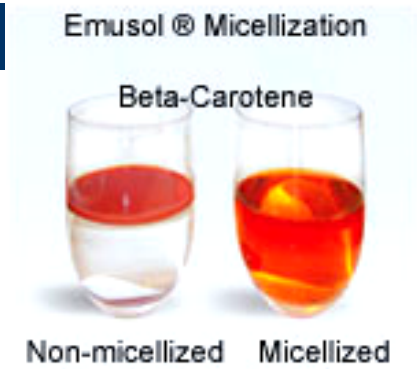


Block copolymers self assemble in solution to form micelles.

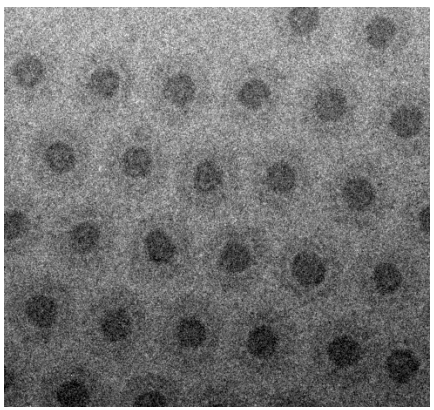


Nishiyama (2006)

Block copolymers entrap poorly soluble drugs in the core of micelles.



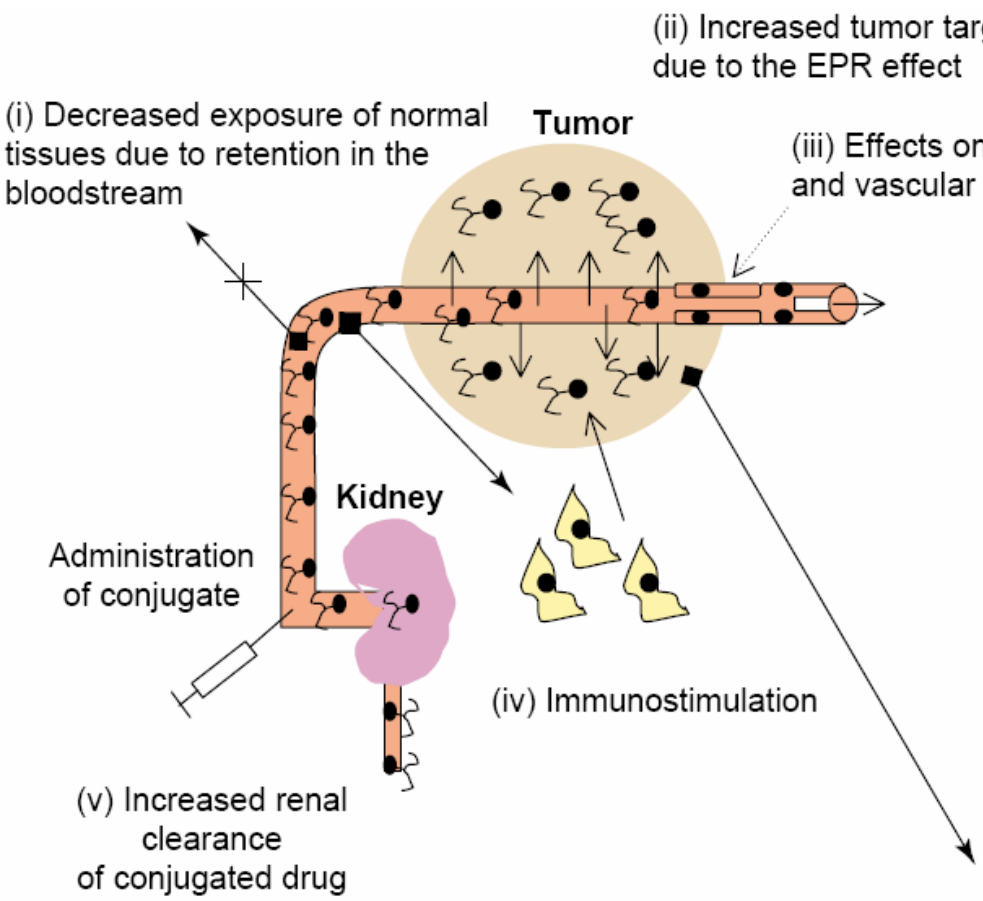
Micelle Labs, Inc.

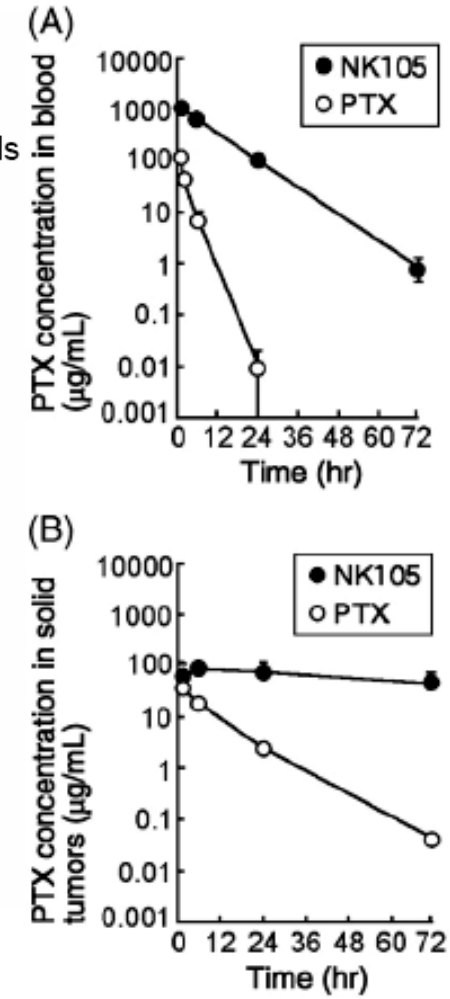


~40 nm diameter

- Improved solubility of poorly soluble drug
- Amprenavair – HIV protease inhibitor
- Formulated with Vitamin E TGPS to improve pharmacological properties (solubility, permeability, etc.

Micelles accumulate in tumors through "enhanced permeability and retention."





Vicent (2004), Nishiyama (2006)

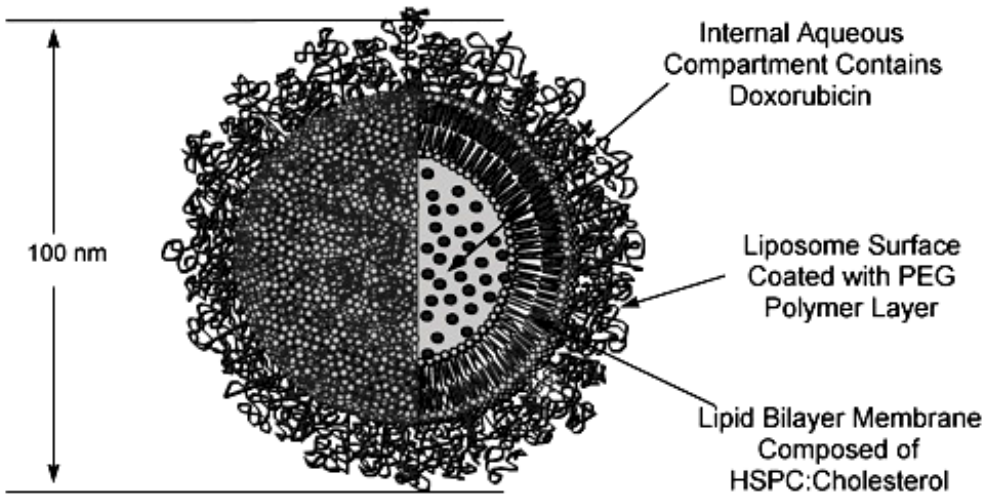
Liposomes/ Polymersomes

Liposomes like the STEALTH® liposome may target delivery.

- PEG coating
  - Reduces MPS uptake
  - Increase residence
  - Plasma stability
- Lipid shell/water core
  - Decent drug load
  - Low permeability
- ~100 nm
  - Increase residence
  - Extravasation
- FRAGILE!
- Been around and minimal products

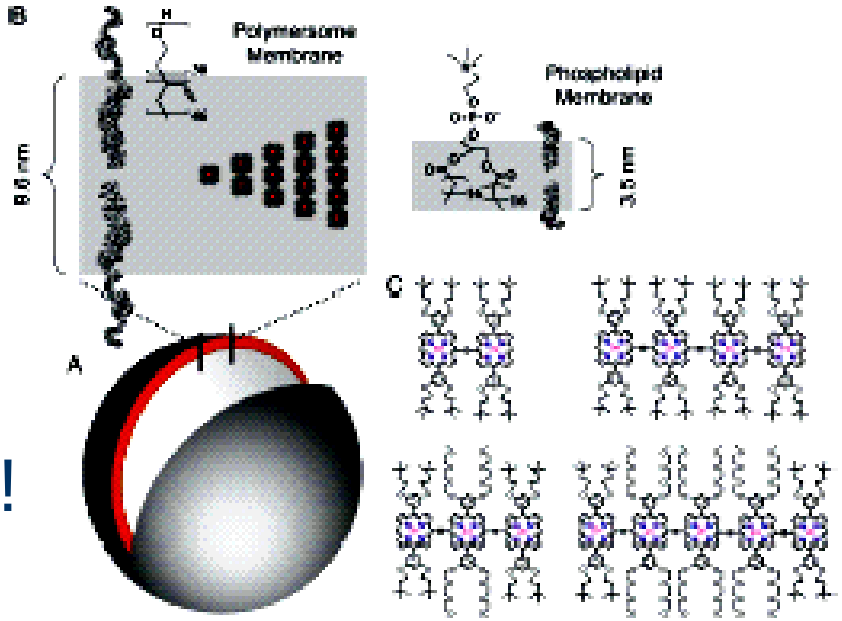
FIGURE 1

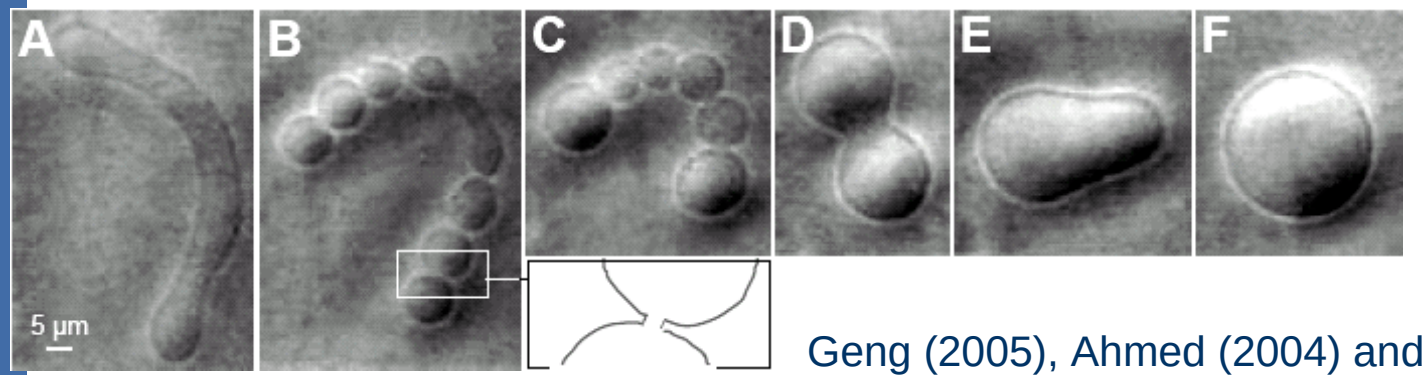
Illustration of a Doxil® liposome. A single lipid bilayer membrane composed of hydrogenated soy phosphatidyl choline (HSPC) and cholesterol separates an internal aqueous compartment from the external medium. Doxorubicin is encapsulated in the internal compartment. Polymer groups (linear 2000 dalton segments of polyethylene glycol) are grafted to the liposome surface (although not shown, the polymer also extends from the inner monolayer of the membrane).



Polymersomes offer an attractive alternative to liposomal formulations.

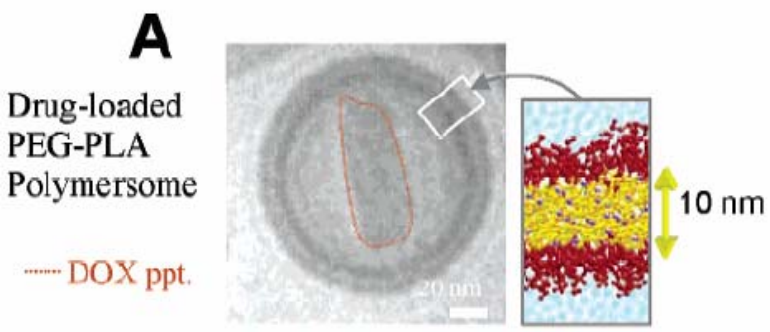
- PCL-PEG (biodeg.)
- Morphology control
- Cross-linked but flexible
- Worms circulate 1 week!



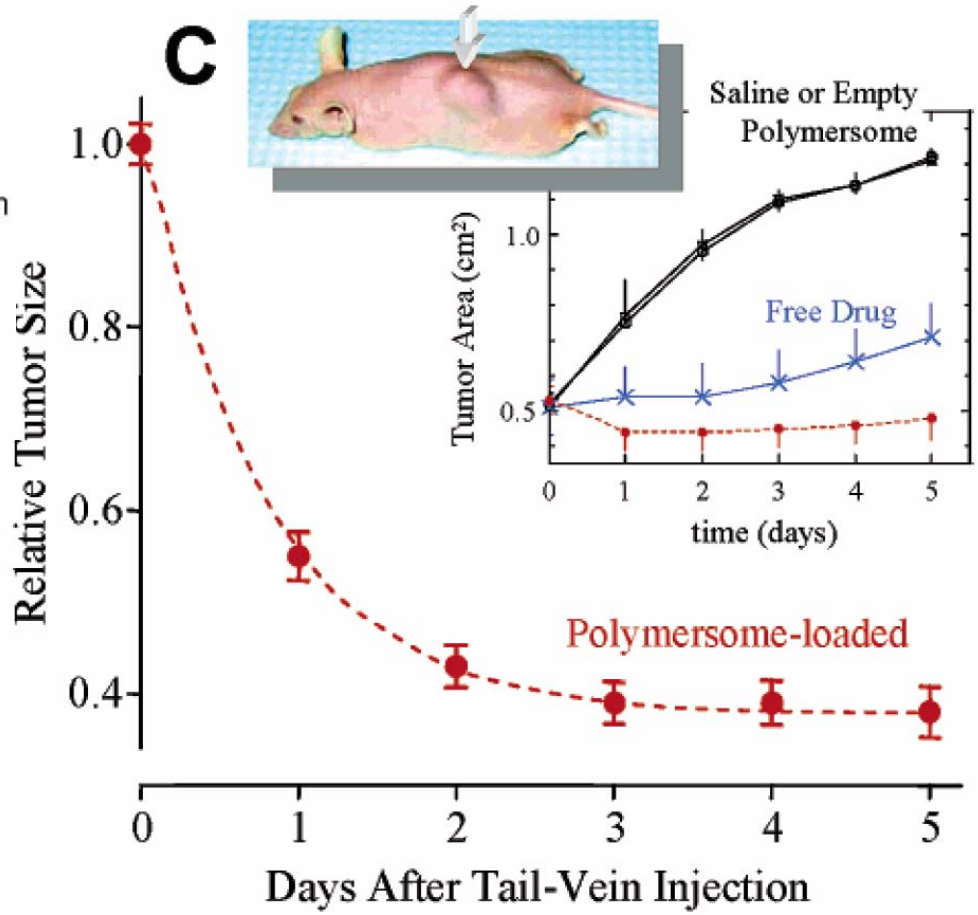


Geng (2005), Ahmed (2004) and Hammer (2001)

Combination chemotherapy reduced tumor size *in vivo*.



Polymersomes loaded with paclitaxel and doxorubicin improve the performance of these drugs by accumulating in the tumor after IV injection and controllably releasing these two drugs.



Ahmed (2006)

Polymer/Drug conjugates

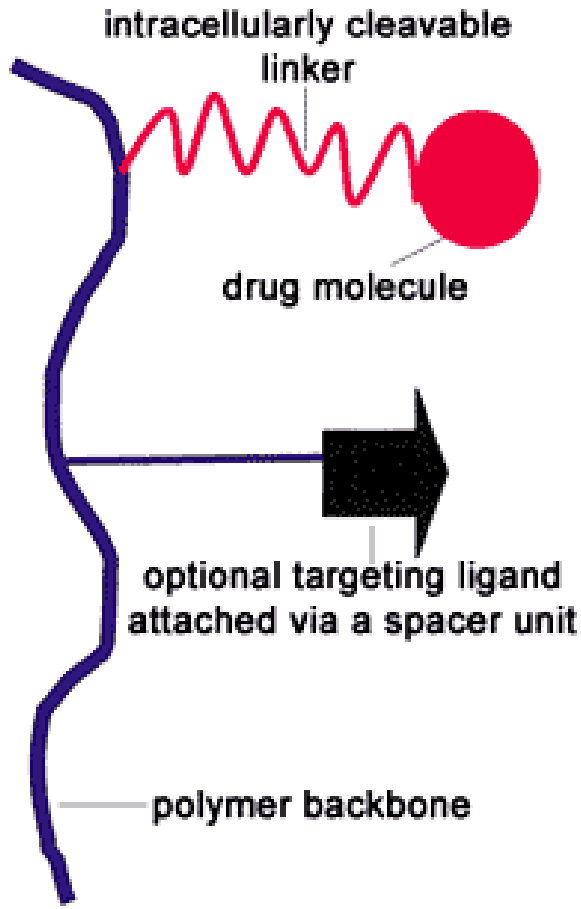


Table 2. Polymer–protein and polymer–drug conjugates

Compound	Name	Status	Comment
Polymer–protein conjugates			
SMANCS	Zinostatin Stimalmer®	Market	Hepatocellular carcinoma
PEG–L-asparaginase	Oncaspar®	Market	Acute lymphoblastic leukamia
PEG–GCSF	Neulasta™	Market	Prevention of neutropenia associated with cancer and AIDS chemotherapy
PEG–interferon α 2a	PEG-Asys®	Market	Hepatitis B and C
		Phase I/II	Melanoma, chronic myelogenous leukemia and renal cell carcinoma
PEG–interferon α 2b	PEG-Intron™	Market	Hepatitis C
		Phase I/II	Melanoma, multiple myeloma and renal cell carcinoma
PEG–arginine deiminase	ADI-PEG20	Phase I	Hepatocellular carcinoma
PEG–glutaminase combined with a glutamine antimetabolite 6-diazo-5-oxo-L-norleucine (DON)	PEG-PGA and DON	Phase I/II	Various
Polymer–drug conjugates			
Polyglutamate–paclitaxel	CT-2103; XYOTAX™	Phase II/III	Various, particularly non small cell lung cancer; ovarian cancer
Polyglutamate–camptothecin	CT-2106	Phase I	Various
HPMA copolymer–doxorubicin	PK1; FCE28068	Phase II	Various, particularly lung and breast cancer
HPMA copolymer–doxorubicin-galactosamine	PK2; FCE28069	Phase I/II	Particularly hepatocellular carcinoma
HPMA copolymer–carboplatin platinate	AP5280	Phase I/II	Various
HPMA copolymer–DACH–platinate	AP5346	Phase I/II	Various
PEG–camptothecin	PROTHECAN™	Phase II	Various

SMANCS: poly(styrene-co-maleic anhydride)-neocarzinostatin; G-CSF: granulocyte colony-stimulating factor; HPMA: N-(2-hydroxypropyl)methacrylamide; PEG: poly(ethylene glycol); DACH: diaminocyclohexane.

Vicent (2004), Nishiyama (2006)

Berkland Lab

- Dry powder aerosols
  - Cystic Fibrosis Foundation, PhRMA Foundation
- Protein stabilization in nanoparticles
  - American Heart Association
- Nanoparticle targeting
  - Juvenile Diabetes Research Foundation
- Intracellular drug delivery
  - NIH, HBC, KMCRI
- Implantable controlled release films
  - Juvenile Diabetes Research Foundation

Particle engineering is critical for pharmaceutical applications.

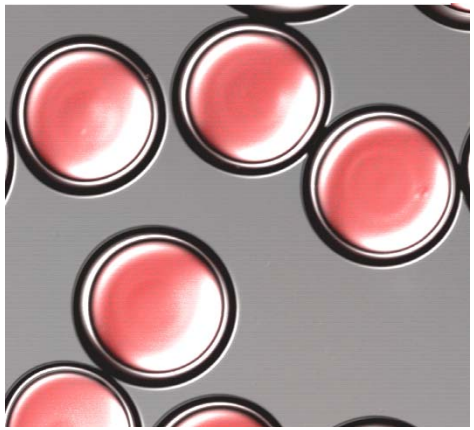
- Control particle…
  - Size and distribution
    - Dissolution rate
      - Control size
    - Pulmonary delivery
      - ~3 microns
    - Nasal delivery
      - ~5-15 microns
    - Embolism
      - ~10-20 microns
    - Avoid RES
      - >150 nm
    - Target “leaky” vessels
      - <250 nm
    - Endocytosis
      - <200 nm
  - Morphology
  - Surface roughness
    - Dispersibility
    - Flowability
  - Surface chemistry
    - Passivate
    - Activate
  - Consistency/quality control
  - Product lifecycle management

Micro- and nanoparticles possess advantages for discrete applications.

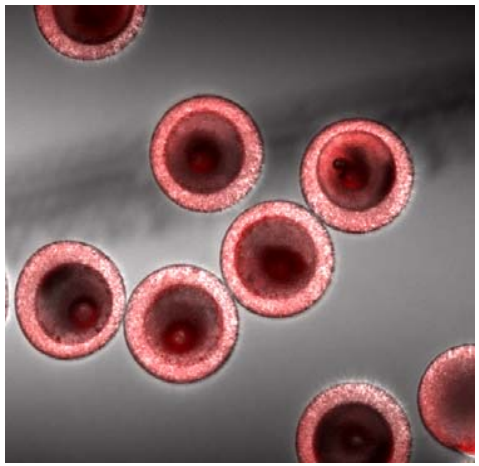
- Microparticles
  - Control release rate
    - Reservoir or matrix type devices
  - Protect drugs
    - Co-encapsulation of excipients
  - Passive localization
    - Depots - ~10-100 μm
    - Nasal - ~10 μm
    - Lung - ~2-10 μm
  - Immune response
    - Vaccine adjuvant ~1-5 μm
- Nanoparticles
  - Enhance dissolution
    - Poorly water soluble drugs
  - Extend circulation
    - >10 nm retained in blood
  - Passive targeting
    - Enhanced permeability and retention (tumors) ~100 nm
  - Enter cells
    - Intracellular drug delivery <200 nm

Exercising control over micro- or nanoparticle structure.

- Microparticles

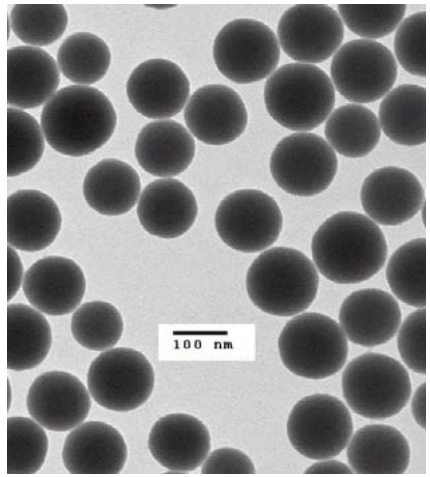


~100 µm aqueous core/ PLGA shell (rhodamine-labeled albumin)

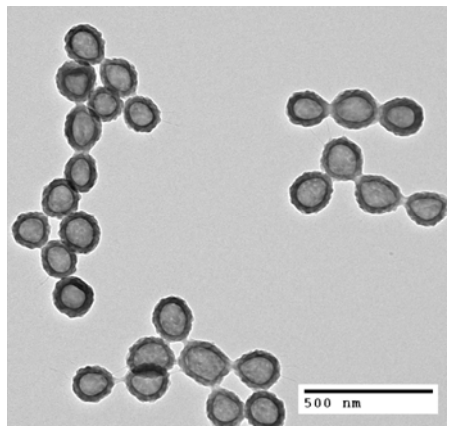


~50 µm PLGA core/ polyanhydride shell (Balaji Narasimhan)

- Nanoparticles



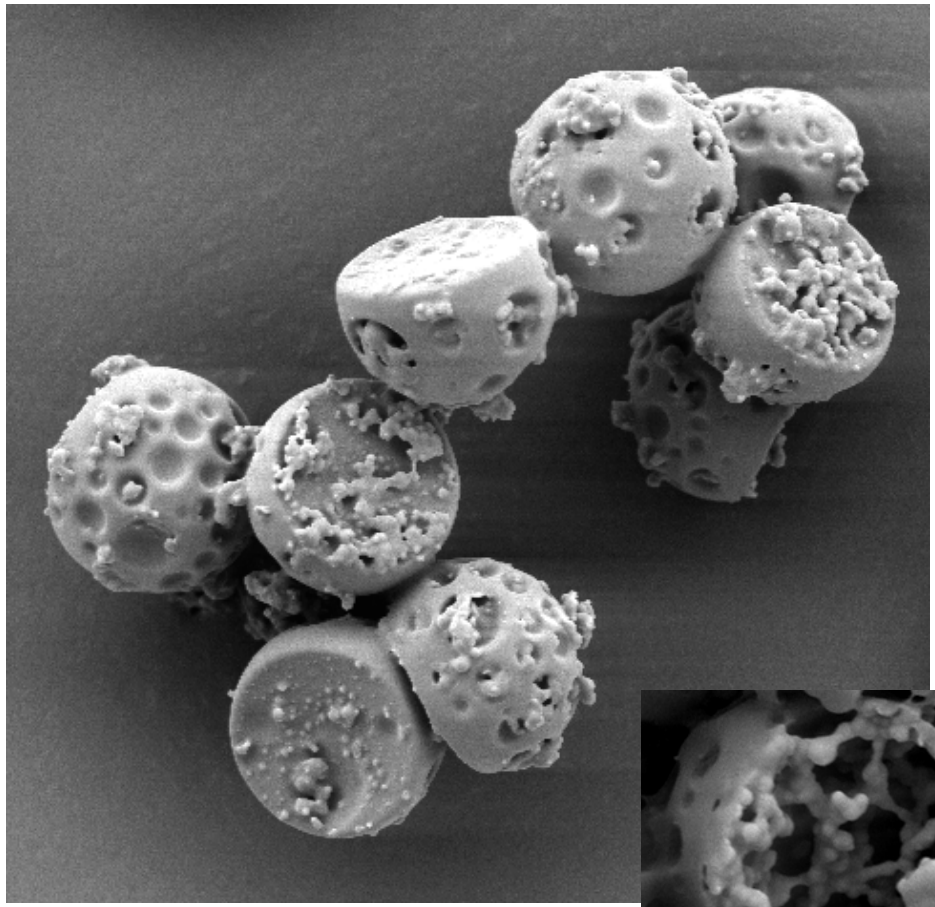
~100 nm silica nanoparticles



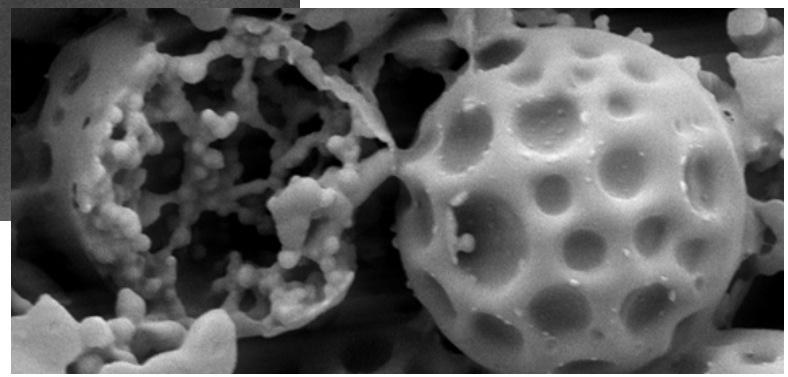
~200 nm poly(vinyl-formamide) pH-sensitive nanocapsules

Ciprofloxacin nanoparticles associated with PLGA microparticle carriers.

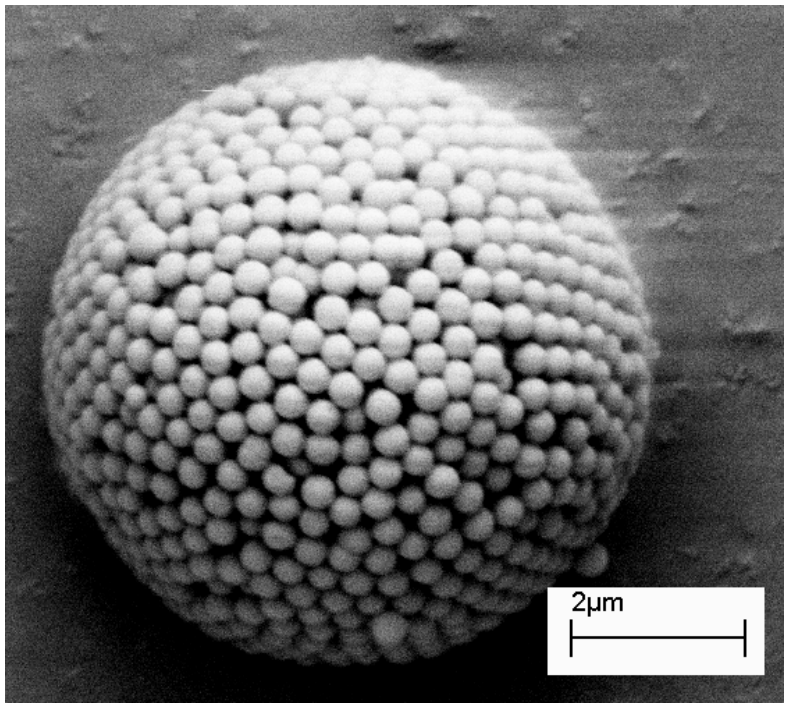
Monodisperse low-density PLGA microparticles for deep-lung delivery of poorly soluble antibiotics.



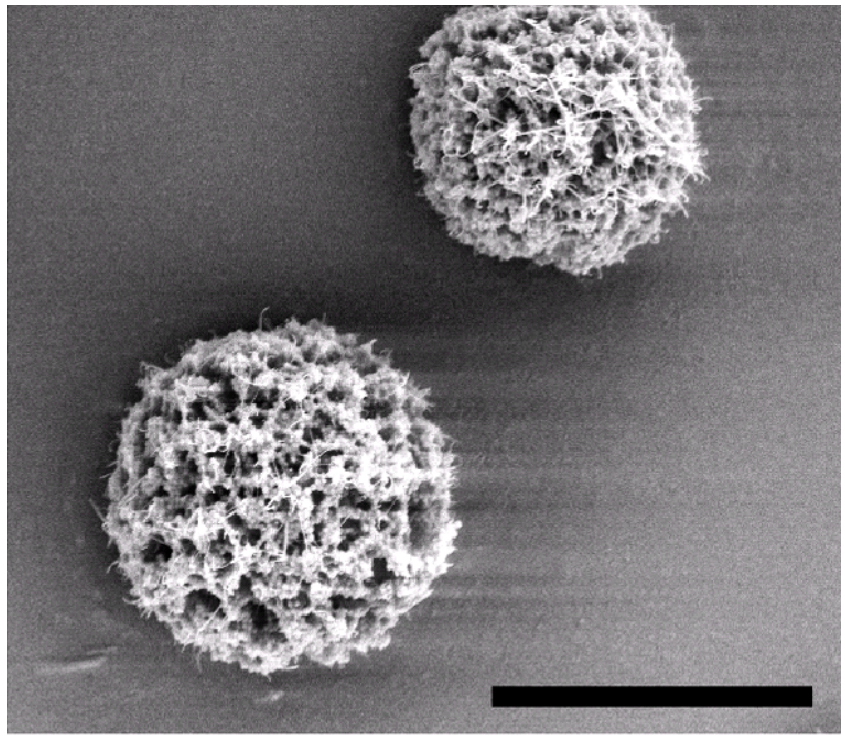
Porous and irregular structure improves aerosol performance.



Exercising control over micro- *and* nanoparticle structure.

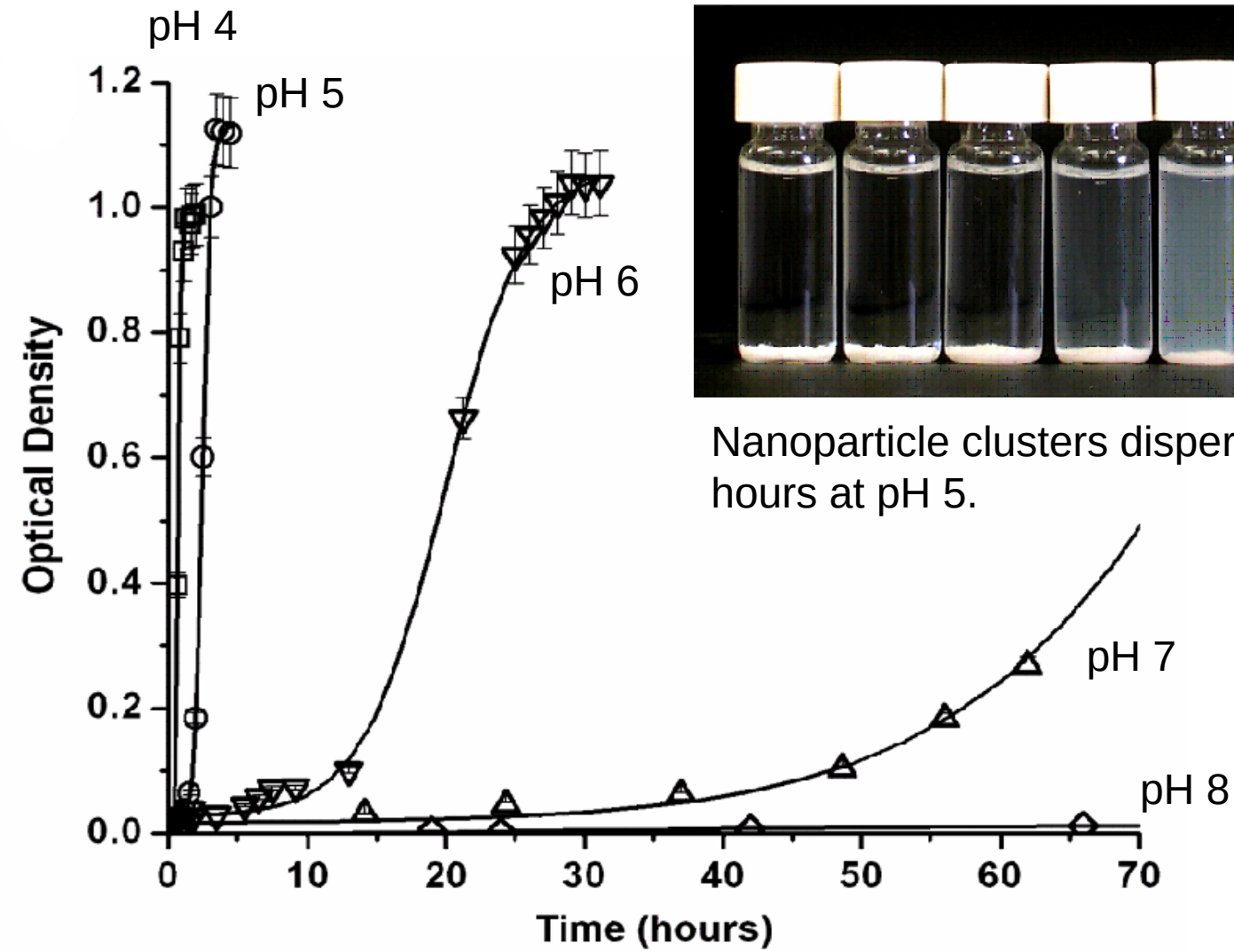


Nanoparticles as building blocks



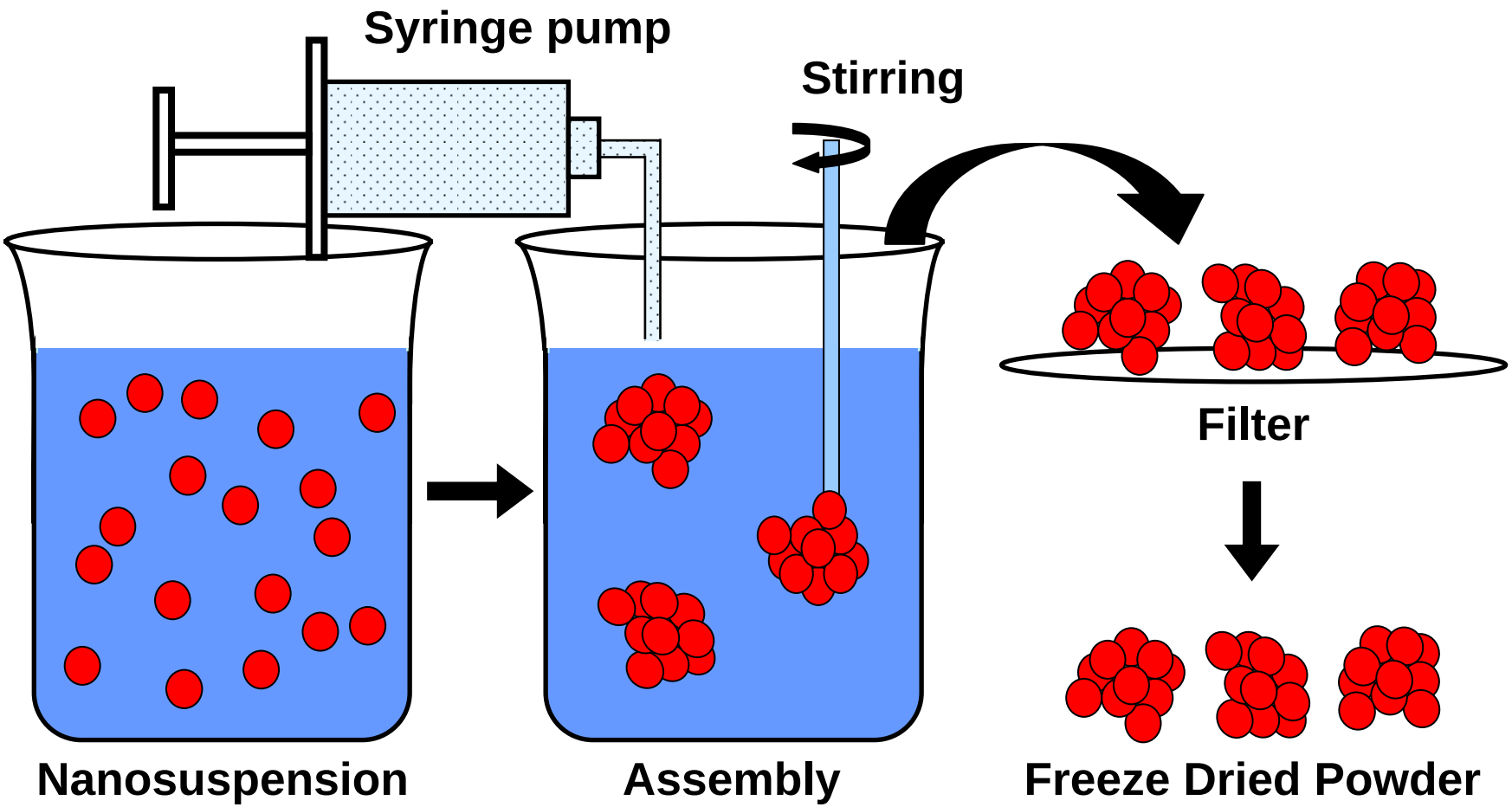
pH-sensitive nanoparticle clusters

Coating nanoparticles with PNVF allowed dispersion in response to pH.

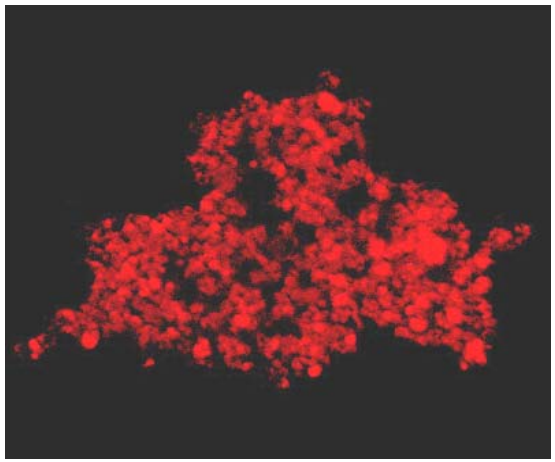


Nanoparticle clusters disperse over a few hours at pH 5.

Controlled agglomeration of nanoparticles for inhaled dry powders.

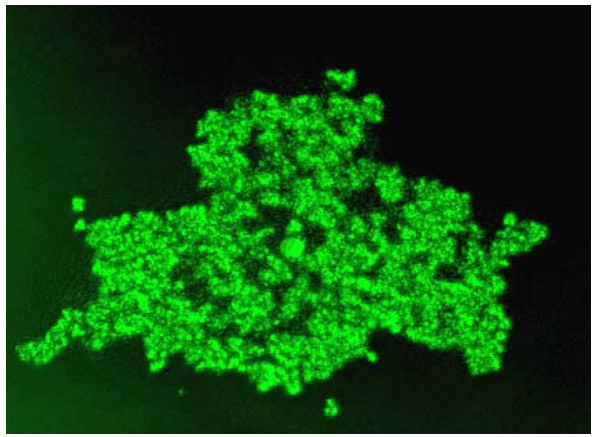


Self assembled PLGA nanoparticles form a low density cluster.

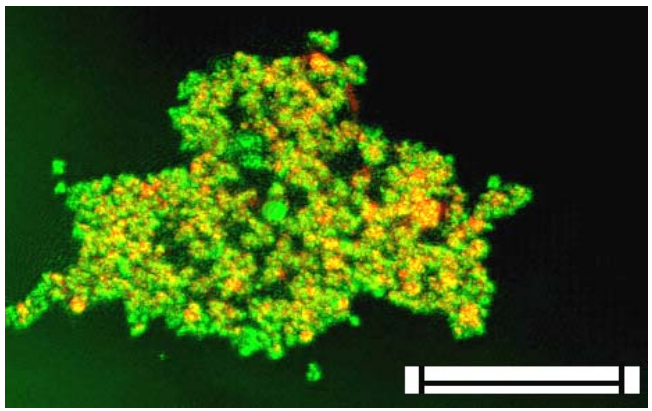


Rhodamine-labeled
(-) PLGA particles

+

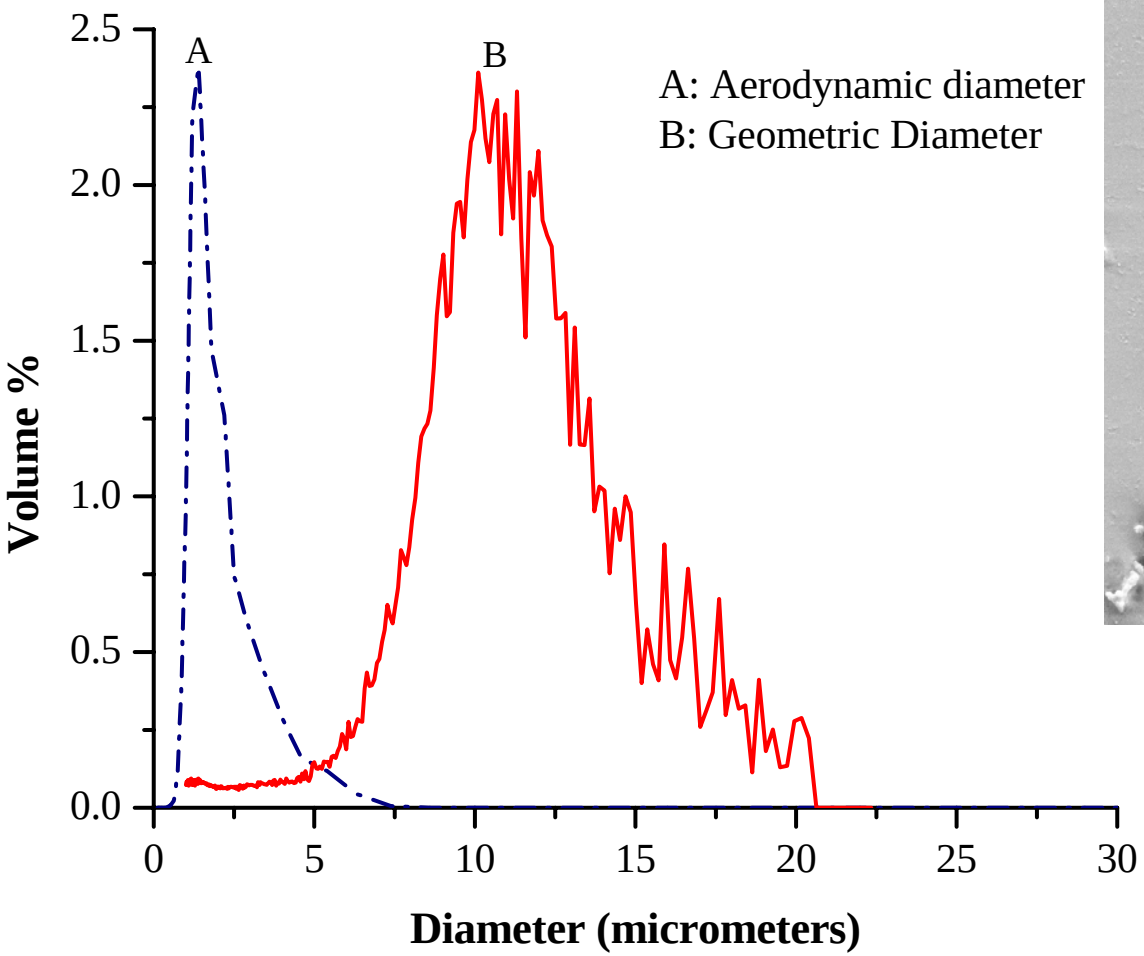


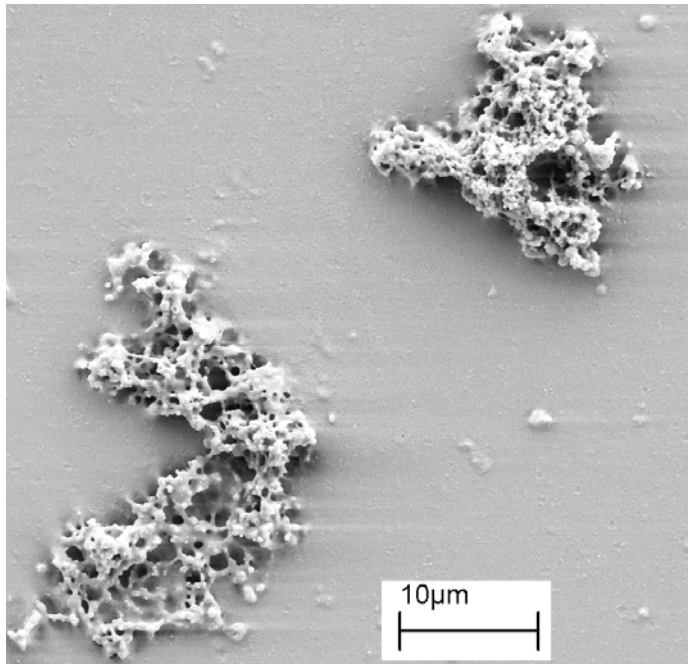
FITC-labeled
(+) PLGA particles



Scale bar = 10 μm

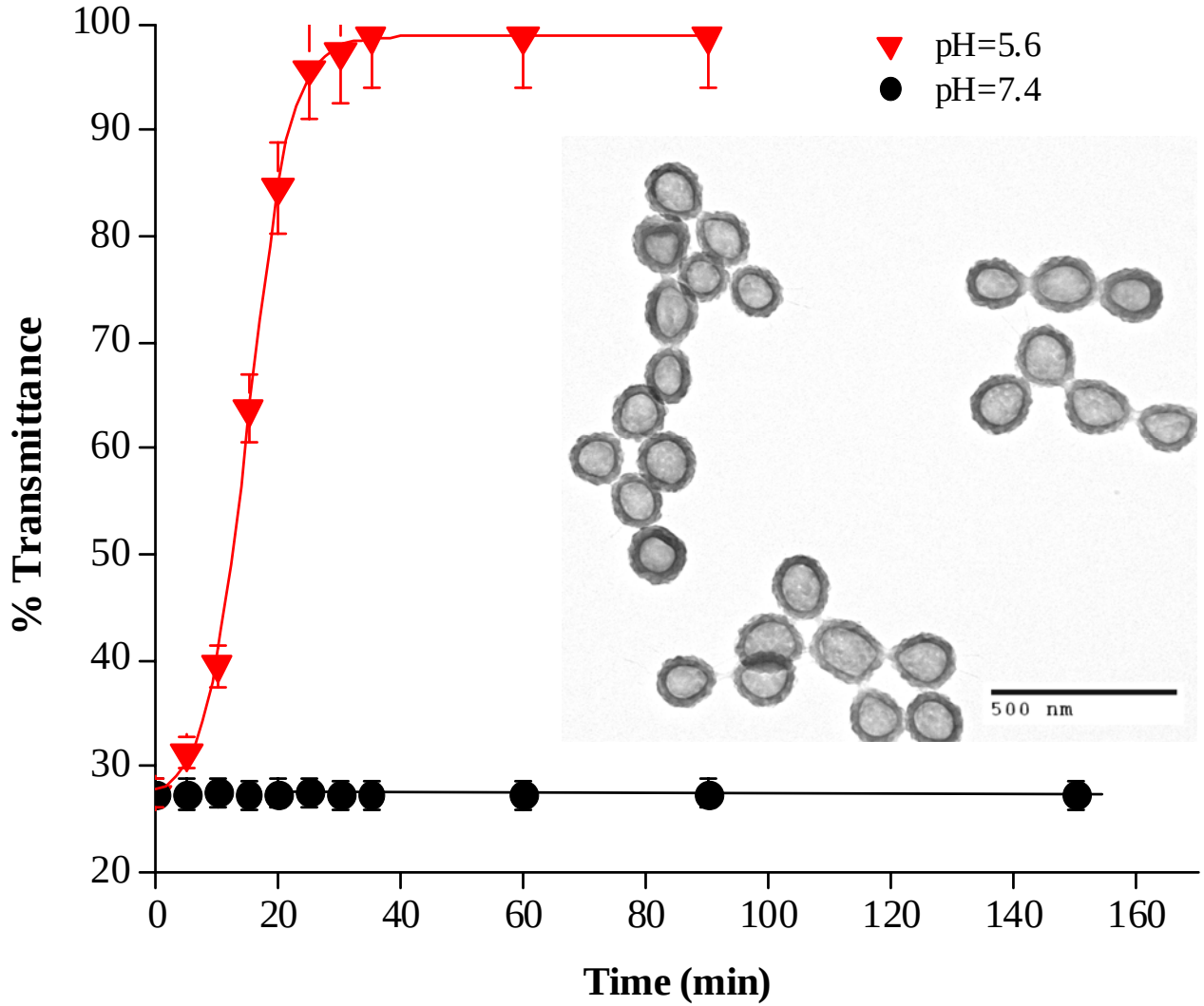
PLGA nanoparticle clusters possess attractive aerodynamic properties.





$$D_{aero} = \frac{d_n(\rho/\rho_{ref})^{0.5}}{\gamma}$$

pH sensitive nanocapsules may selectively deliver drugs intracellularly.



~200 nm poly(vinyl-formamide) pH-sensitive nanocapsules

Conclusions

Table 1. Current advanced approaches to enhancing delivery of poorly water-soluble drugs.

Advanced Approaches	Concept
Solid Dispersions	Intimate mixture of drug substance and diluent, such as polyethylene glycol or polyvinylpyrrolidone. The modified drug is often in an amorphous, more soluble state. Due to the higher energy state, there is a potential for recrystallization.
Microemulsions	Micellular dispersion of oil/solvent-dissolved drug as nanometer size droplets in water. The drug can be directly absorbed from the droplets. There are some concerns about toxicity of high surfactant and cosolvent levels and the possibility of precipitation. Administered as a liquid.
Self-Emulsifying Systems	Mixture of drugs, oils, surfactants, and cosolvents that form an emulsion upon administration. Phase inversion may further promote drug release. Can be administered as a solid dosage form.
Complexation	Formation of a reversible, noncovalent chemical complex of a drug with a "carrier" compound. Cyclodextrins are the most common complexing agents used to enhance drug absorption.
Liposomes	Encapsulation of a drug in uni- or multilayered vesicles of phospholipids. Specific sites can be targeted and certain drugs can be protected from inactivation.
Particle Size Reduction (attrition)	Increased particle surface area enhances rate of solubilization.
Wet Milling	Particle size reduction to nano-sized particles through attrition in the presence of stabilizing agents.
Homogenization	Particle size reduction by high-shear processing of an aqueous slurry of drug and stabilizing agents.
Controlled Particle Formation	Growth of drug particles with controlled morphology.
Supercritical Fluid-Based Approach	Engineered particle growth using supercritical fluid as a solvent.
Multifaceted Approaches	Engineered particle growth using a wide variety of solvents and stabilizers under several conditions, including precipitation, cryogenics, and the use of hydrophobic media.

- Particle technology is developing rapidly!
- May need to match method to particular API formulation.
- A portfolio of approaches improves chances of success (*e.g.* Dow's BioAqueous).

Conners, *et al.* (2004)

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