Nano-drug delivey systems

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Nano Drug Delivery System

- **Outline of the presentation**
  - Introduction
  - Formulation engineering
    - Case study
  - Process engineering
  - Process modelling

Introduction

Introduction: The Scale of Things

'Nano' in Greek means 'dwarf'

- **Wavelength of visible light: 300 – 700 nm**
- **1 nm = one-billionth of a meter = 1 x 10^{-9} m (1 x 10^{-7} cm)**
- **1 nm = 1/50,000th diameter of a human hair**
- **1 nm = 1/100,000th thickness of printing paper**
- **100 nm = 0.1 micron (µm)**
- **1000 nm = 1 micron (µm)**
- **1000 µm = 1 mm**

Introduction: Evolution of the Scale of Things

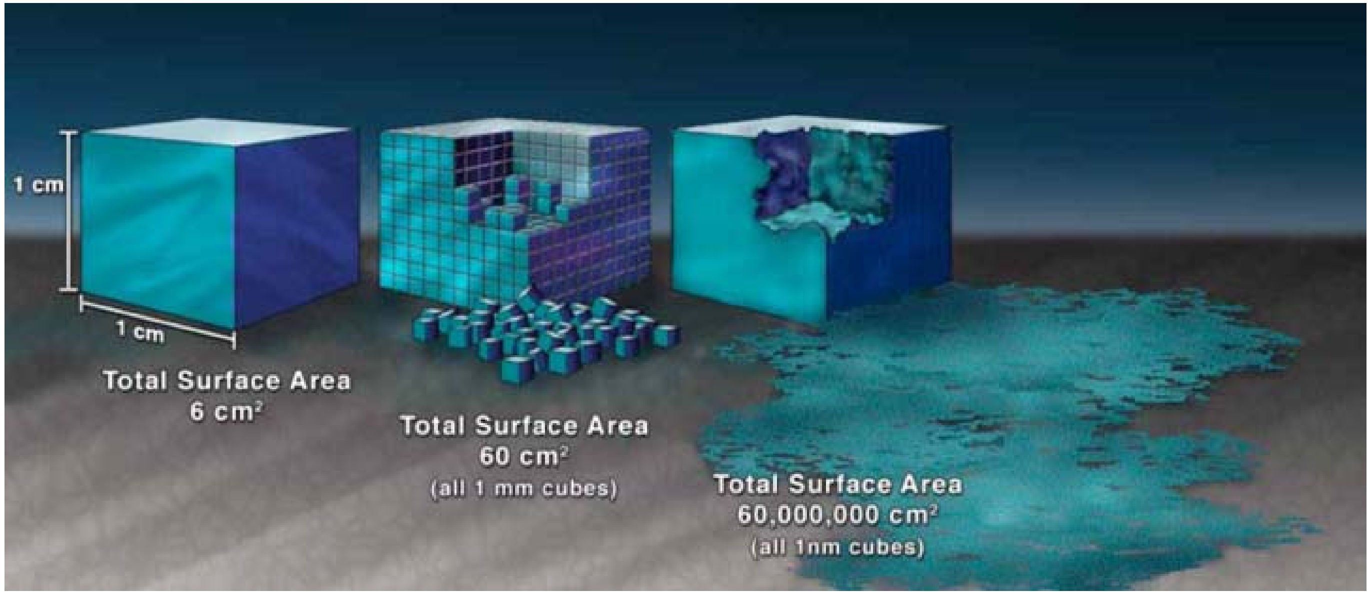
- The "Micron" world is the one that we are most familiar with
- Over the years, the trend in materials and devices has been toward smaller and smaller length scales
- Nanotechnology is a sudden transition from the micron/sub-micron regime to 1 to 100 nm scale

Introduction: Definition of Nanotechnology

- Nanotechnology refers to "understanding and controlling matter" at dimensions of roughly 1 to 100 nm
- Nanotechnology involves imaging, measuring, modeling, and manipulating matter at this length scale
- Nanotechnology encompasses all things that are synthesized by deliberately manipulating matter at the nanoscale and introducing a desired functionality
- Nanotechnology NOT to be confused with "miniaturization"

Introduction: What is Special About Nanoscale

- **High specific surface area**



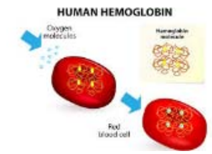
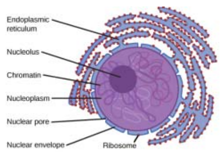
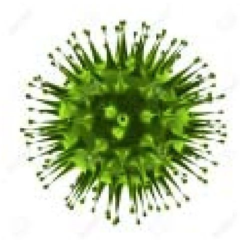
  - Improved reactivity
    - Better catalysts (catalytic converter in a car, which reduces the toxicity of the engine's fumes)
    - nanostructured membranes and materials ideal candidates for water treatment and desalination

Introduction: What is Special About Nanoscale

- **Change of materials properties Valid for particles created with dimensions of about 1–100 nanometers**
  - the materials' properties change significantly from those at larger scales
  - melting point, fluorescence, electrical conductivity, magnetic permeability, and chemical reactivity change as a function of the size of the particle.

  - Nanoscale gold particles are not the yellow color
  - nanoscale gold can appear red or purple.
    - At the nanoscale, the motion of the gold's electrons is confined
    - Because this movement is restricted, gold nanoparticles react differently with light compared to larger-scale gold particles.

Introduction: Many of the inner workings of cells naturally occur at the nanoscale.

	Object	Size (nm)
	•DNA double helix (dia.)	3
	•Hemoglobin	5.5
	•Ribosome	10
	•Virus	100

Introduction: Examples from History

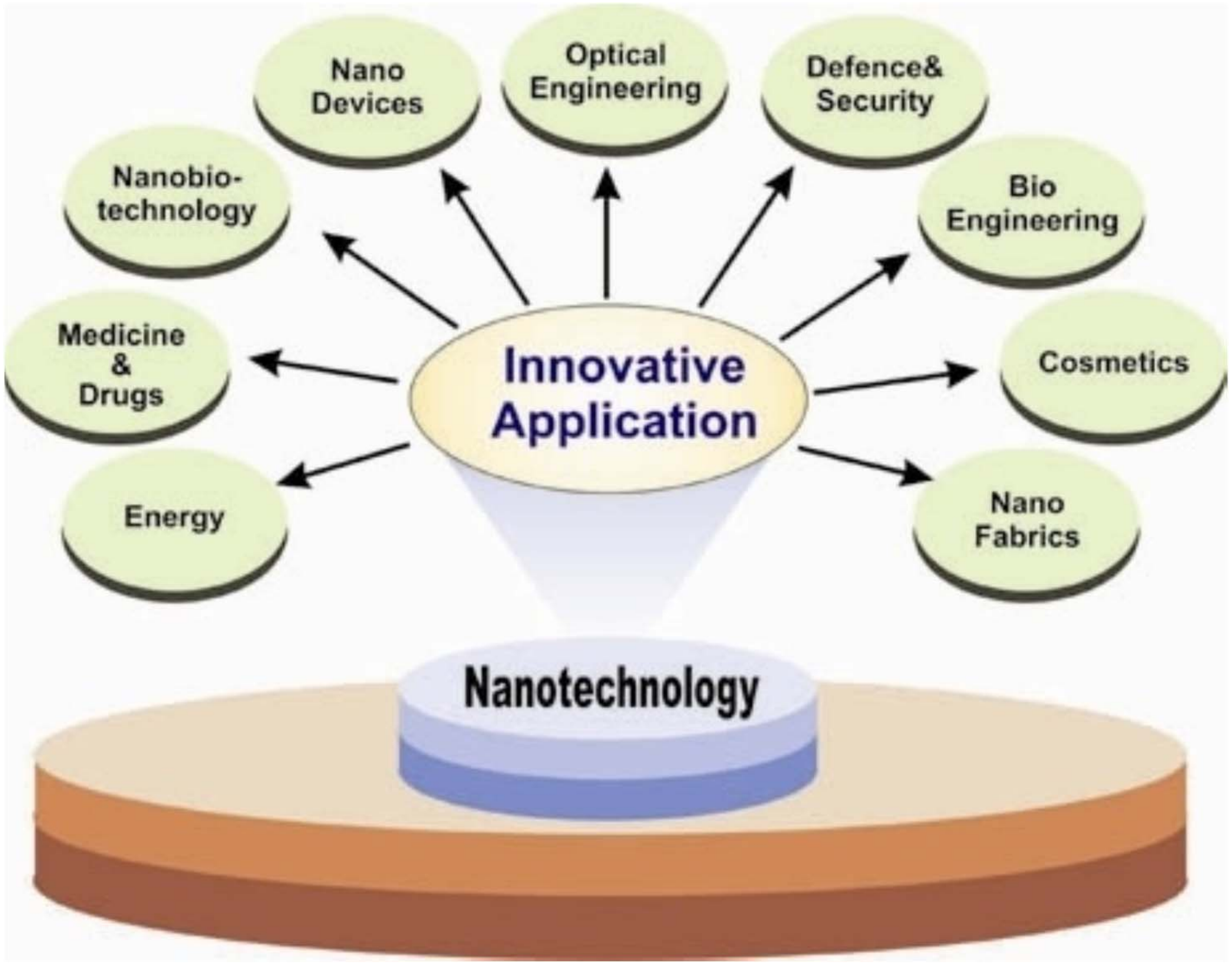
- **Nanoscale particles are not new in either nature or science**
- **Nanotechnology was used inadvertently by our ancestors**
  - **Medieval Stained Glass**
  - **Potters of Renaissance Italy (town of Deruta) in 15th and 16th centuries**
  - **The ceramic plate was shown to contain nanoparticles of Cu and Ag***

Lycurgus cup

Introduction: Materials used for the nanomaterials

- **Several materials are used for the preparation of nano-dispersions. Some examples are listed below :**
  - Drugs
  - ZnO (for sunscreen applications)
  - TiO_2 (for sunscreens)
  - SiO_2 (for coatings)
  - Al_2O_3 (for paints and coatings)
  - Metals, e.g. Ag, Au and Cu for applications in the electronic industry
  - Organic and Inorganic Pigments (for paints)
  - Magnetic Materials such as Fe_3O_4
- **Most of the above materials need to be prepared as nano-dispersions covering the size range 10 – 100 nm.**

Introduction: Nanotechnology application



Introduction: Nanotechnology application

- **Medicine and biomedical devices:**
  - diagnosis, treatment, monitoring, and control of biological systems;
    - nanoscale gold particles selectively accumulate in tumors, where they can enable both precise imaging and targeted laser destruction of the tumor by means that avoid harming healthy cells.
- **Composites materials:**
  - Manufacturing using carbon nano-tubes that present both flexibility and significant tensile strength (about 100 times stronger than steel);
- **Electronic circuits:**
  - Manufacturing by using carbon nano-tubes for cables and electronic connexion miniaturization.
  - Flat screens manufacturing (brighter and more energy effective than LCD and plasma screens) by using carbon nano-tubes;

Introduction: Nanotechnology application

- **Textile:**
  - Nano-particles (metal oxides or carbon nanotubes or clay) can be mixed with polymers before extrusion, thus introducing new material functionality (improved mechanical properties, reduced shrinkage, anti-bacterial effect, flame retardants capacity, UV stability, conductivity, wear resistance, reduced creep, etc.);
- **Energy:**
  - Batteries in which components are made of nano-particles would be longer-lasting and would have a higher energy density than those we use nowadays.
  - Nano-particles may also open the way for more practical and renewable energy. They have already demonstrated many times the ability to improve solar panel efficiency many times over.
  - Nano-particles are used as catalysts in combustion engines, they have shown properties that render the engine more efficient and therefore more economic;

Introduction: Nanotechnology application

- **Thermal:**
  - Specifically engineered particles could improve the transfer of heat from collectors of solar energy to their storage tanks. They could also enhance the cooling system currently used by transformers in these types of processes;
- **Mechanical:**
  - Nano-particles could provide improved wear and tear resistance for almost any mechanical device. They could also give to these devices previously unseen anti-corrosion abilities, as well as creating entirely new composites and structural materials that are both lighter and stronger than nowadays ones.

Introduction: Nanotechnology application

- **Optical**
  - Nano-particles could be engineered and used for anti-reflection product coatings, producing a refractive index for various surfaces, and also providing light based sensors for use e.g. in diagnosing cancer.
- **Cosmetic:**
  - The best known and most widely used nanomaterial is titanium dioxide (TiO2): ability to reflect, scatter and absorb ultra-violet (UV) and to protect against the deleterious effects caused by prolonged sun exposure

Introduction: Context of using nano drug delivery systems in pharmaceutical industry

Context of using nano drug delivery systems in pharmaceutical industry

- **Solubility issue**
  - **Leads identification has fundamentally changed in the last decades**
  - **High throughput screening has dramatically changed the physico-chemical properties of drug candidates**
    - **Compounds are becoming less water soluble**
    - **More lipophilic**
    - **Of higher molecular weight**
    - **Of higher molecular complexity**
  - **Consequences**
    - **Poor bioavailability for orally administered drug**
    - **Food effect**
    - **High in vivo variability**
    - **Formulation and application limitation for parenteral route (e.g dose limitation related to excipient toxicity)**

Context of using nano drug delivery systems in pharmaceutical industry

- **Injectability of high drug dose**
- **Controlled release and targeting**
  - **Prolonged systemic circulation, and avoid undesired liver deposition**
- **Protection of drugs against rapid in vivo metabolism.**
- **Reduction of toxic side effects, especially for potent chemotherapeutic drugs**

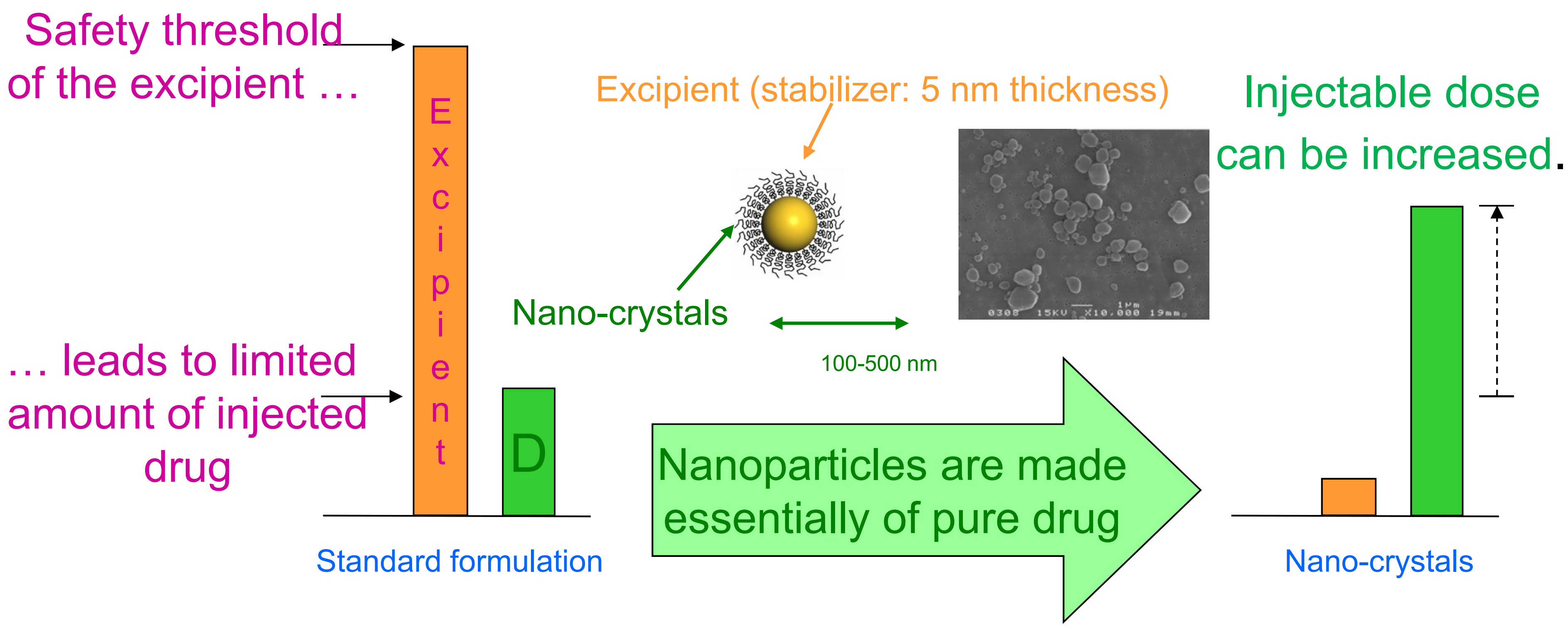
Introduction: Nanocolloidal drug delivery systems

- Conventional surfactant
  - Micelles
- Pure drugs
  - Nano-crystalline
- Lipids
  - Emulsions
  - Liposomes targeted or not
  - Solid lipid nanoparticles
- Non covalent matriced nanoparticles (NP)
  - Polymeric NP Targeted or not
  - Non polymeric matriced NP
  - Polymersomes targeted or not
- Conjugated drugs
  - Squalene conjugate
  - Polymeric conjugate targeted or not
  - Immuno-conjugate
- Inorganic materials
  - Carbon nanotubes
  - Colloidal gold nanoparticles
  - Calcium phosphate nanoparticles
  - Silica nanoparticles

Introduction: Pharmaceutical nano drug delivery systems / Biopharmaceutical added value

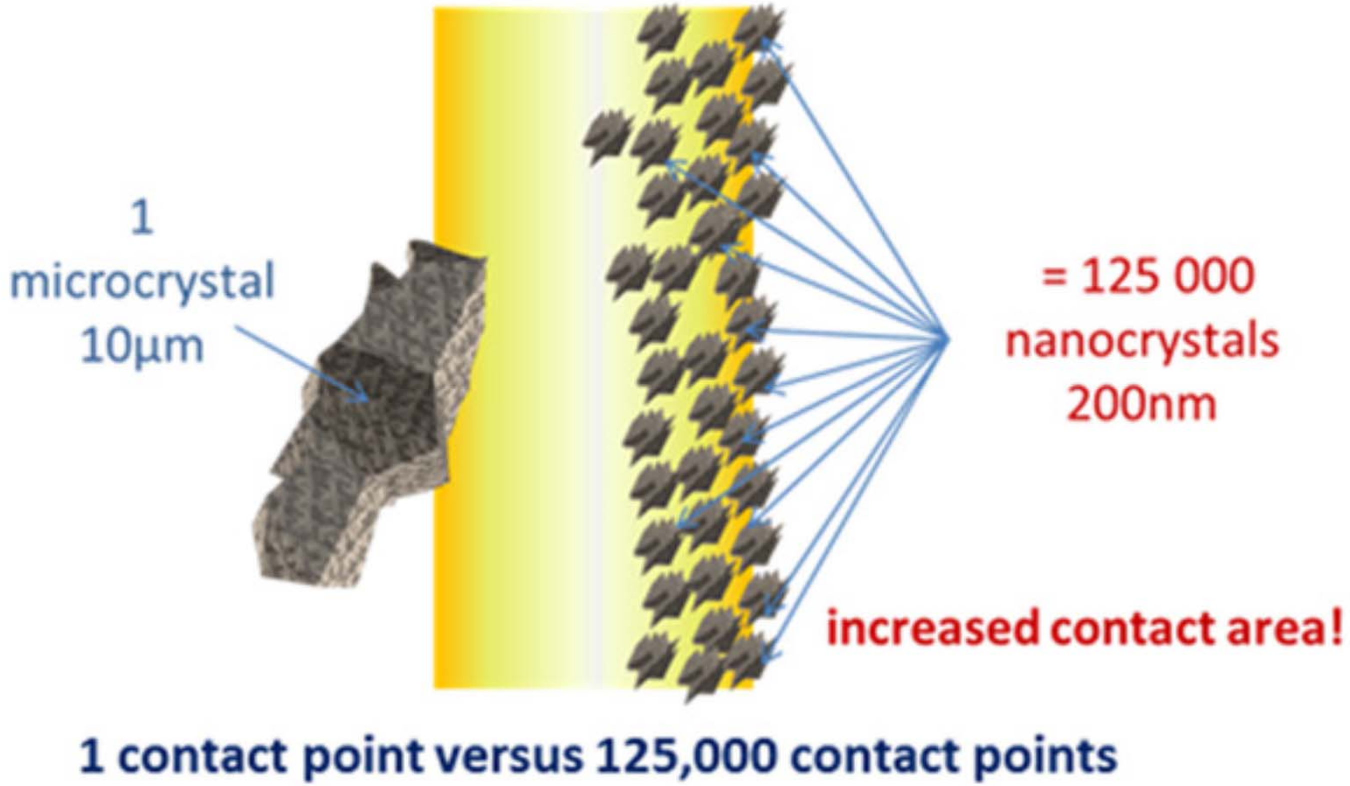
Why too small (2/8): Nanocrystalline suspension

- **Parenteral administration: Injection of high dose of insoluble drugs**



Why too small (3/8): Nanocrystalline suspension

- **increased adhesiveness to surfaces/cell membranes**



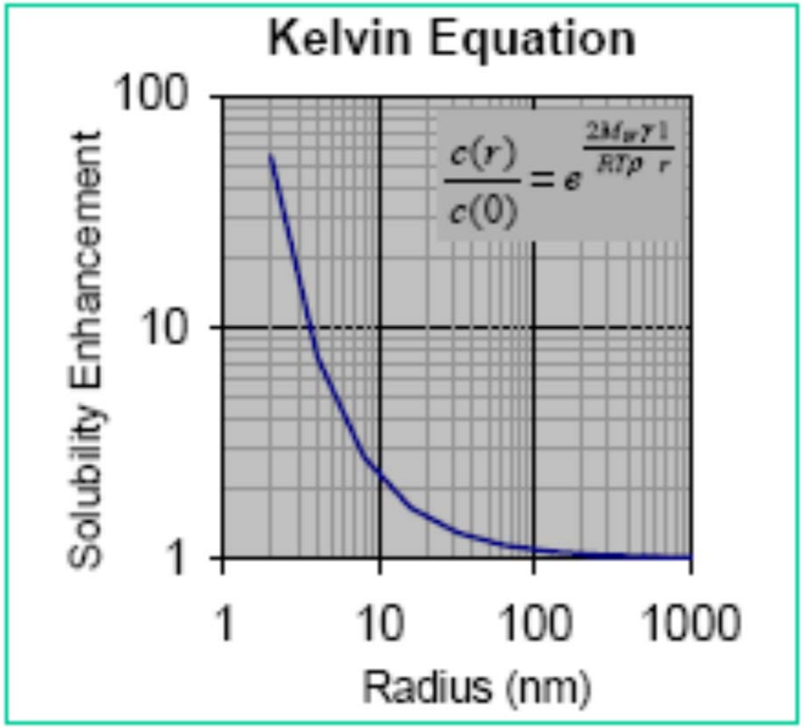
State of the art of nanocrystals – Special features, production, nanotoxicology aspects and intracellular delivery
Rainer H. Müller, Sven Gohla, Cornelia M. Keck
European Journal of Pharmaceutics and Biopharmaceutics 78 (2011) 1–9

Why too small (1/8): Nanocrystalline suspension

- **Oral administration: Increasing of dissolution rate due to an increase of the surface specific area**

$$\frac{dc}{dt} = \frac{K * SSA}{h}(C_s - C)$$

Noyes-Whitney equation



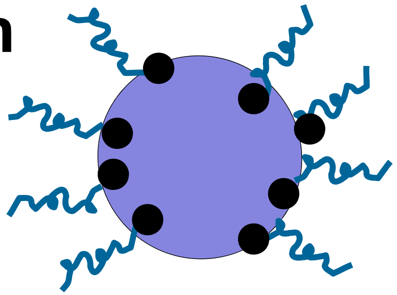
Cs : saturation solubility = f(d_{50})

when d50 is lower than 100

Why too small (4/8): Pegylated polymeric nanoparticles

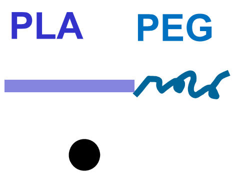
- **increase systemic circulation time and avoid accumulation of the drug in the macrophages (liver and spleen)**

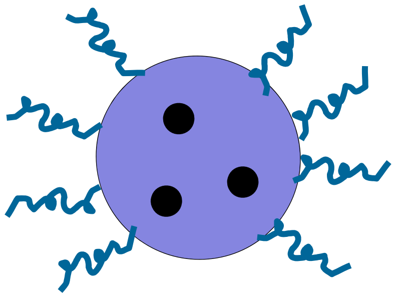
Covalent approach

- They are stealth ➔ EPR effect, passive targeting of the tumors
- They can be chemically functionnalized ➔ active targeting of organs or tumors is possible

Non Covalent approach

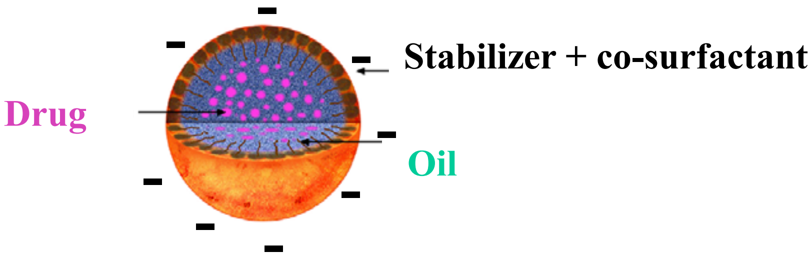




- Drug release is delayed ➔ drug is released mostly when the NPs are in the targeted organ or tumor

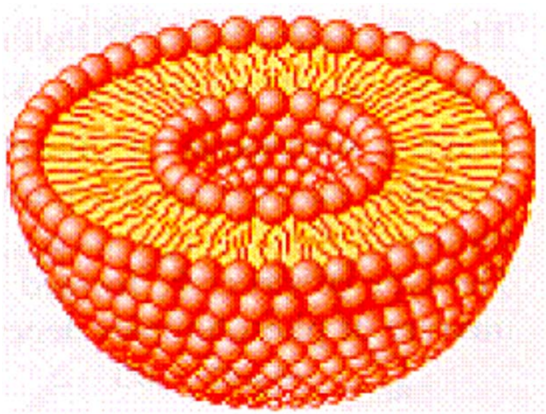
Control release

Why too small (6/8): nano-Emulsion and liposomes



Mean size : 130 – 200 nm

- Make poorly soluble molecules drugable

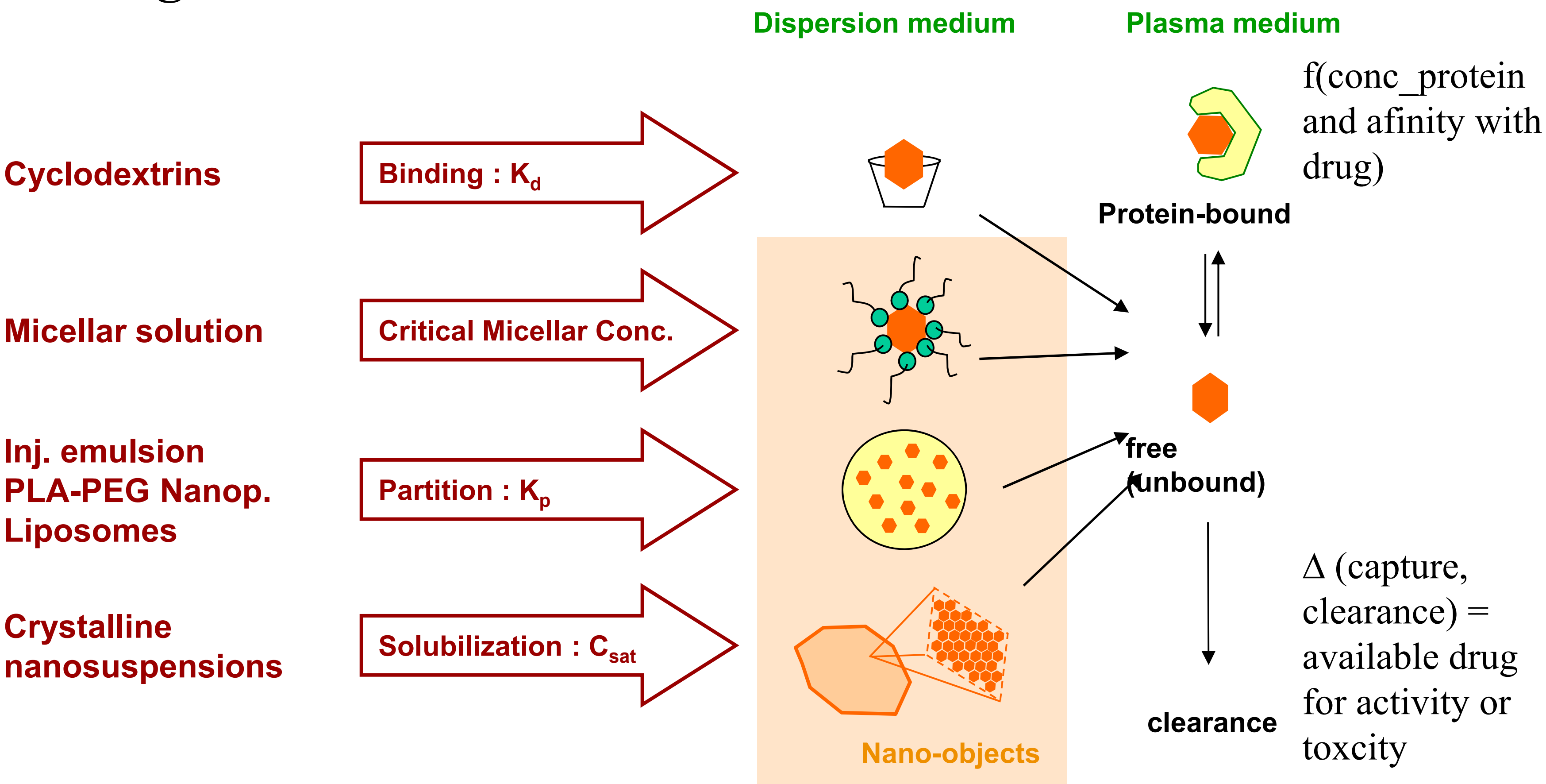


Mean size : 50 – 200 nm

- Improvement of Pharmacokinetic profile

Why small ? (7/8)

- **Quick equilibrium shift from dispersed drug to free drug.**

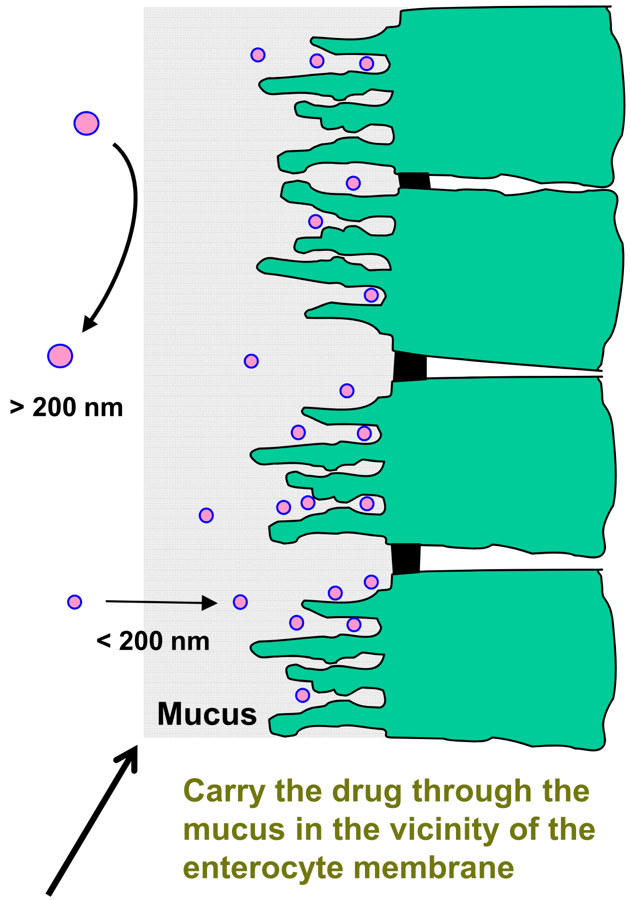


Why small ? (8/8)

Oral route

Elimination by excretion

INTESTINAL TRACT



EPITHILIAL BARRIER

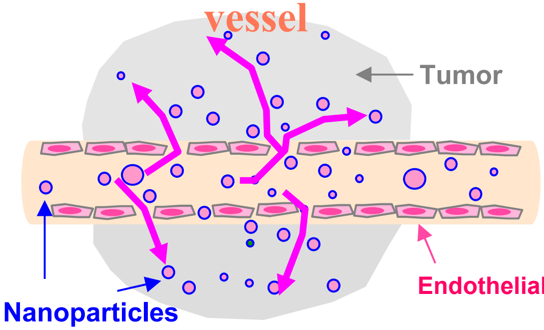
Carry the drug through the mucus in the vicinity of the enterocyte membrane

Enzymatic activity + Protein + Electrostatic charges

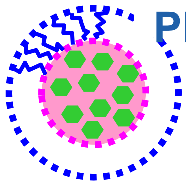
Intravenous route

Excessive vascularization + leakage through blood vessel

Tumor accumulation (EPR effect)



Passive targeting

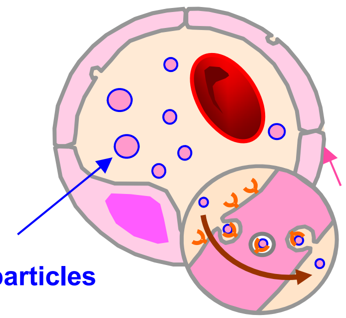


PEG

High water bound barrier

Blood Brain Barrier crossing through R/L recognition

High surface area



Active targeting

Targeting agent able to identify receptors on endoththelial cell

Nano-crystalline suspension: Biopharmaceutical advantages

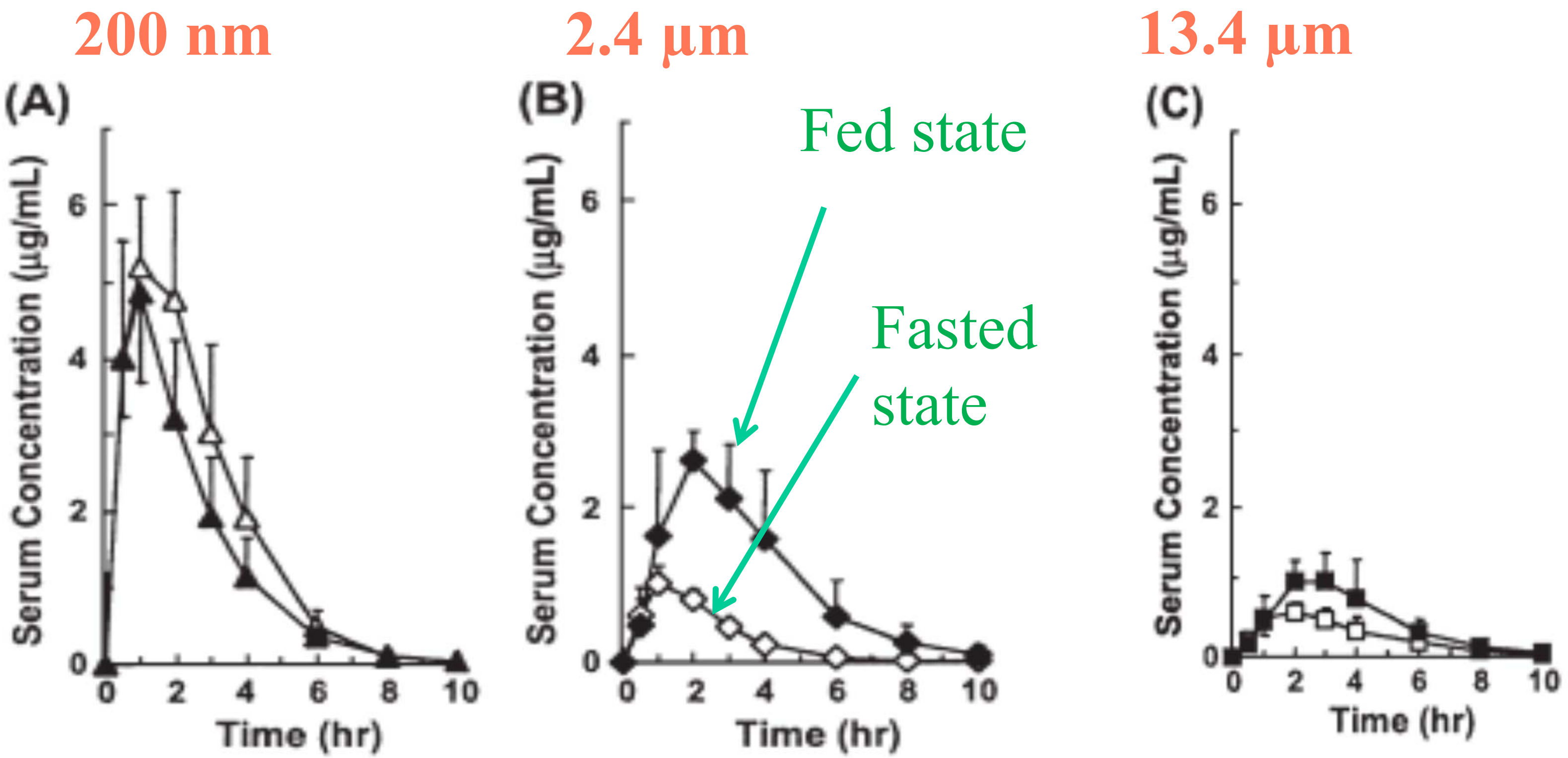
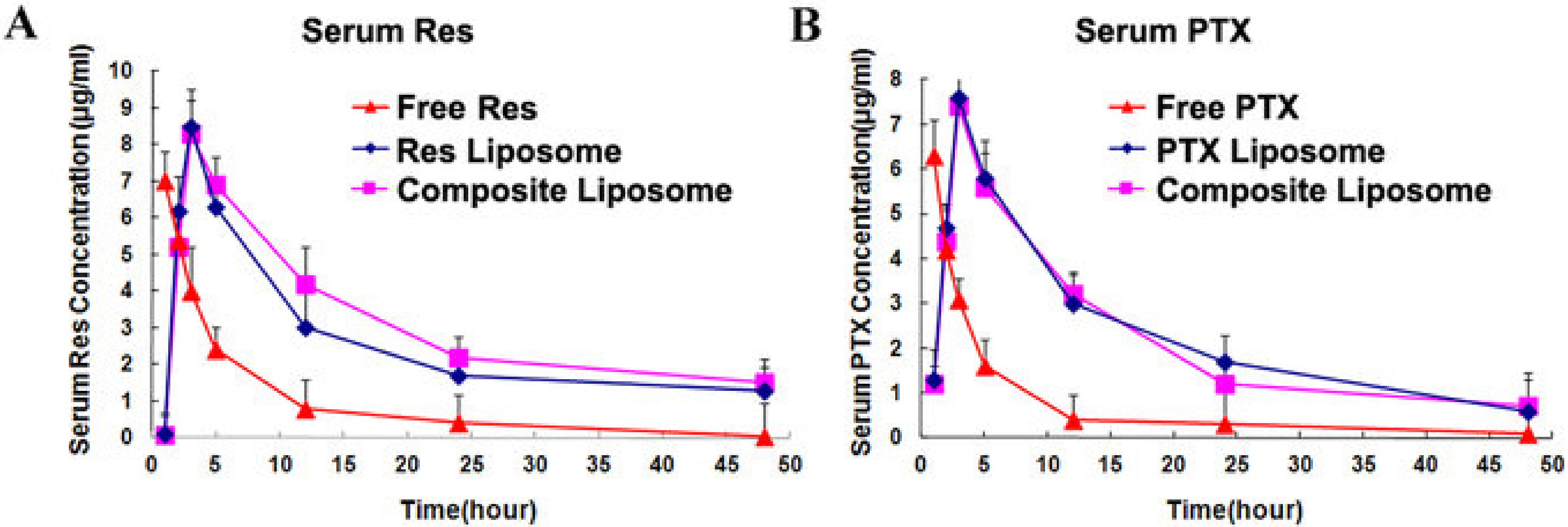


Fig. 3. Effect of food on bioavailability of nanosized and micronized API for cilostazol. (A) Nanocrystal suspension (220 nm); (B) jet-milled suspension (2.4 µm); (C) hammer-milled suspension (13.4 µm). Open symbols represent fasted state, filled symbols represent fed state (reprinted from Journal of Controlled Release, 111 (1–2), Jinno, J., et al., Effect of particle size reduction on dissolution and oral absorption of a poorly water-soluble drug, cilostazol, in beagle dogs, 56–64, Copyright (2006), with permission from Elsevier).

F. Kesisoglou et al. / Advanced Drug Delivery Reviews 59 (2007) 631–644

Liposomes: Biopharmaceutical advantages

Resveratrol and Paclitaxel in Liposomes



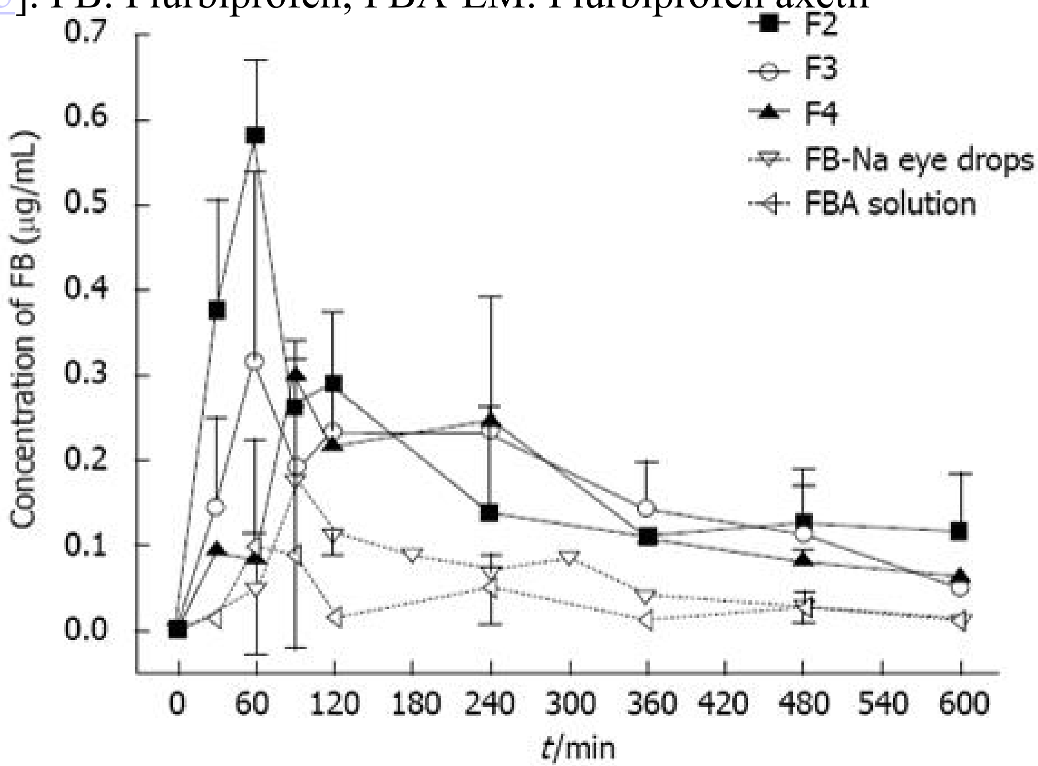
Combination Therapy using Co-encapsulated Resveratrol and Paclitaxel in Liposomes for Drug Resistance Reversal in Breast Cancer Cells*in vivo*

Jie Meng, Fangqin Guo, Haiyan Xu, Wei Liang, Chen Wang & Xian-Da Yang

Emusion: Biopharmaceutical advantages

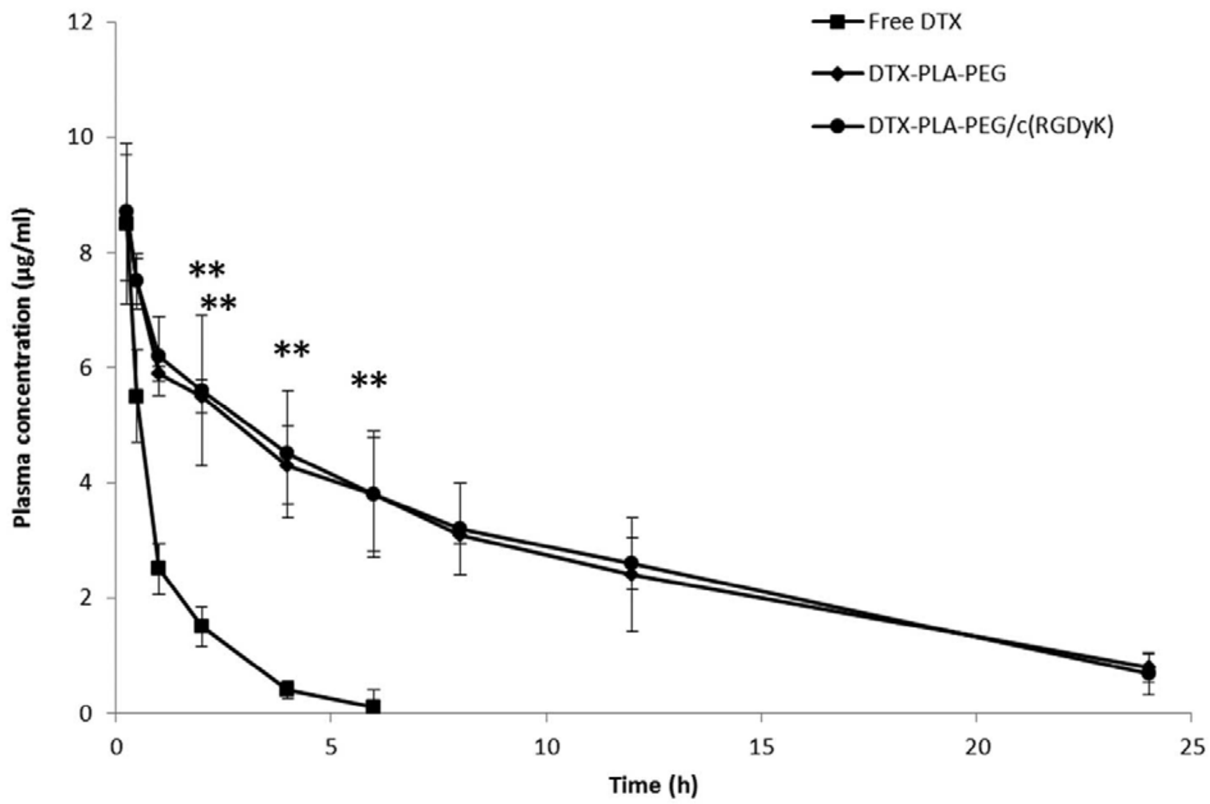
Figure 2 Concentration-time profiles of flurbiprofen (in the aqueous humor after instillation of flurbiprofen axetil emulsion F2-F4, FB-Na eye drops and flurbiprofen axetil-oil solution in rabbits. F1 = 0.1 wt% of castor oil, 0.08 wt% of tween-80; F2 = 0.5 wt% of castor oil, 0.4 wt% of tween-80; F3 = 1.0 wt% of castor oil, 0.8 wt% of tween-80; and F4 = 2.5 wt% of castor oil, 4.0 wt% of tween-80 with 2.2 wt% and 0.1 wt% of glycerol and flurbiprofen respectively. Reproduced with permission from reference Shen et al[25]. FB: Flurbiprofen; FBA-EM: Flurbiprofen axetil emulsion.

Flurbiprofen



Ocular drug delivery systems: An overview
Ashaben Patel, Kishore Cholkar, Vibhuti Agrahari, Ashim K Mitra
World J Pharmacol. Jun 9, 2013; 2(2): 47-64

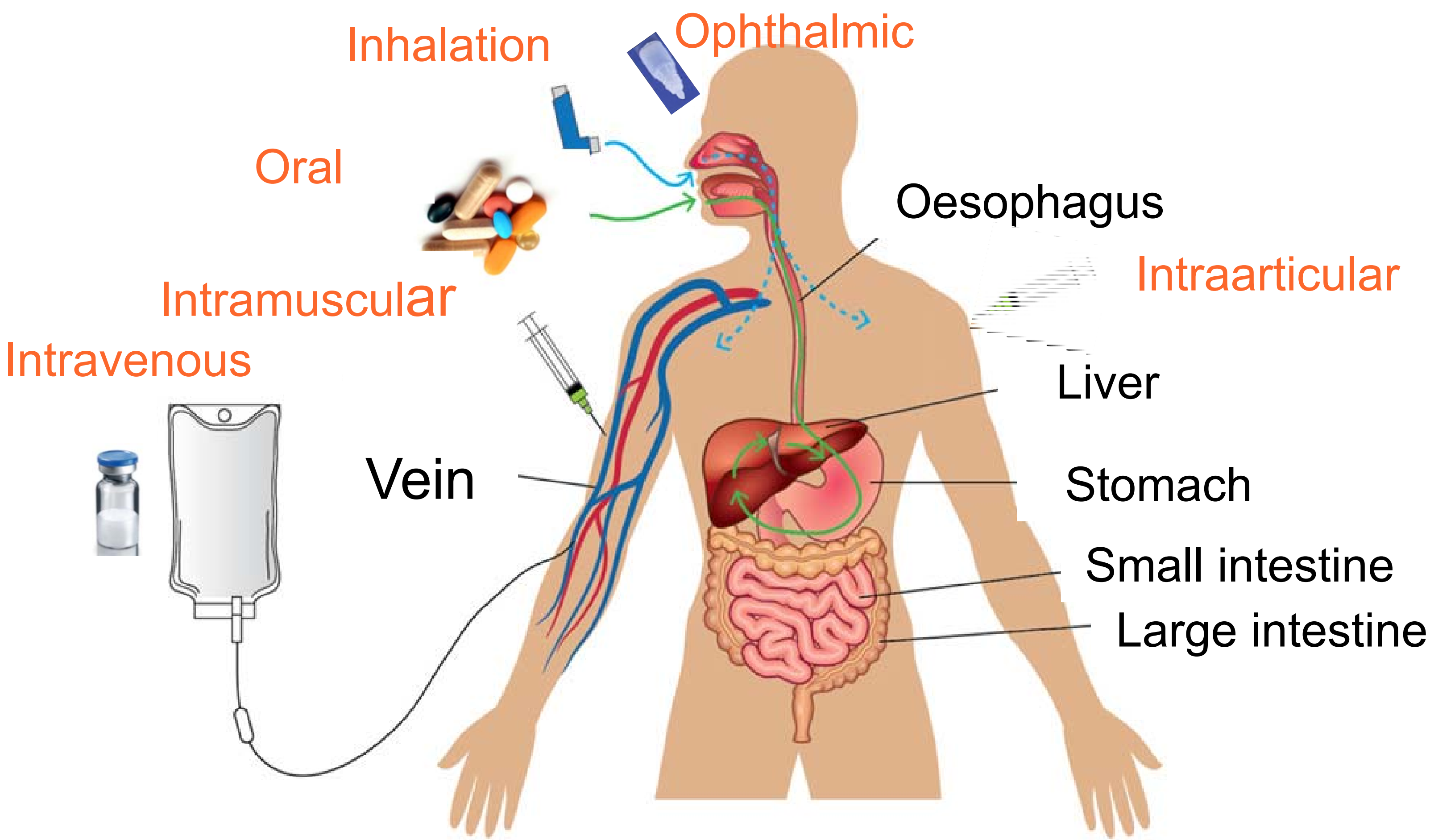
Polymeric naoparticles: Biopharmaceutical advantages



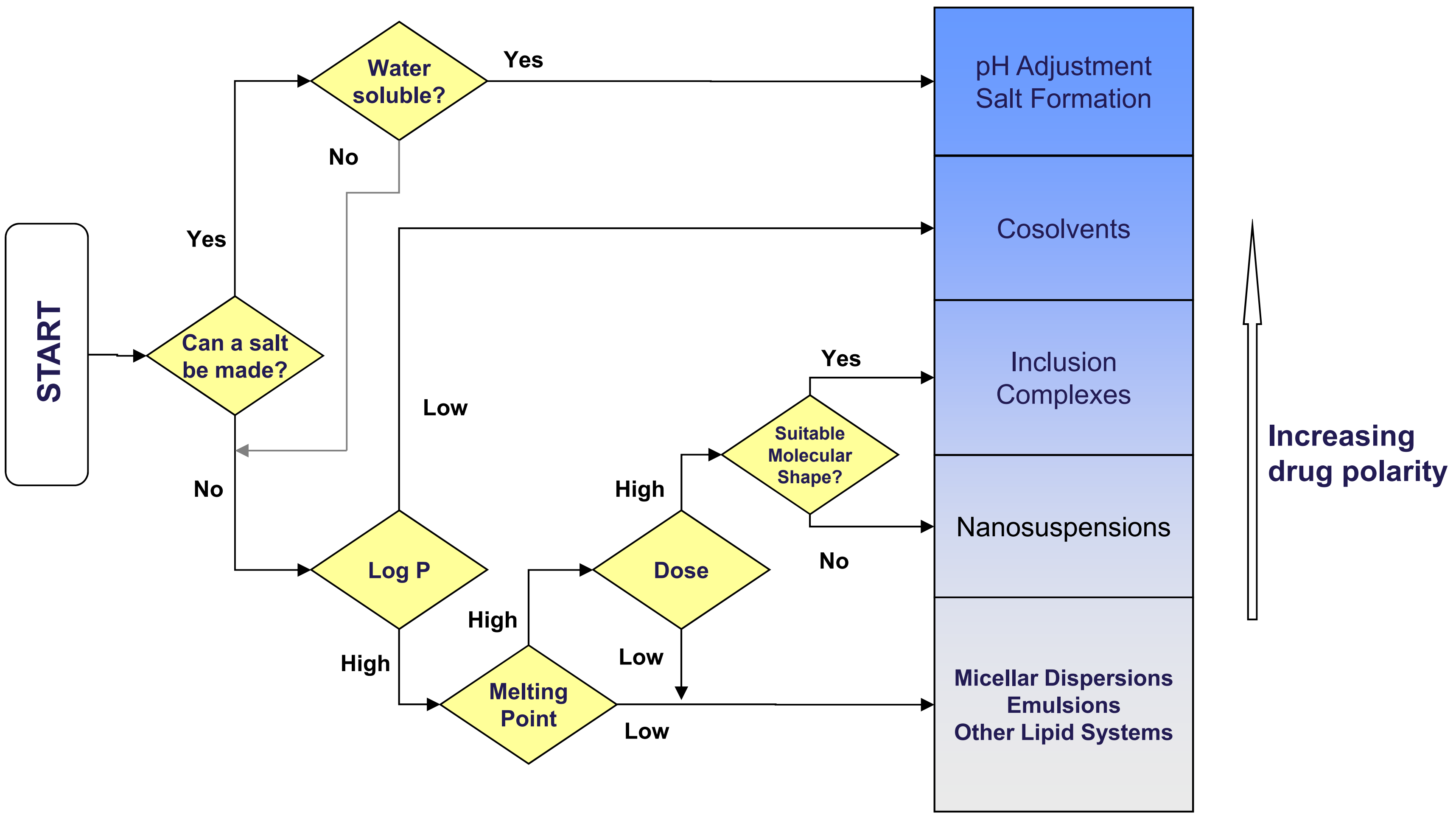
Efficient delivery of docetaxel for the treatment of brain tumors by cyclic RGD-tagged polymeric micelles

Ai-Jun Li Yue-Hua Zheng Guo-Dong Liu Wei-Sheng Liu Pei-Cheng CaoZhen-Fu Bu

Introduction – administration routes of nanodrug delivery systems

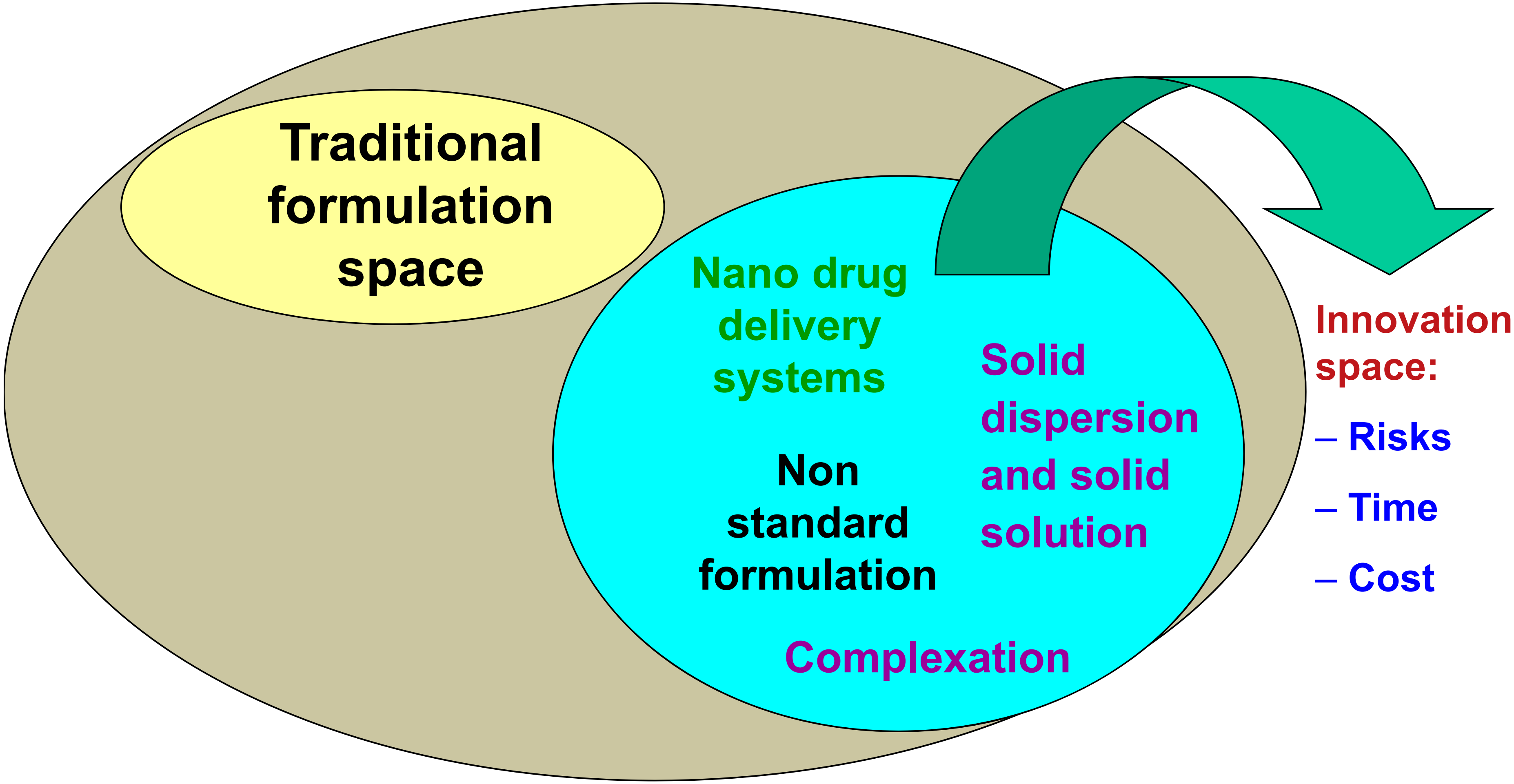


Criteria of formulation enhancement

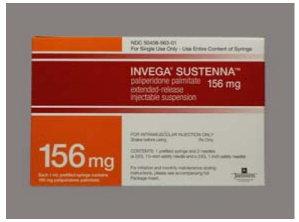


Overcoming of the basic hurdles

- **Formulation ability space is expanded**



Marketed products



Nanocrystalline suspensions

Liposomes

Emulions

Deals in naomedecine in 2013

> 1 Bio. $

Map of involved organization in nanomedecine

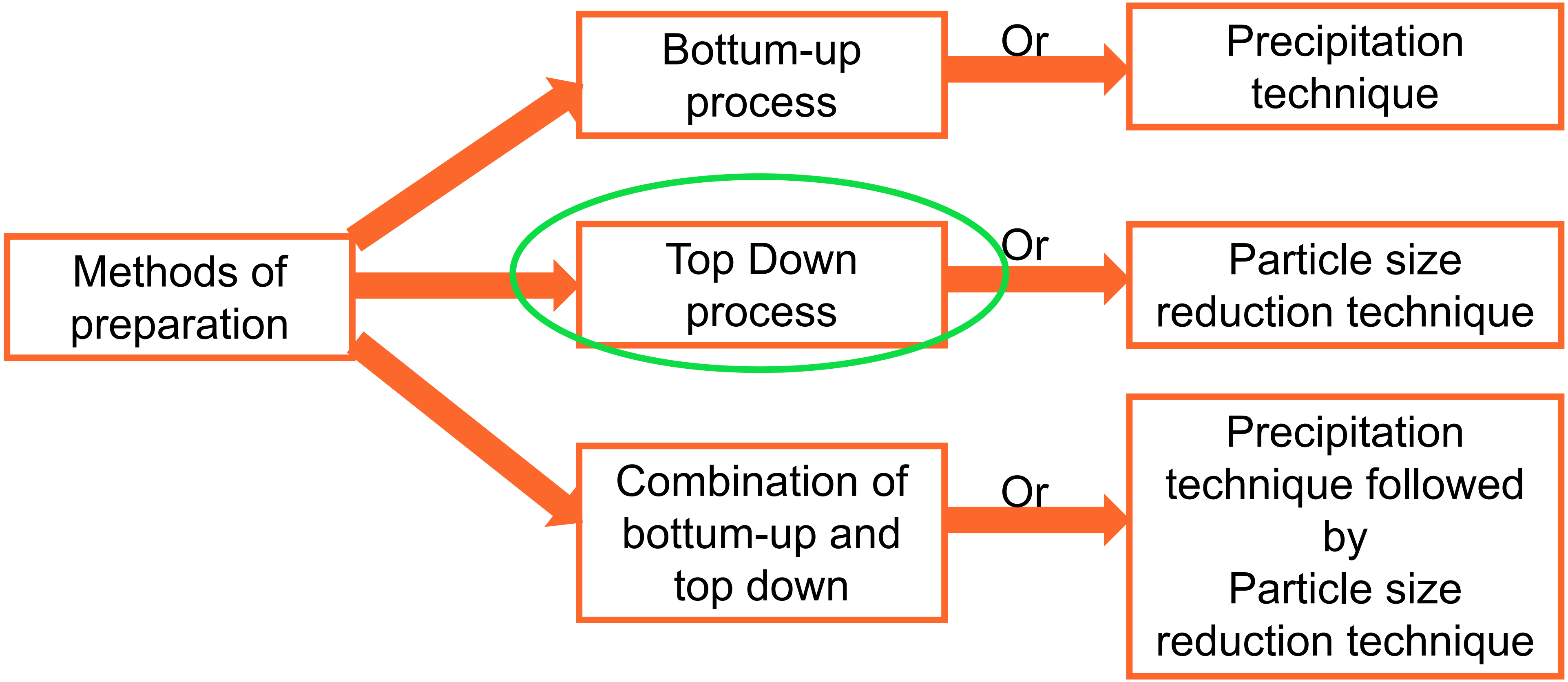
- **1500+ Organisation directly or indirectly involved in the Nanomedicine Field, covering research, industry, healthcare providers, public authorities**

Initiative to set-up a structured network European Technology Platform on Nanomedicine

nanomedicine
EUROPEAN TECHNOLOGY PLATFORM

- **European network of academic, industrial, public actors**
- **Address nanotechnology breakthroughs in healthcare**
- **Coordinate the joint research efforts of members and improve the coordination amongst them as well as towards the European Commission and the Member States**
- **Liaising Academia with industry**
- **Establishing a supply chain of innovative SMEs**
- **Support of the early preclinical or clinical proofs of concepts before transfer to large companies**

Introduction: Different Process Options for manufacturing of nano drug delivery systems



Introduction: Different Process Options for nano drug delivery systems

Different Process Options for Nano-suspensions

Bottom up process: From the molecular scale to the nano scale (solubilization in a solvent and precipitation in a non-solvent)

- Precipitation followed by evaporation (spontaneous formation)
- Solvent emulsification followed by evaporation (requirement of energy)

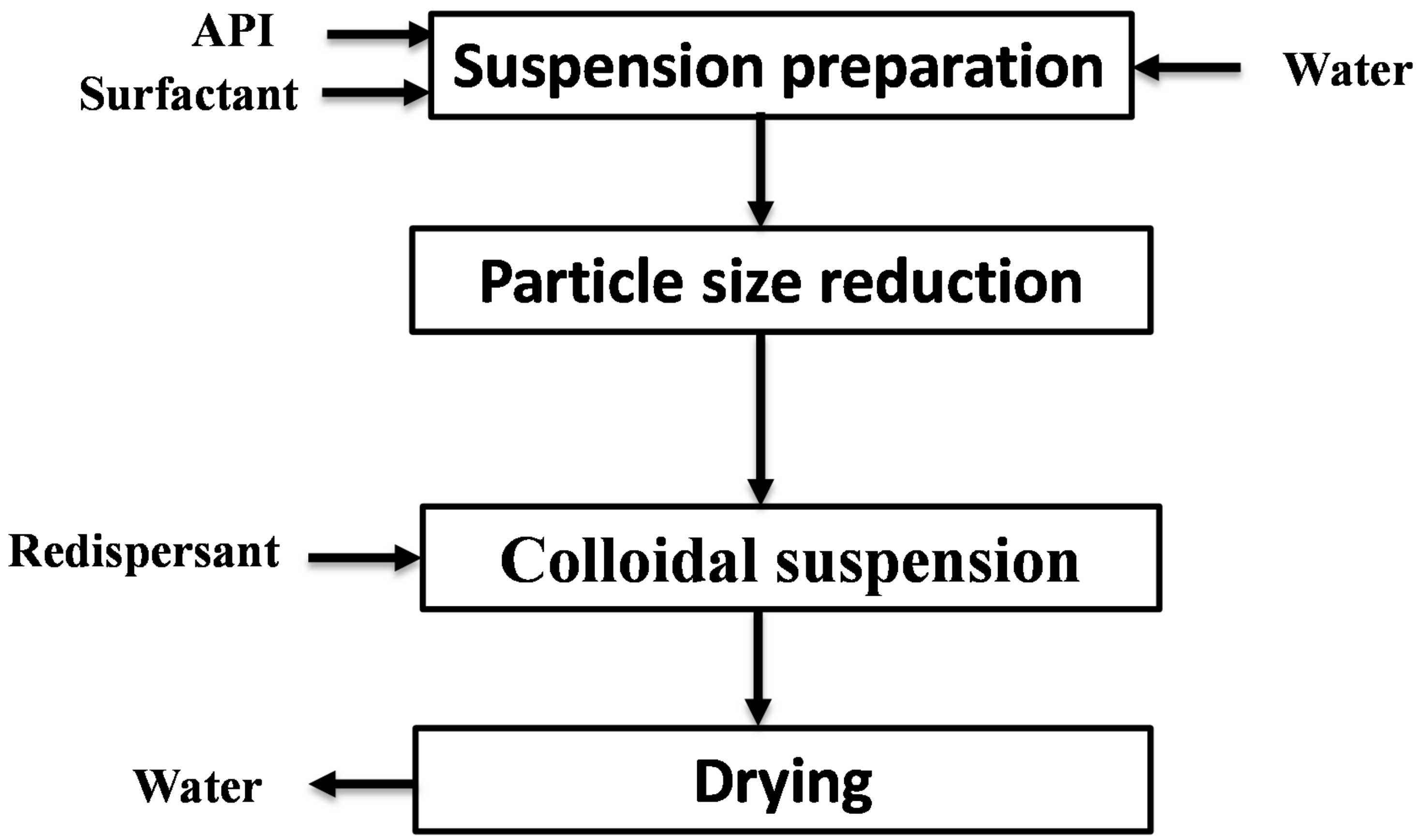
Particles could be below 100 nm.
Positive impact on the solubility.
Could be an issue for crystallinity and stability

Top down process: From the micron scale to the nano scale (particles disintegration using high energy → Milling)

- High pressure homogenization (HPH)
- Bead milling

Particles cannot be below 100 nm: no impact on the saturation solubility

Introduction: manufacturing of nanocrystalline suspension using top down process



Introduction: manufacturing of nanocrystalline suspension or nanopolymeric particles using bottom-up process (1/2)

Organic solvent → Organic solution
Surfactant → Organic solution
API → Organic solution
Polymer → Organic solution

Water → Aqueous solution
Ingredient (*) → Aqueous solution

Organic solution + Aqueous solution → Precipitation

Precipitation → Solvent removal by extraction or evaporation → Solvent

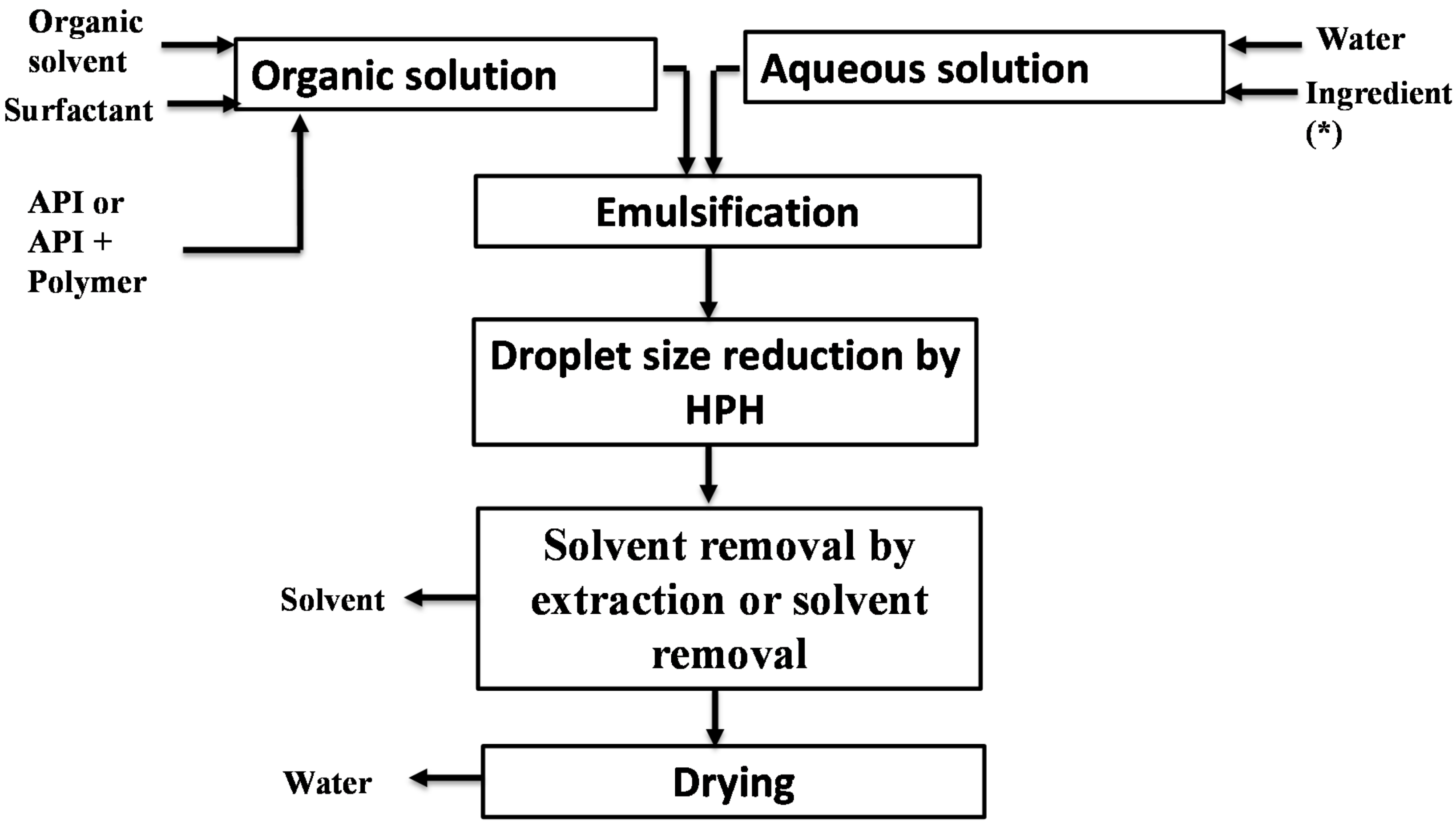
Solvent removal by extraction or evaporation → Drying → Water

Sonication Transducer

Micromixing

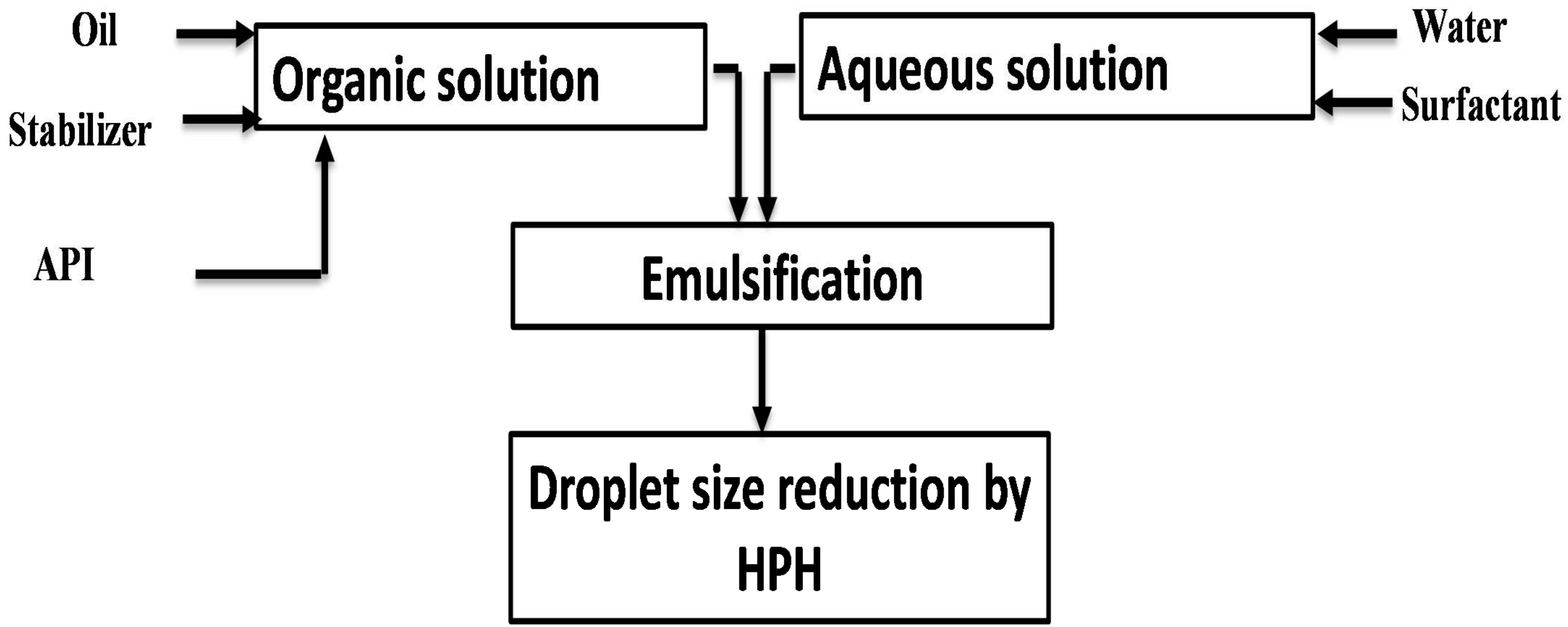
(*) could be viscosity reducer and/or cryprotector and/or stabilizer

Introduction: manufacturing of nanocrystalline suspension or nanopolymeric particles using bottom-up process (2/2)

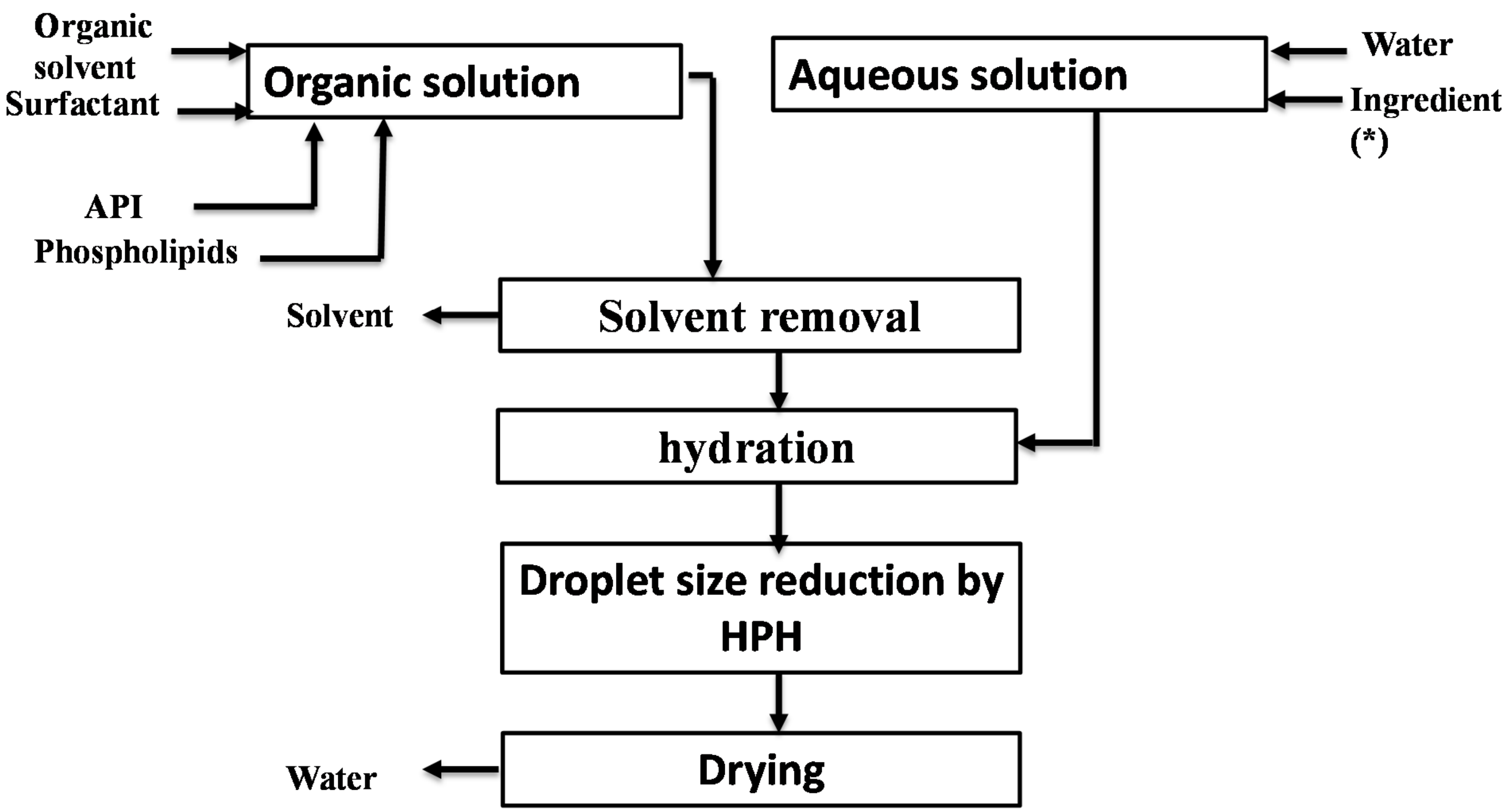


(*) could be viscosity reducer and/or cryprotector and/or stabilizer

Introduction: manufacturing of emulsion



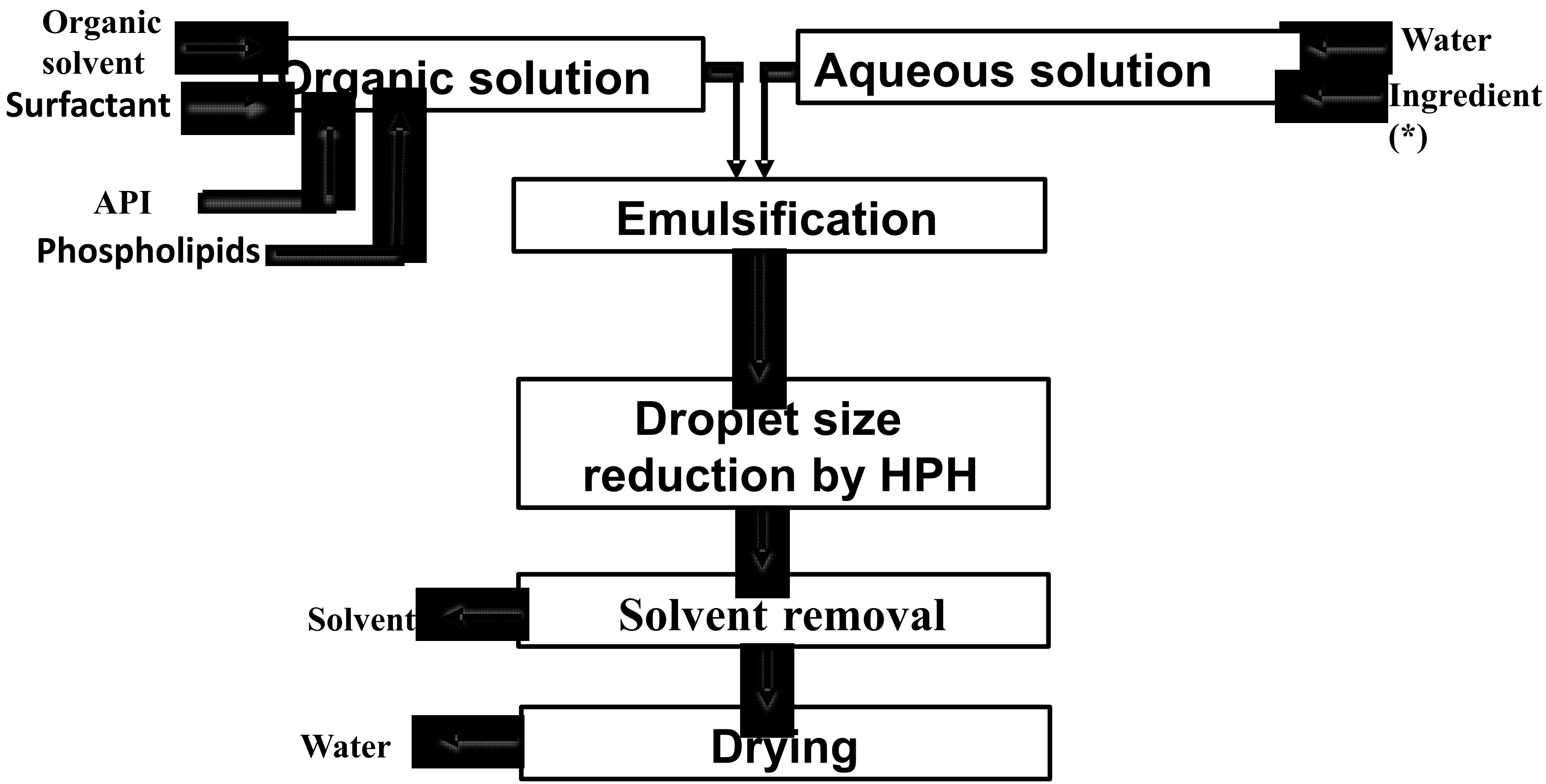
Introduction: manufacturing of Liposomes using Bangham method



(*) could be viscosity reducer and/or cryprotector and/or stabilizer

Academic manufacturing process

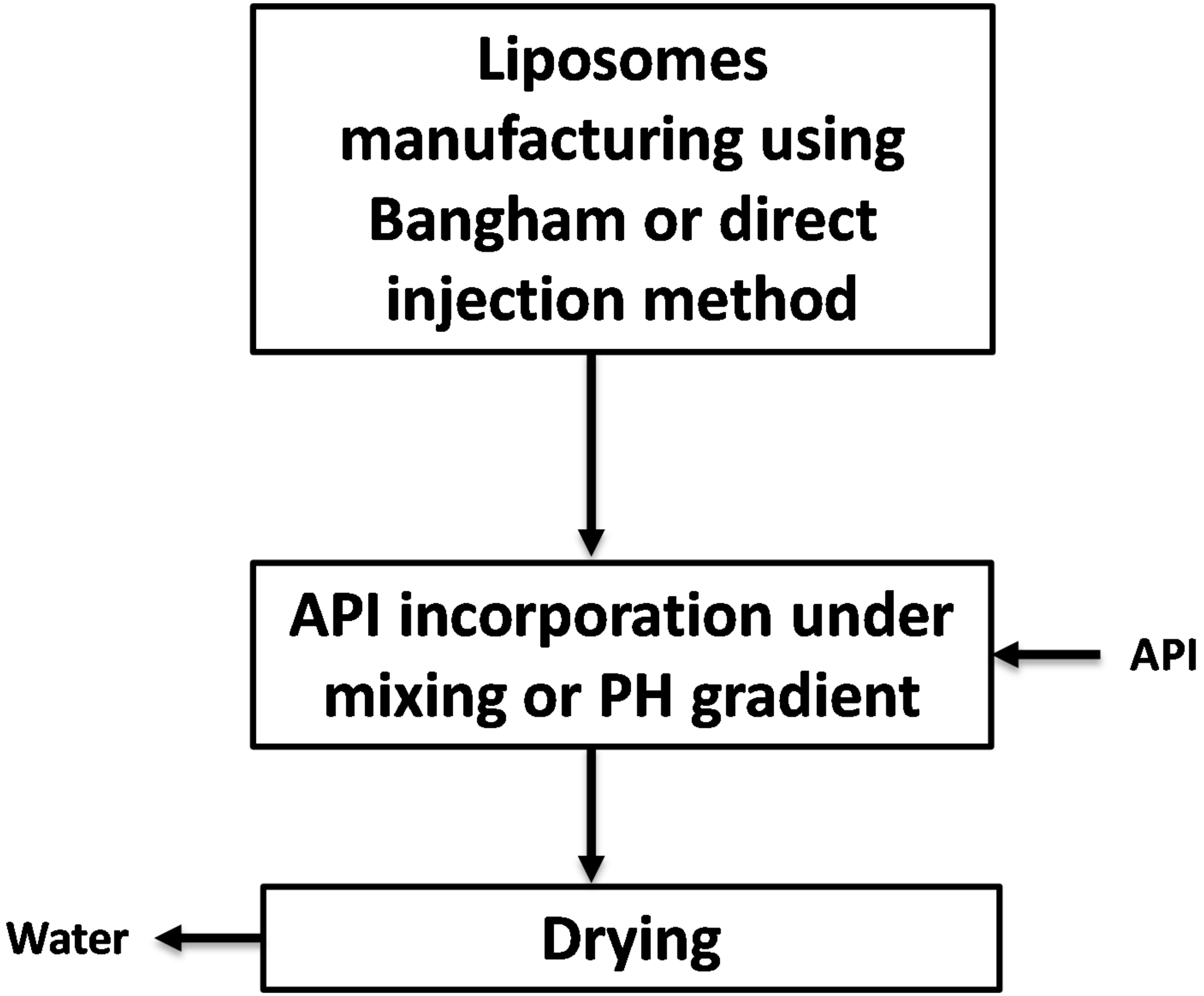
Introduction: manufacturing of Liposomes using direct injection method



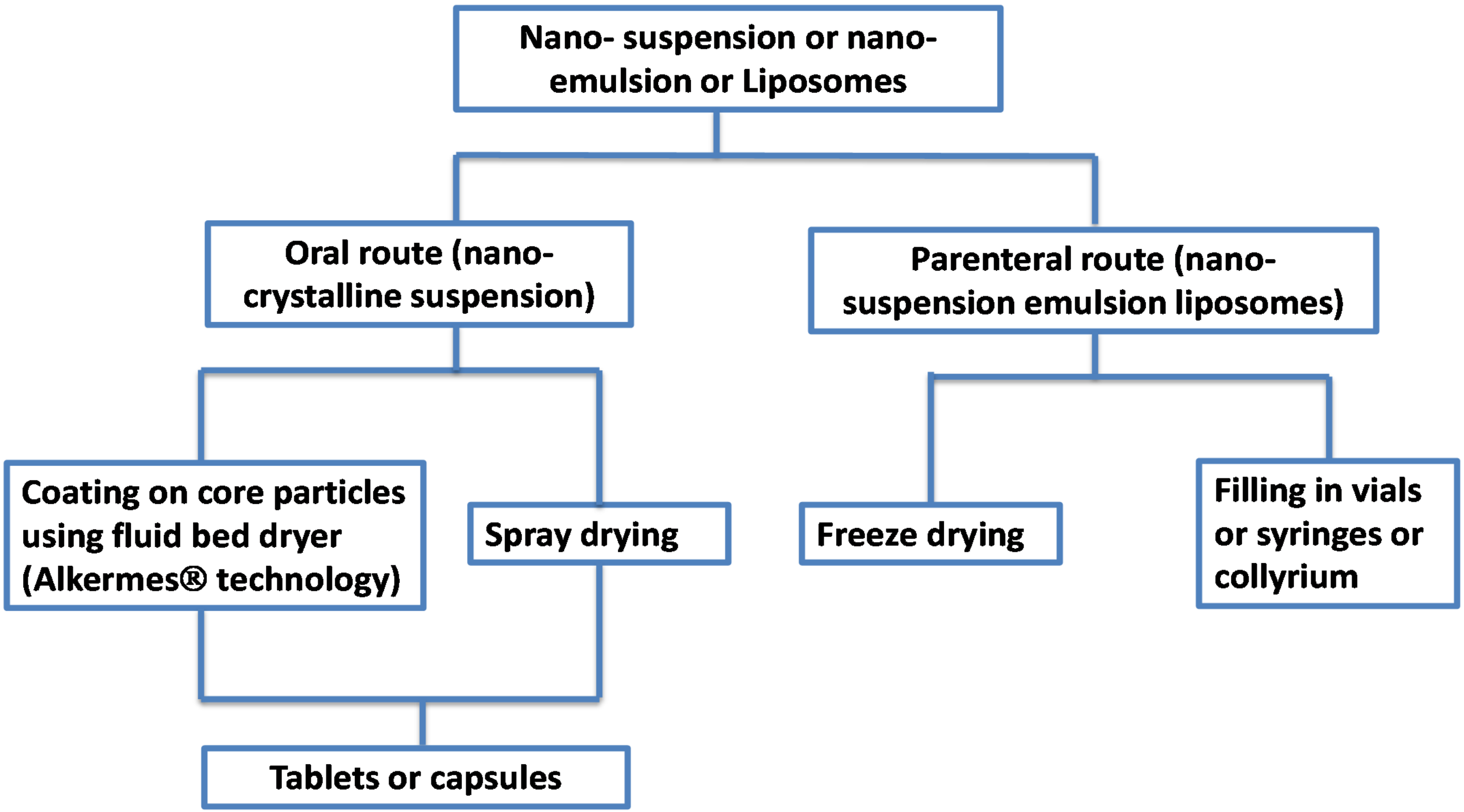
(*) could be viscosity reducer and or cryprotector

industrial manufacturing process

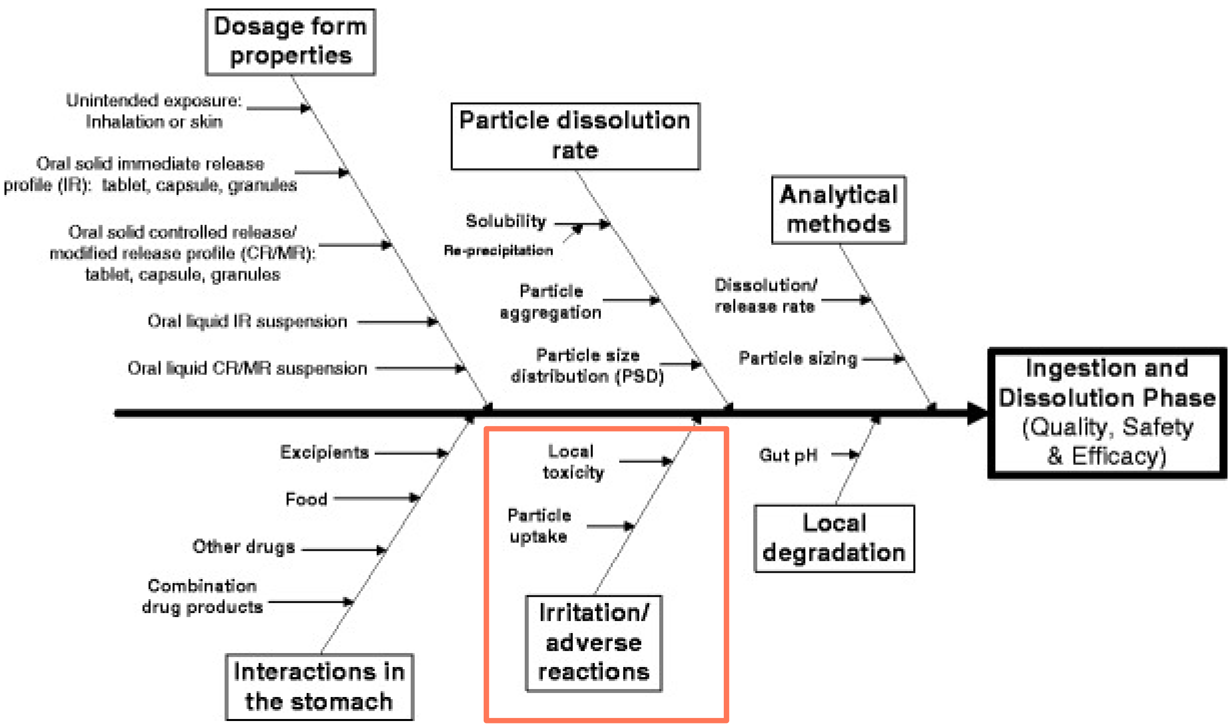
Introduction: manufacturing of Liposomes using direct encapsulation



Introduction: : Down-stream processing of nano-drug delivery systems



Nanotoxicity aspects:



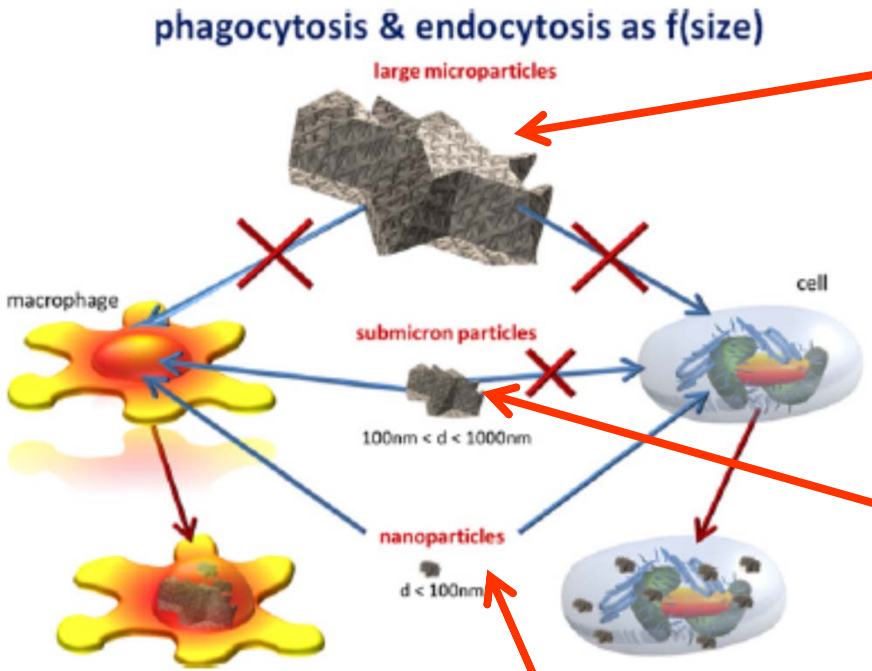
CDER Risk Assessment Exercise to Evaluate Potential Risks from the Use of Nanomaterials in Drug Products

AAPS J. 2013 Jul; 15(3): 623–628.

Celia N. Cruz, Katherine M. Tyner, Lydia Velazquez, Kenneth C. Hyams, Abigail Jacobs, Arthur B. Shaw, Wenlei Jiang,Robert Lionberger, Peter Hinderling, Yoon Kong, Paul C. Brown, Tapash Ghosh, Caroline Strasinger, Sandra Suarez-Sharp, Don Henry, Maat Van Uitert, Nakissa Sadrieh, and Elaine Morefield

Nanotoxicity aspects:

Uptake of particles is a function of size



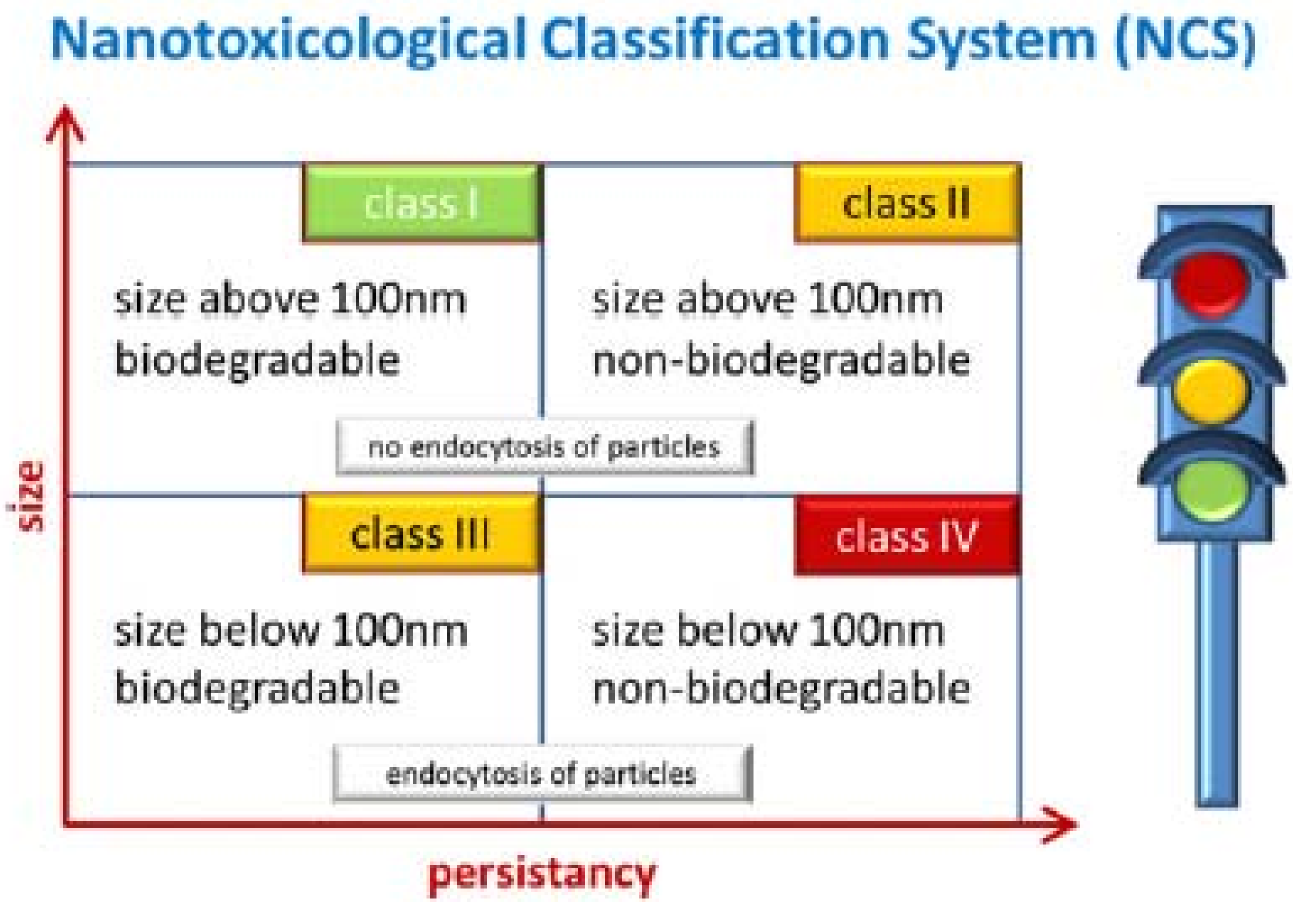
cannot be taken up by cells

Can be taken up by cells with phagocytic activity by the macrophages e.g. in the liver, spleen and the lung: Toxic risk is limited

Particles can be taken up by all cells by endocytosis: Toxic risk is high

State of the art of nanocrystals – Special features, production, nanotoxicology aspects and intracellular delivery
Rainer H. Müller, Sven Gohla, Cornelia M. Keck
European Journal of Pharmaceutics and Biopharmaceutics 78 (2011) 1–9

Nanotoxicity aspects:



State of the art of nanocrystals – Special features, production, nanotoxicology aspects and intracellular delivery
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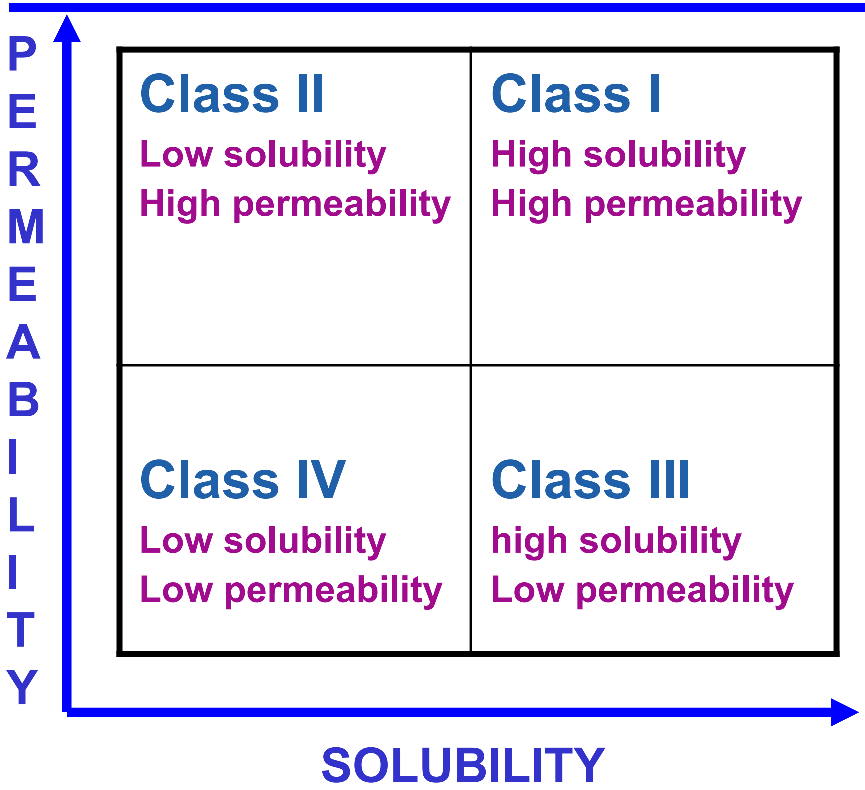
Nano-Crystalline suspensions

Biopharmaceutical classification



Description	Approximate weight of solvent (g) necessary to dissolve 1g of solute	Solubility (% w/v)
Very soluble	< 1	10 - 50
Freely soluble	1 - 10	3.3 - 10
Soluble	10 - 30	1 – 3.3
Sparingly soluble	30 - 100	0.1 – 1
Slightly soluble	100 - 1000	0.01 – 0.1
Very slightly soluble	1000 – 10000	0.01 – 0.1
Practically insoluble	> 10000	< 0.01

•Highly permeable = Extent of absorption in human is ≥ adminestred dose
•Highly soluble = highest dose is soluble in volume ≤ 250 ml at pH of 1-7,5 at 37°C

- BCS1: conventional formulation
- BCS2: Enhancement by optimal formulation design
- BC3: conventional formulation + absorption enhancer
- BCS4: Enhancement by optimal formulation design + absorption enhancer

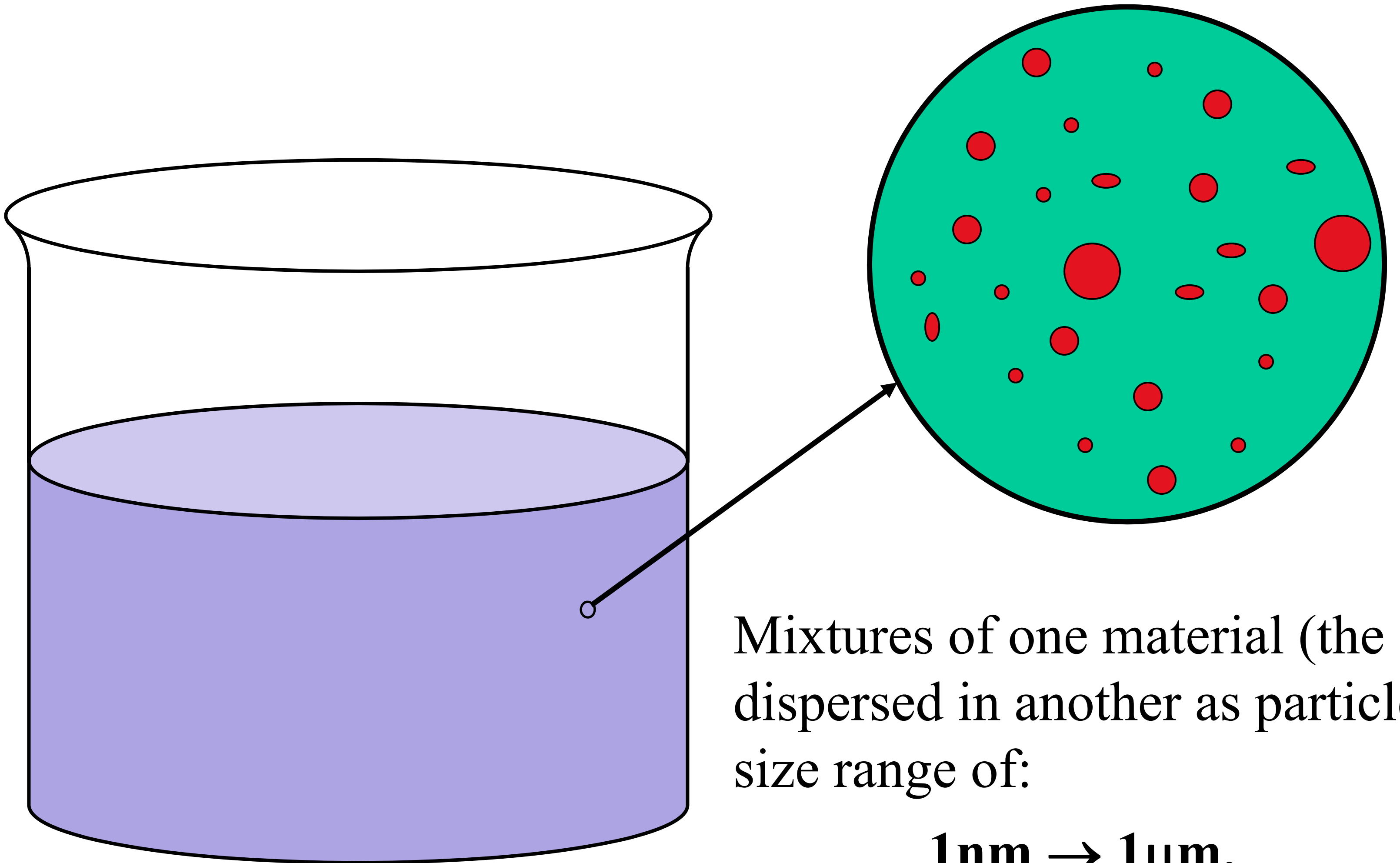
Formulation enhancement

- **Formulation enhancement: Physical modification**
  - **Polymorph and pseudo-polymorphs**
  - **Crystal modification: Co-crystal, amorphous drug**
  - **Complexation (e.g. Cyclodextrin)**
  - **Solubilization (use of surfactant)**
  - **Lipid formulation: Emulsion, liposome**
  - **Solid dispersion and solid solution**
  - **Particles size reduction**
    - **Dry milling or Micronization**
      - **Limited efficiency because only a small fraction is below 1μm**
      - **Leads to partial amorphization**
    - **Nanonization**
      - **all particles could be below 1 μm**
      - **Crystal structure unchanged if wet milling is applied**

Potential Benefits of nanocrystalline For Poorly Soluble Drugs

Route of administration	Potential benefits
Oral	Rapid onset , Reduced fed/fasted ratio Improved bioavailability.
Intravenous	Rapid dissolution, high adhesiveness
Ocular	Higher bioavailability, More consistent dosing.
Inhalation	Higher bioavailability, More consistent dosing
Subcutaneous/ intramuscular	Higher bioavailability, Rapid onset.

DEFINITION OF A COLLOID



Mixtures of one material (the “dispersoid” dispersed in another as particles in the size range of:

1nm → 1μm.

Suspension: Formulation

- **Basic ingredients**
  - API (hydrophobic drug compound)
    - There are usually particle size requirements to ensure proper bioactivity.
    - Stable crystal form within suspension vehicle
  - Dispersion medium (Usually water)
  - Wetting agent
    - Most fine particles are not easily wet by water because of occluded air and/or natural hydrophobicity. This is a particular problem at high concentrations.
    - The wetting agent molecule has a portion with an affinity for the particle surface and a portion with an affinity for water. It facilitates intimate contact of the liquid with particle surfaces.

Suspension: Formulation

- Stabilizer (adsorb at the surface of drug particle with water)
  - The dispersant keeps the wetted particles separated and mutually repulsed.
    - anionic: one portion has an affinity for the particle, and the hydrophilic anionic group extends into the water.
    - Polymeric or copolymeric
- Antifreezing agent (glycol)
  - Glycol, e.g. propylene glycol, is added to depress the freezing point if the dispersion will be stored or transported in a sub-freezing environment.

– Preservative agent
  - A preservative is used when the concentrate's organic ingredients (wetting agent, dispersant, suspending agent) are susceptible to degradation by bacteria or fungi.

Suspension: Formulation

– Antifoaming agent
  - The surfactants used as wetting agents are often sufficiently surface active to form air bubbles in the concentrate, which suspending agents can make difficult to remove. An ,antifoam is used to inhibit bubble formation.
– Suspending Agent(s)
  - Some dispersions are made without a suspending agent because the particle size is extremely fine, the concentration is very high, or the viscosity is high.
  - The insoluble particles remain separated and suspended. A suspending agent is usually a thickener as well,.

Introduction: process flow chart for the manufacturing of nano-crystalline drug product

API → Suspension preparation ← Water

Wetting agent (ionic small molecule) → Suspension preparation ← Stabilizer (large molecule)

Suspension preparation ↓

Particle size reduction ↓

Redispersant (heating or freezing protectant) → Colloidal suspension ↓

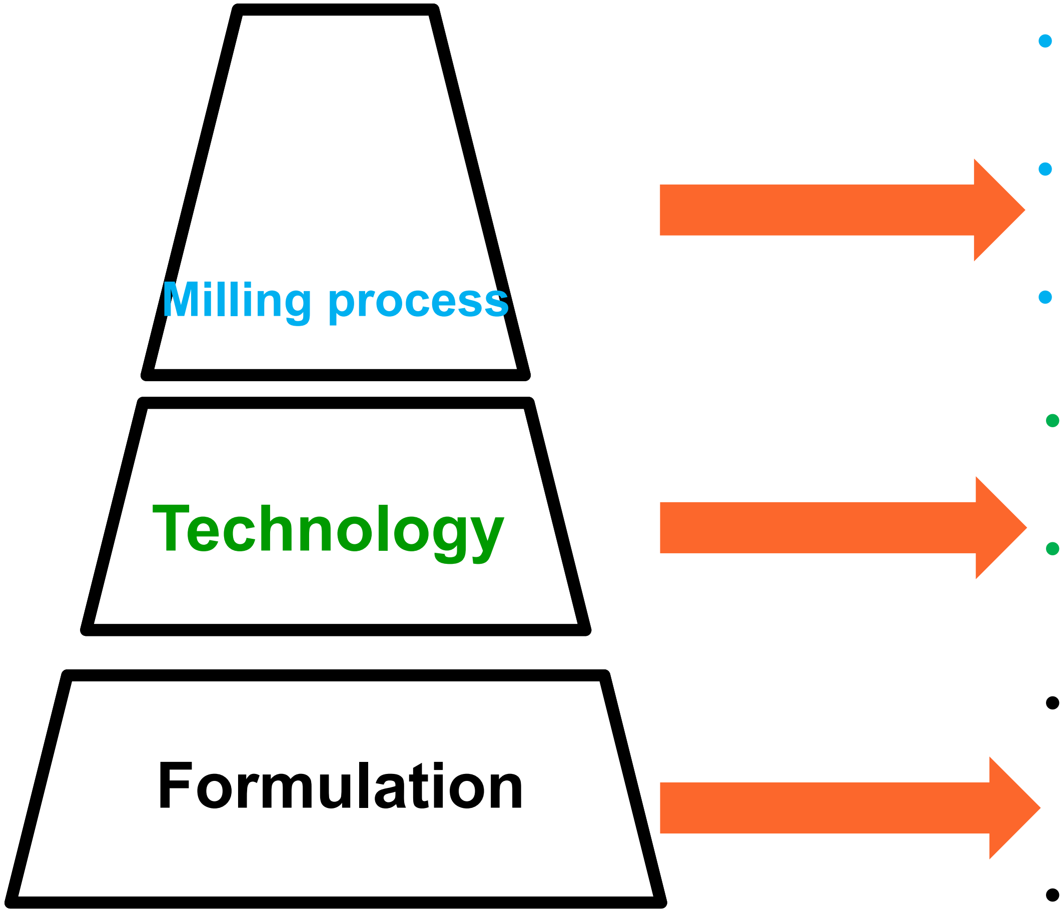
Down-stream processing (Final dosage form) ↓

Packaging

Formulation process engineering and technologies

Must be stable during administration

Introduction: What has to be considered to make Nano-crystalline suspension successful?



- Development based on scale-down & scale-up appoach
- Higly robust within the designed space
- Modelled for any prediction and simulation

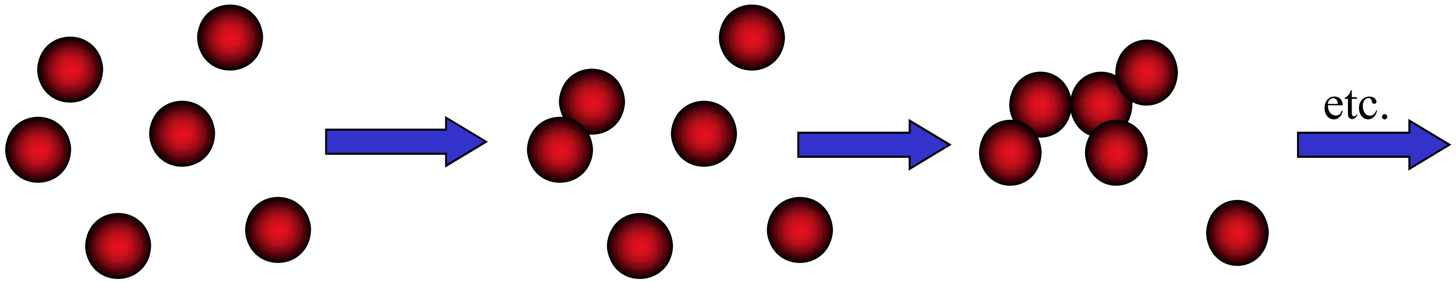
- Able to achieve the desired particle size
- Needs to be compliant with pharmaceutical regulation

- Proper selection of ingredients controls particle size during milling
- Stabilization during storage and administration

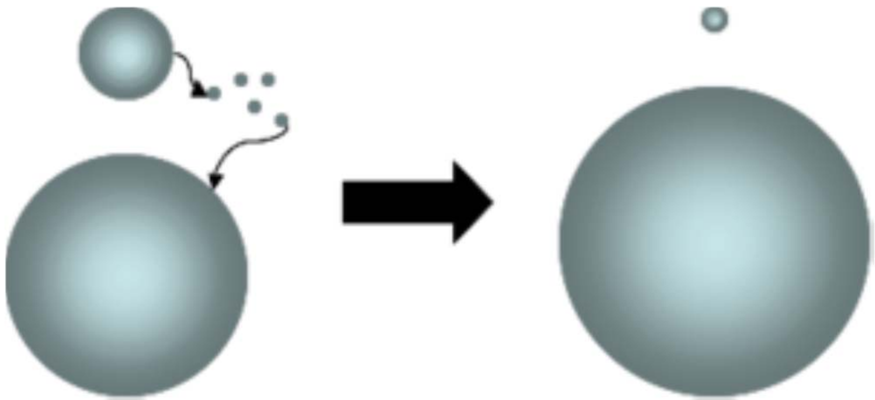
Nano-Crystalline suspensions: formulation engineering

Mechanism of colloids instability

Aggregation:



Ostwald ripening



Theoretical considerations

- **Interfacial properties**
  - **Nanosuspension implies development of large amount of interface which affect the stability of suspension**
  - **Interfacial proprties paly acritical role in modifying the physical cahracteristics od dispersion**
    - **Surface free energy**

$$\Delta G = \gamma_{S/l} * \Delta SSA$$

- **The smaller G is , the most thermodynamically stable is the suspension**
- **High SSA → high G → the syspension tend to agglomerate to reduce G**
- **Reduction of Gamma by adding a weeting agent to the suspension**

Theoretical considerations

- Surface Potential
  - The stability of suspension can be generally explained by presence or absence of surface potential
  - Surface potential exists when solid particles possess charges in relation of their environmental liquid
  - Charges may become through different way
    - **Suspension contains electrolytes -→ charged particles by specific adsorption of ionic species**
    - **Ionization of functional group of the particle: The total charges is a function of pH of environmental liquid**

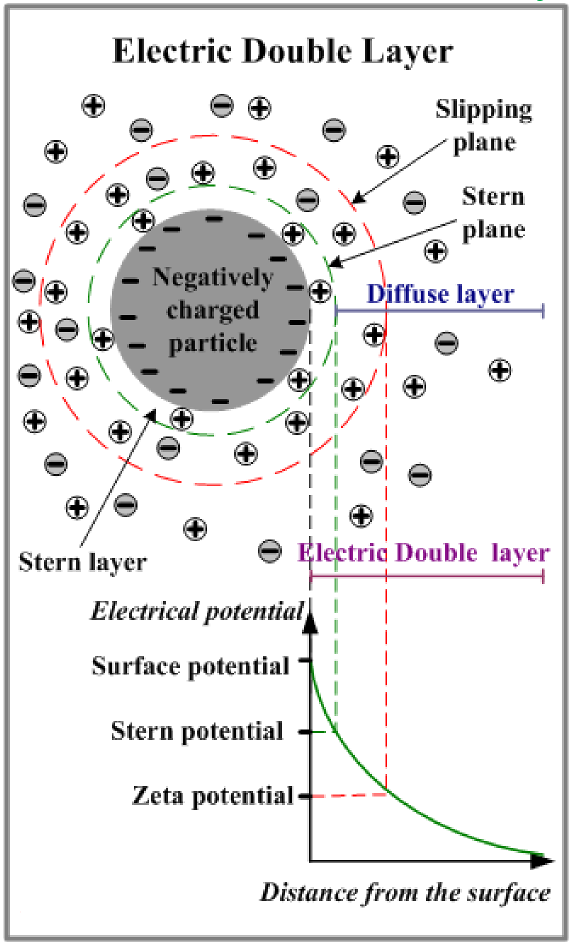
$$\text{Total charges} = Z \times N_g \times f_i$$

- **Z= ion valency**
- **N_g: ionizable group number/surface (obtained from crystallography on the exposed face)**
- **f_i: Ionizable fraction**

$$= \frac{10^{pK_a - pH}}{1 + 10^{pK_a - pH}}$$

Theoretical considerations

- Electric double layer



- Surface charges: charged ions (commonly negative) adsorbed on the particle surface.
- Stern layer - counterions (charged opposite to the surface charge) attracted to the particle surface and closely attached to it by the electrostatic force.
- Diffuse layer - a film of the dispersion medium (solvent) adjacent to the particle. Diffuse layer contains free ions with a higher concentration of the counterions. The ions of the diffuse layer are affected by the electrostatic force of the charged particle.
- The electrical potential within the Electric Double Layer has the maximum value on the particle surface (Stern layer). The potential drops with the increase of distance from the surface and reaches 0 at the boundary of the Electric Double Layer.
- When a colloidal particle moves in the dispersion medium, a layer of the surrounding liquid remains attached to the particle. The boundary of this layer is called slipping plane (shear plane).
  The value of the electric potential at the slipping plane is called Zeta potential, which is very important parameter in the theory of interaction of colloidal particles.

Forces between particles (surfaces)

Attractive Forces

Van der Waals forces that are universals for all materials

Repulsive Forces

Electrostatic repulsion due to:

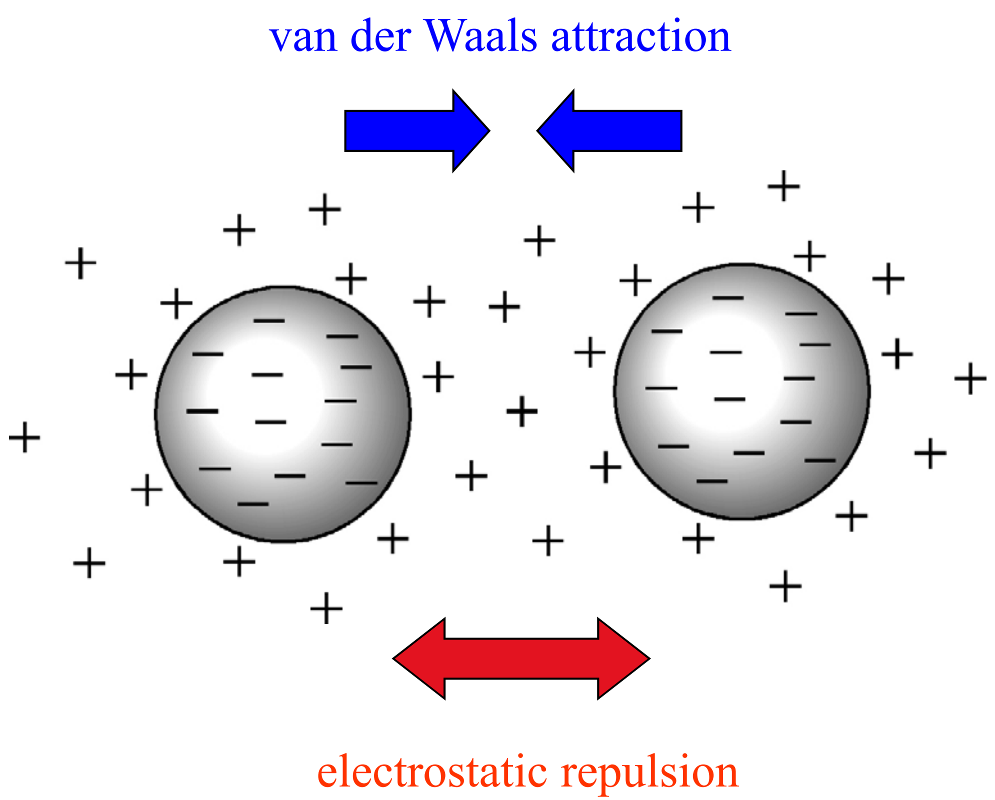
- Surface ionization as function of pH
- Ions adsorption on the surface

DLVO

Steric interaction

Electrosteric

ELECTROSTATIC REPULSION RETARDS AGGREGATION

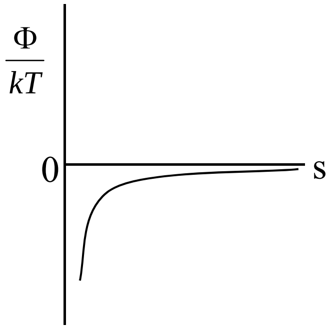


–**Electrostatic repulsive energy produced by the presence of electrical double layers around the particles - produced by charge separation at the solid/liquid interface.**

Forces between particles (surfaces)

Attractive forces

- van der Waals forces

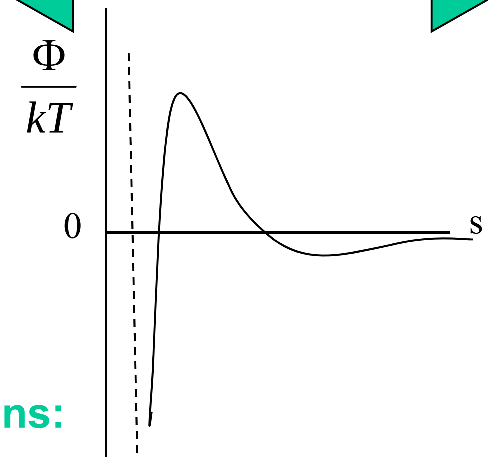


Attractive contribution inversely proportional to particles radius

When $(\Phi/kT) \geq 25$,
→ "stability"

Derjagin Landau Verwey Overbeek

DLVO Theory 1941



3 regions:

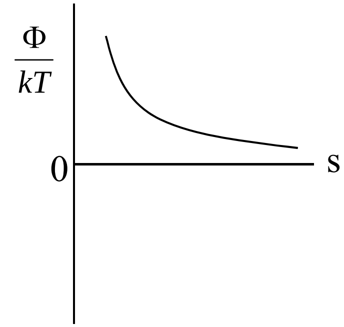
Primary minimum

Barrier with potential dependant on ionic force

Secondary minimum

Repulsive forces

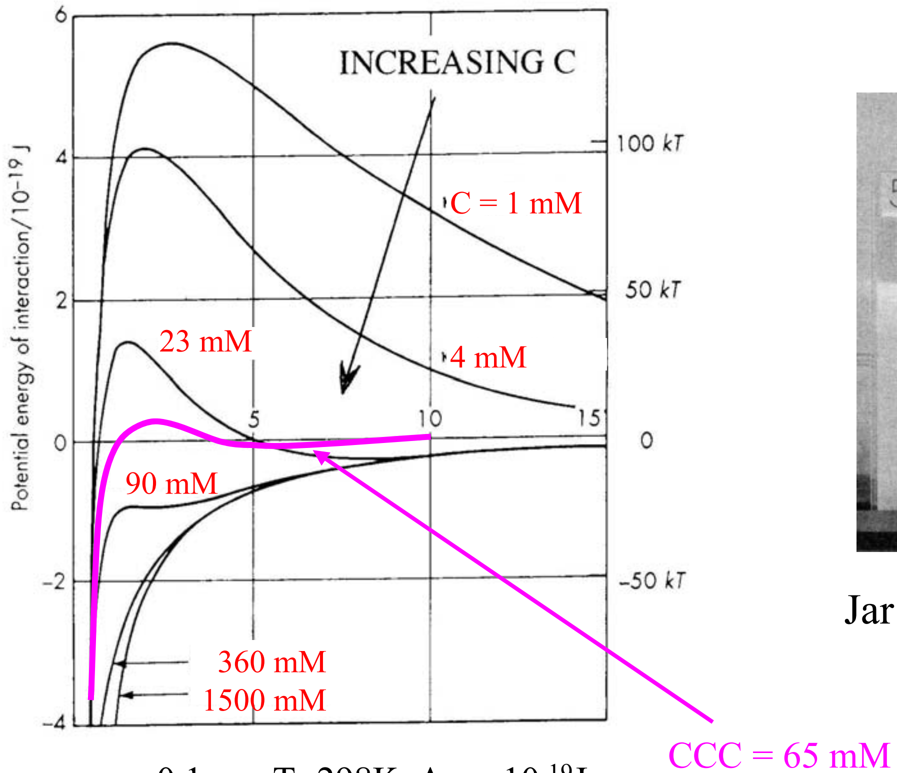
- electrostatic forces



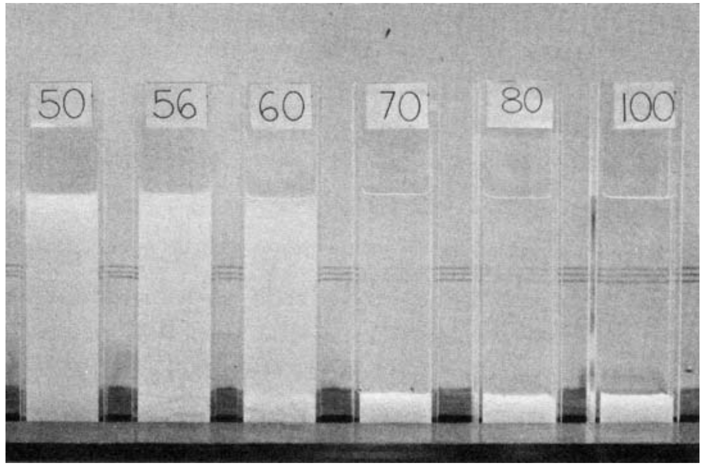
Repulsive contribution exponential of distance betwenn particles

Controlling electrolyte: valence and concentration

Ions in solution screen the charges of the particles. At the Critical Coagulation Concentration (CCC) the colloids will coagulate



a = 0.1μm; T=298K; A_{212}=10^{-19}J; ψ_δ=50mV; z = 1; ε=78.5



Jar testing of As_2S_3 sol with 1-1 electrolyte.

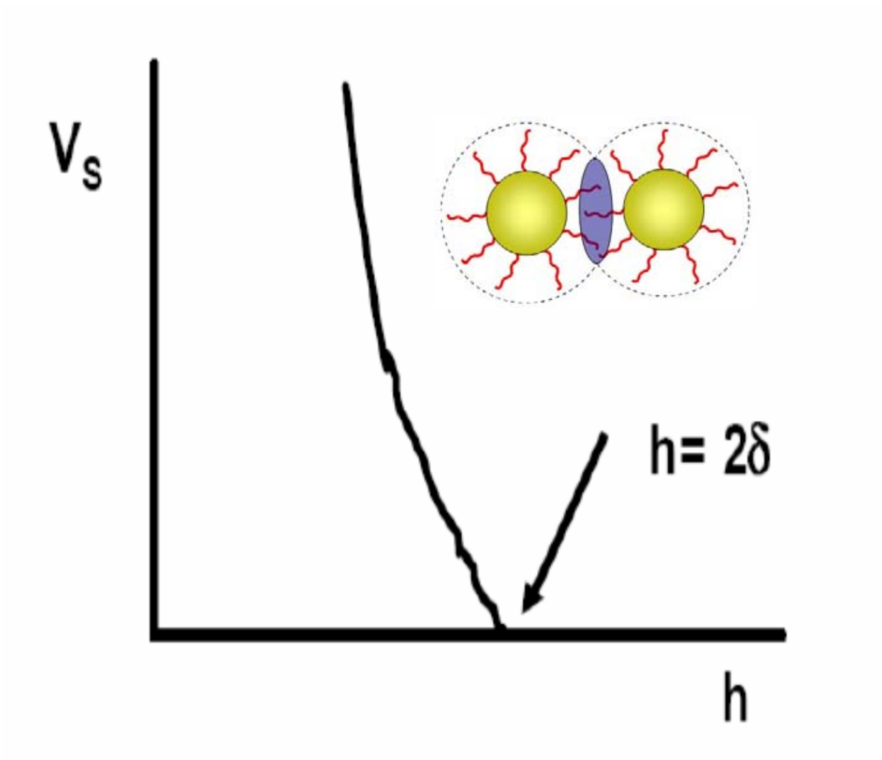
For z =2, divide all C's by 64; for z =3, divide all C's by 729

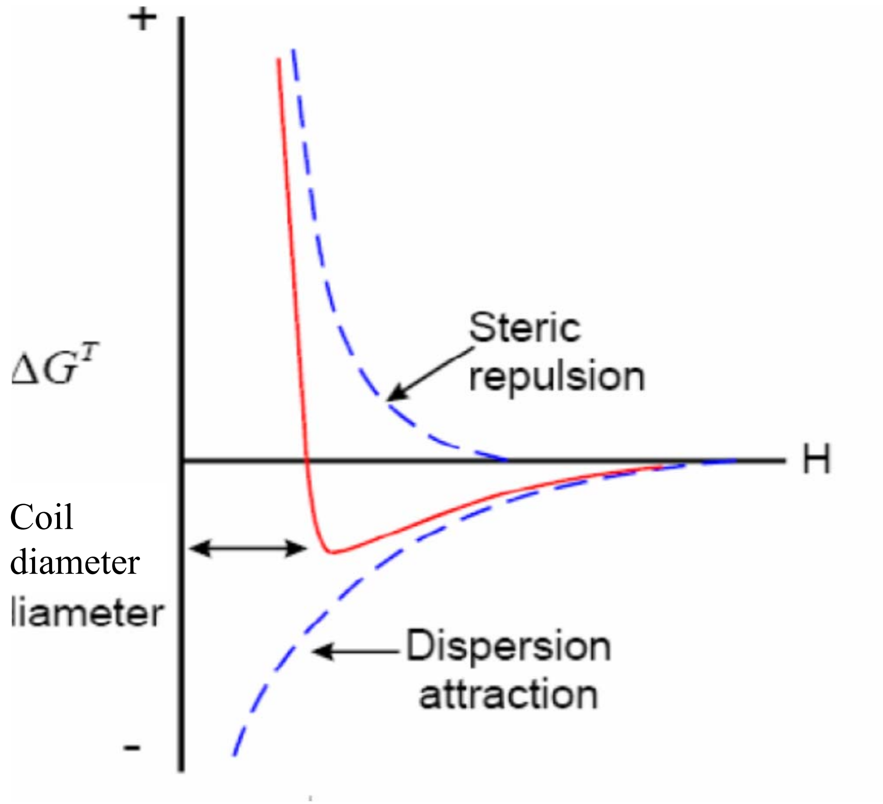
Schulze-Hardy Rule:

$$CCC \propto \frac{1}{z^6}$$

Steric interaction

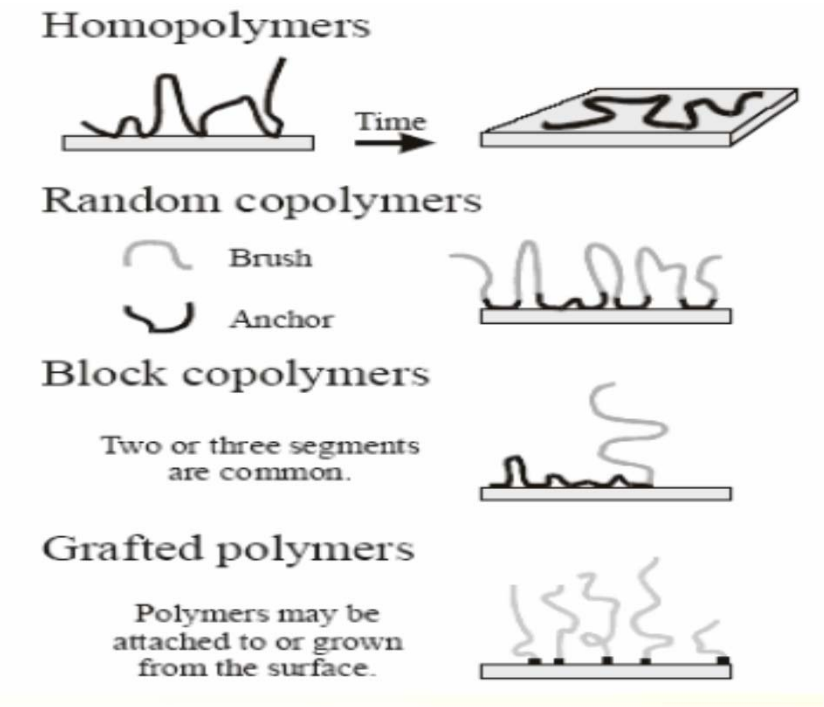
- **Steric repulsive energy produced by the presence of adsorbed (or grafted) layers of surfactant or polymer molecules.**
- The total energy-distance curve shows only a shallow minimum at particle-particle separation distances h compared to twice the adsorbed layer thickness 2δ, but when h < 2δ a sharp increase in the interaction energy occurs with further decrease of h



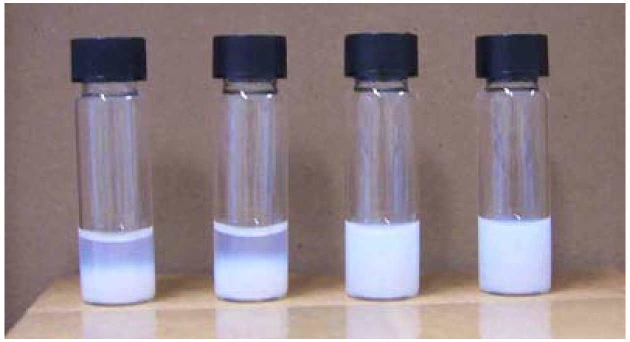


Steric interaction

- **Maintenance of the particles in the dispersed state, i.e. without any aggregation: Stabilization**
  - **Full coverage of the particles by the stabilizer**
  - **Strong adsorption of the stabilizer**
  - **Strong hydration of the stabilizer chain**
  - **The thickness of the adsorption layer > 0.05 * particle radius**



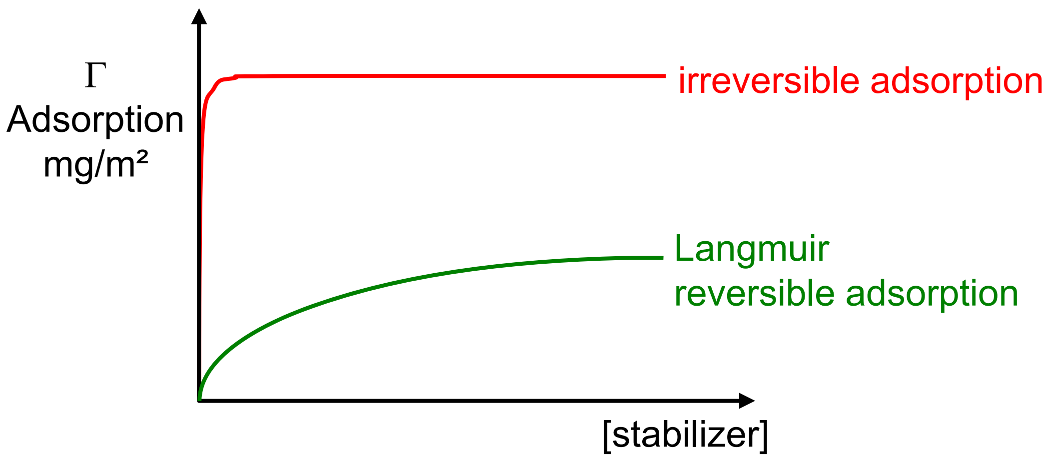
Adsorption Isotherm



Silica dispersions with different PEO concentrations in presence.

- **It is very important to establish adsorption isotherm (direct surface analysis of equilibrium solution analysis)**

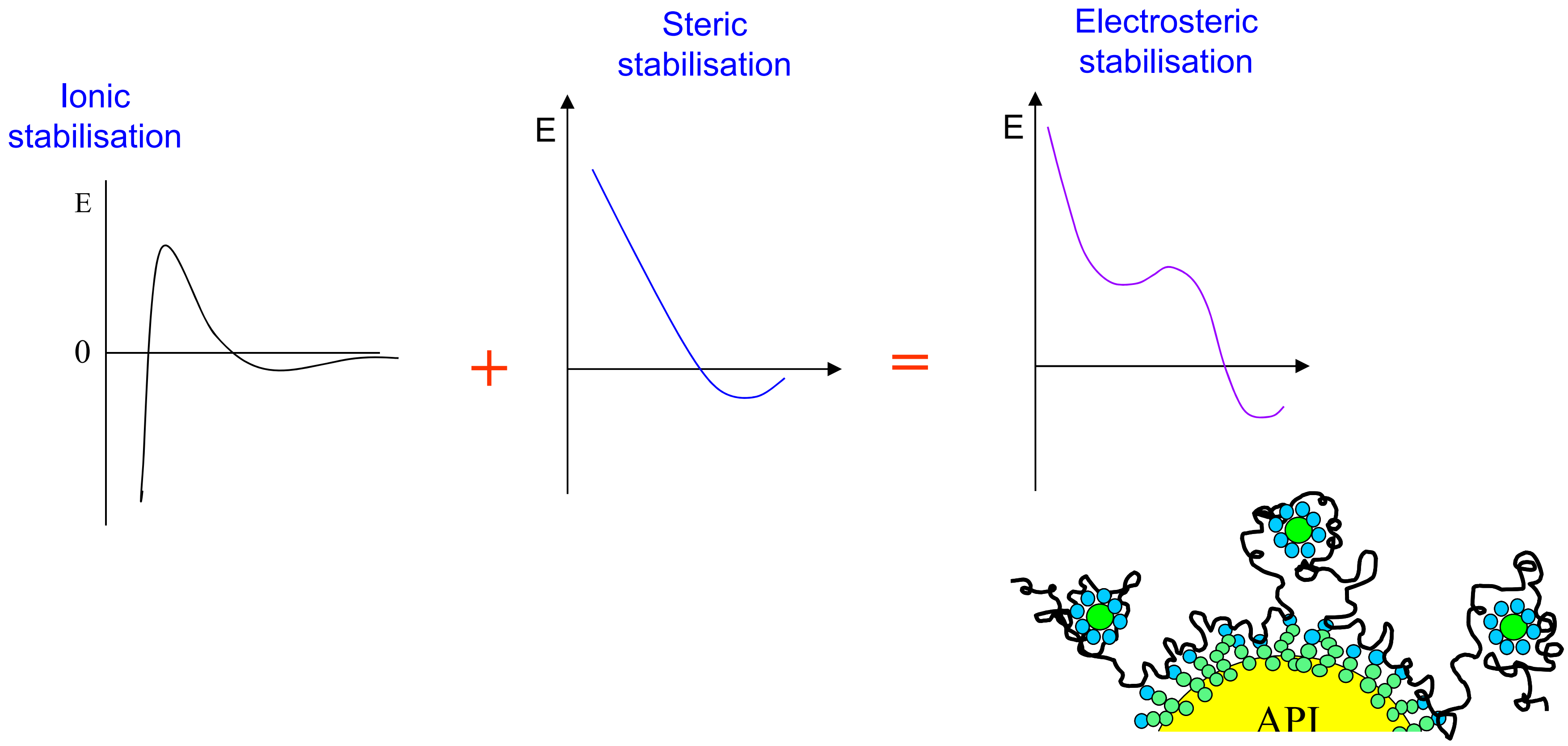
- **It is also useful to determine the layer thikness (DLS)**



•The high affinity isotherm obtained with polymeric surfactants implies that the first added molecules are virtually completely adsorbed and such process is irreversible.

Electrosteric vs steric or electrostatic

- **Coupling both stabilization mechanisms ensures a more robust stability**



Characterization for formulation screening

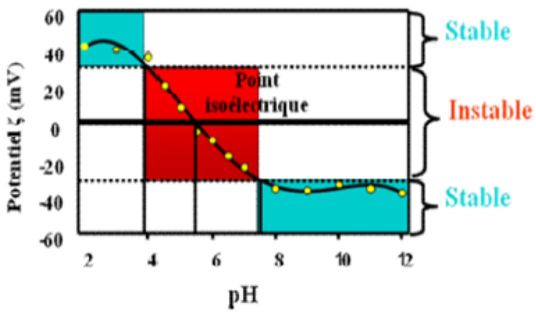
Zeta potential

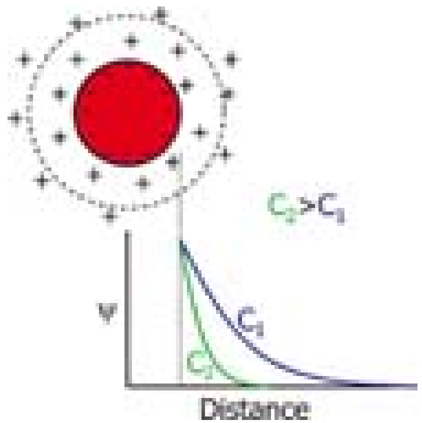
- Can be applied for charged molecules such as sodium dodecyl sulphate.

$$\xi = \frac{3\mu\eta}{2\varepsilon f(\kappa r)}$$

μ Is the molecular mobility
η Is the viscosity of dispersion medium
ε Is the dielectric constant
f(κr) is the Henry function, κ is Deby parameter, and r is particle radius
1/κ is the teckiness of double layer

- Can be affected by particles environmental (pH, electrolyte concentration)



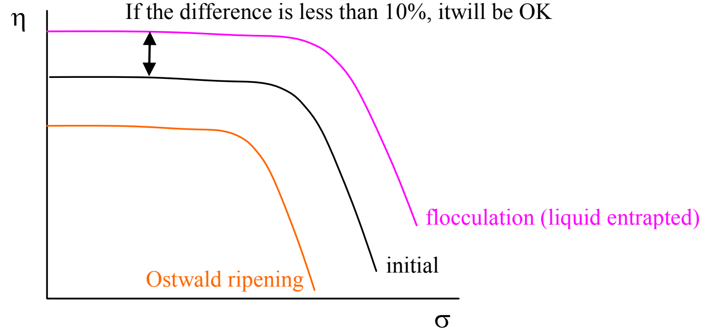


$|\zeta| > 30$ mv

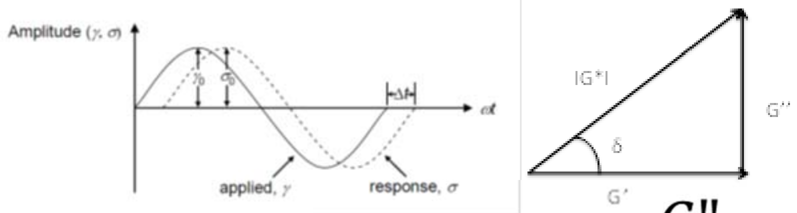
Characterization for formulation screening

Rheology

- It's the most informative techniques for assessment and selection of a dispersant.
- Could assess the flocculation phenomena by using temperature scan (correlation to adsorption and hydration of the stabilizer)
- Use constant stress (creep):



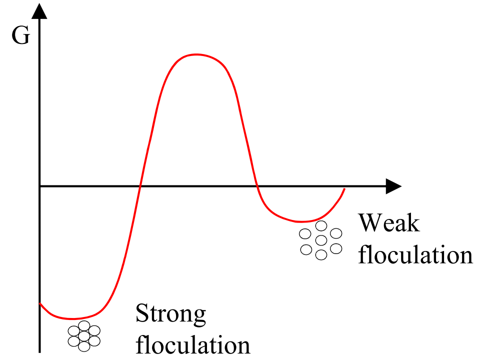
- use oscillatory measurements (strain sweep method)



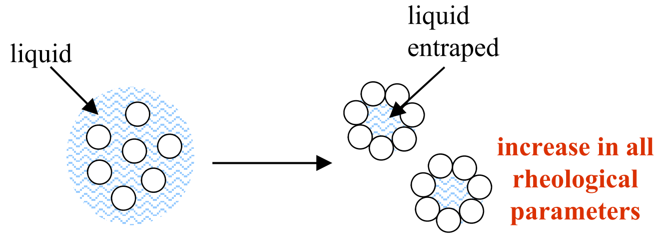
$$\tan(\delta) = \frac{G''}{G'}$$

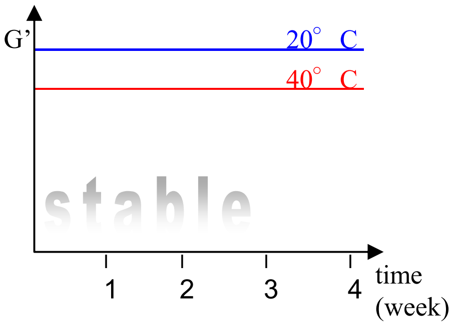
When δ=0° the material is elastic solid. When δ= 90°, the material is visco fluid and when 0< δ<90, the material is viscoelastic system.

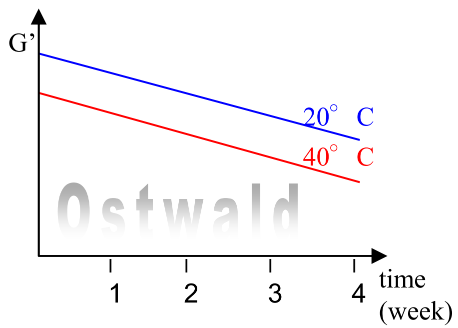
Stability indicating method : complex rheology

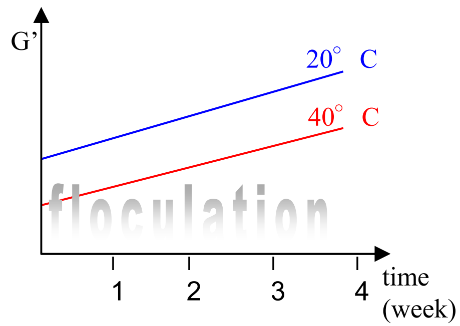


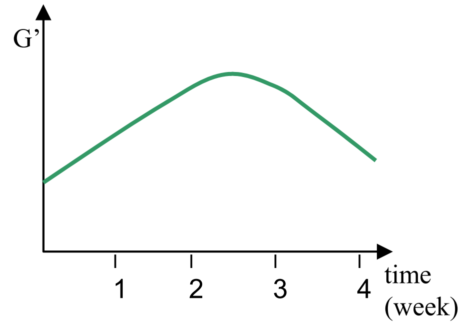
- It is mandatory to predict long term prediction based on short term data
  - Floculation
  - Ostwald ripening
- Particles size measurement
- Proposed method ➔ complex rheology
  - 3 temperatures
  - For few weeks
  - Rheological parameters











Methodology for top-down process

Stabilizer adsorption

- **Adsorption measurement**
  - **Micronized drug**
  - **Surface area determination**
  - **Adsorption evaluation**

$C_1 - C_2 = \Delta C$ ← amount of stabilizer adsorbed per unit mass

amount of stabilizer adsorbed (L x g/L)

$$\frac{V\Delta C}{mA} = \Gamma \Longleftarrow \textbf{adsorption (g/m}^2\textbf{)}$$

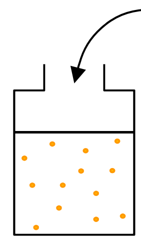
mass of API (g)

specific surface area (m^2/g)

Stabilizer

20 50 70 100 120 150 180 200 ppm C_1

Add 1-2g of API



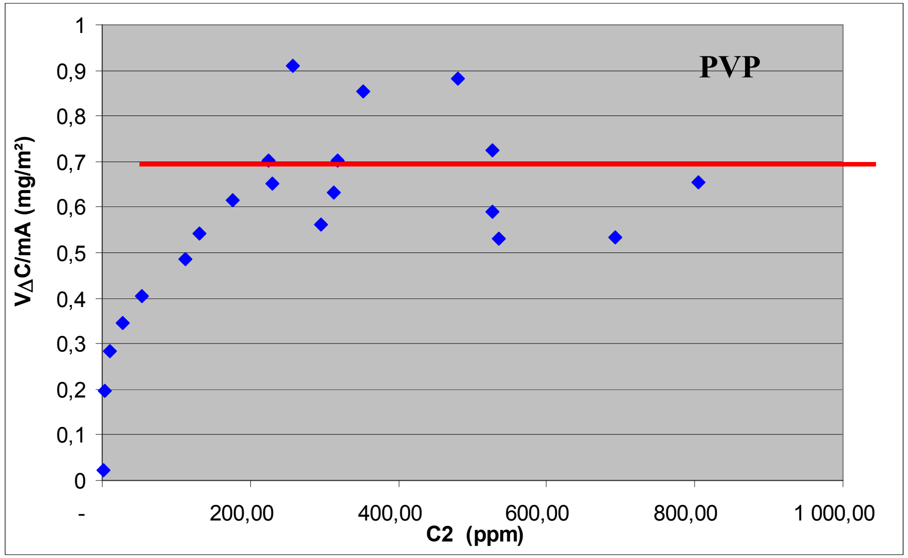
50 mL of stabilizer solution

(In order to assay, minimum need = 10mL)

Sonication to ensure good dispersion of the API powder

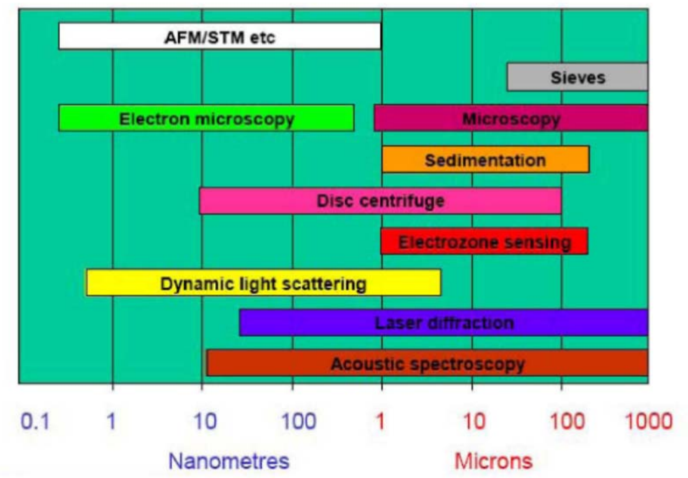
Centrifugation

Determination of the final stabilizer concentration C_2



Characterization of the suspension

- **Particle size measurement**
  - Laser diffraction
  - Photo correlation spectroscopy (dynamic light scattering)
  - Size exclusion chromatography



- **API content and impurities profile**

Settling/sedimentation

- Sedimentation: Stokes' equation law

$$v = \frac{d^2(\rho_s - \rho_l)g}{18\eta}$$

  - d is particle diameter
  - ρ_s and ρ_l are the density of dispersed phase and dispersion medium, respectively
  - η is the viscosity of dispersion medium
  - g is the acceleration due to gravity
  - Smaller particles lead to a low rate of sedimentation
  - Reduction of settling can be done by increasing the viscosity by adding cellulose derivative
  - Reduction of settling can be done by increasing the density of dispersed phase by adding sorbitol or mannitol
- Stokes' equation low is valid for dilute pharmaceutical suspension: $\leq$ 2 % of solid

$$v_{cor} = v * (1 - 6{,}55 * \emptyset) \text{ pour } \phi < 0.15 \qquad \emptyset = \frac{\textit{Solid volume}}{\textit{Volume of suspension}}$$

Floculation/Defloculation

- Flocculated suspensions (b) show a rapid sedimentation exhibiting a loose sediment
- Deflocculated suspensions (a) show a slow sedimentation but compact sedimentation

$$F = \frac{V_u}{V_0}$$

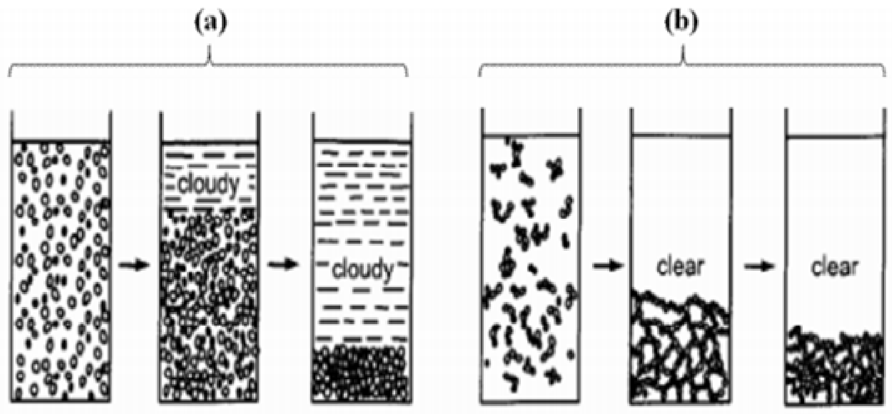
F is the sedimentation volume

V_u is the volume of the sediment at the equilibrium

V_0 is the volume of total suspension

F ranges from 0 to 1

$$\beta = \frac{F}{F_\infty} = \frac{V_u}{V_\infty}$$



β Is degree of flocculation (comparison to a standard deflocculated suspension

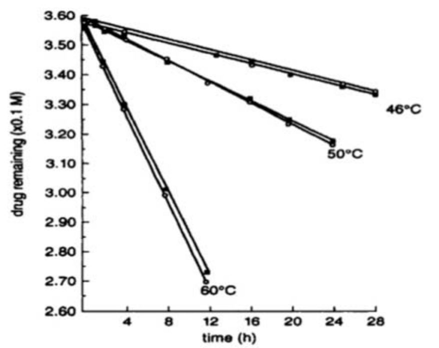
V_∞ volume of sediment of suspension when it is defloccualted

Characterization of the suspension

- Morphology
  - Scanning electron microscopy
- Polymorph and crystallinity
  - Milling of drug → creation of new surface → can cause change in the crystal
  - X ray diffraction
- Dilution in relevant media
- Long term stability if the suspension is selected as final product
- Tox and PK profile

Process ability assesssement

- **Process the stable physical form in vehicles**
  - Polymorphic conversion
  - Conversion of crystall form to amorphous form
  - Conversion of anhydrous form to hydrate form
- **Check chemical suspension stability at different temperature**
- **Check Physical and chemical stability at different stress**
- **Check milling ability**



Chemical degradation kinetic at different temperature

$$v(t) = (A/2)\cdot\omega\cdot\cos(\omega t)$$

$$Ek(t) = \frac{1}{2} * m * v(t)^2$$

Formulation screening case study

API X

- **Characteristics**
  - Molecular weight = 497 g/mol
  - Melting point = 158°C
  - Density = 1.4 g/ml
  - Log P = 6.9
  - Solubility = 0.2 µg/ml at neutral pH
  - pKa: NA
  - Morphology: needle shaped
  - Particle size: d_{10} = 9; d_{50} = 24; d_{90} = 61 µm
  - Crystalline form : stable crystal can be transformed to amorphous form
- **Requirement for nano-crystalline suspension**
  - Crystalline form: identical to unmilled API
  - Particle size: < 500 nm
  - Administration route: oral
  - Final dosage form: Tablet

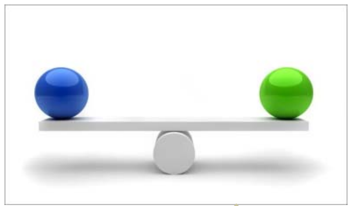
New strategy in Pharmaceutical industry

- **Test a new API in a target patient population as quickly as possible in order to validate the proof of concept**

Fast to patient

- **Product and process development based only on physico-chemistry and engineering science would provide a lot of scientific information but would be very time and resources consuming**
- **In contrary, a purely empirical methodology (e.g. design of experiment, trial error approach) may provide a quick solution with poor scientific information.**
  - Due to the lack of scientific understanding, long term stability or process robustness are not anticipated

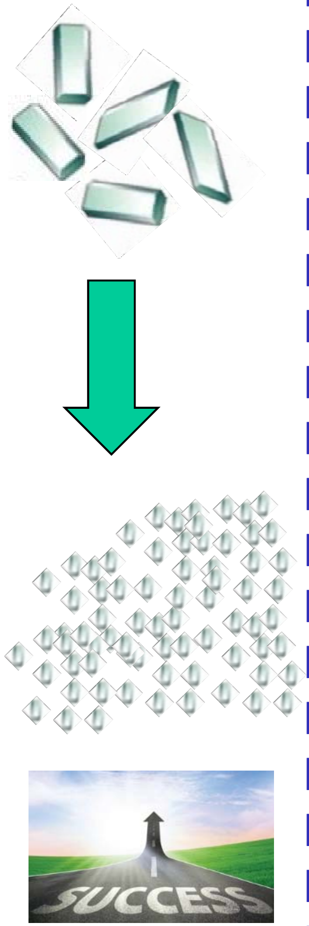
Scientific information



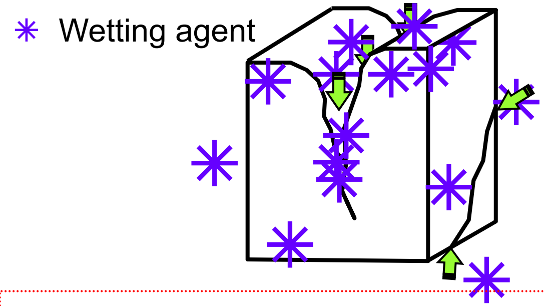
Timeliness & effectiveness

Pragmatic approach: compromise between purely scientific and purely empirical methodology to ensure economic and scientific criteria

Formulation engineering: Role of formulation ingredients



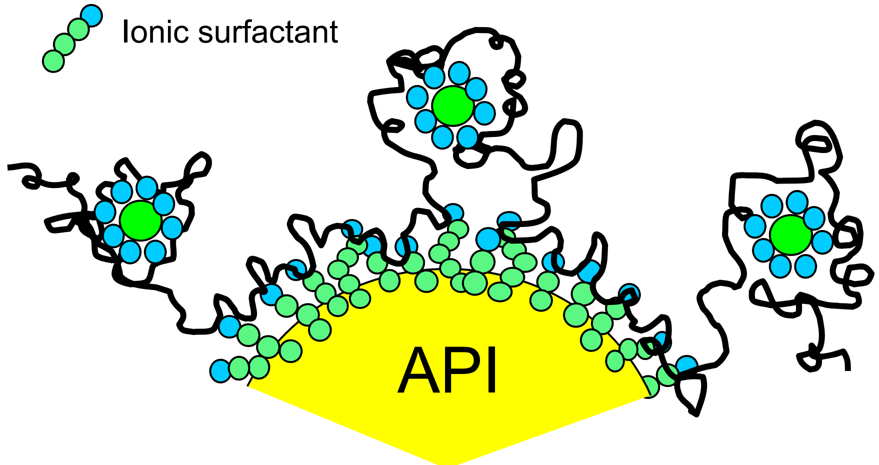
- **Wetting agent: Makes easy the milling process**



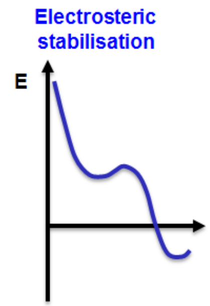
$$\Delta G = \gamma_{S/l} * \Delta SSA$$

- **Adsorbs at the surface of particles.**
- **Diffuses fast within the cracks to facilitates their propagation**

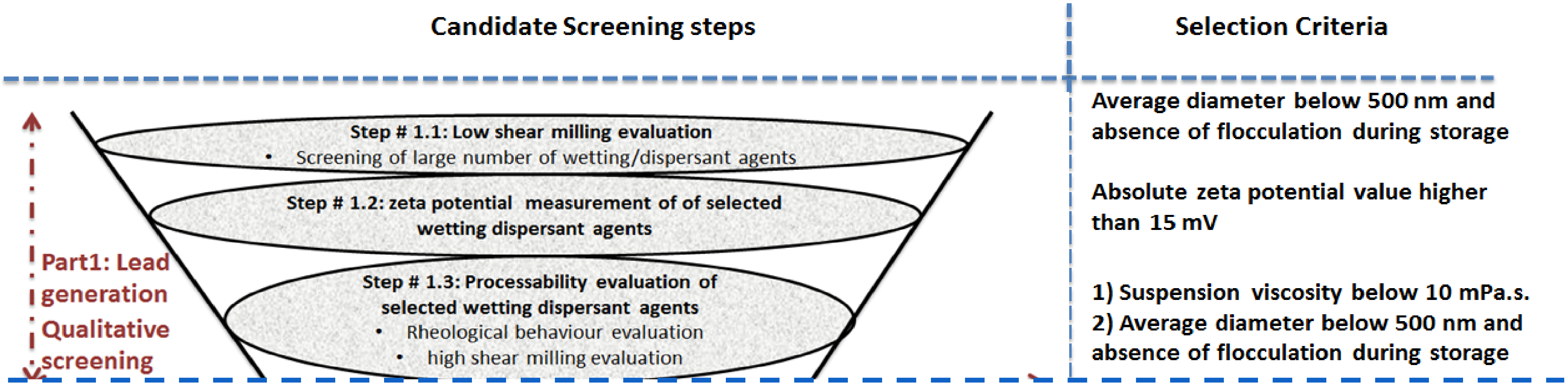
- **Dispersant agent: ensure effective stabilization during storage and administration**



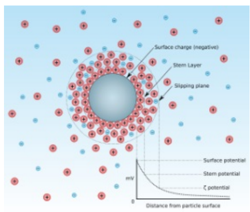
- **Electrosteric Coupling both stabilization mechanisms ensures a more robust stability**



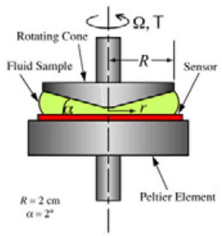
Formulation engineering – Step by step approach
(Part 1: Lead generation)



Milling ability: medium throughput Screening: Step # 1.1



High Zeta potential: Step # 1.2



Rheology: Step # 1.3a

High shear milling: Step # 1.3b

More than 20 wetting dispersant agent were investigated

Qualitative screening results: milling ability using low shear mill (Step #1.1)

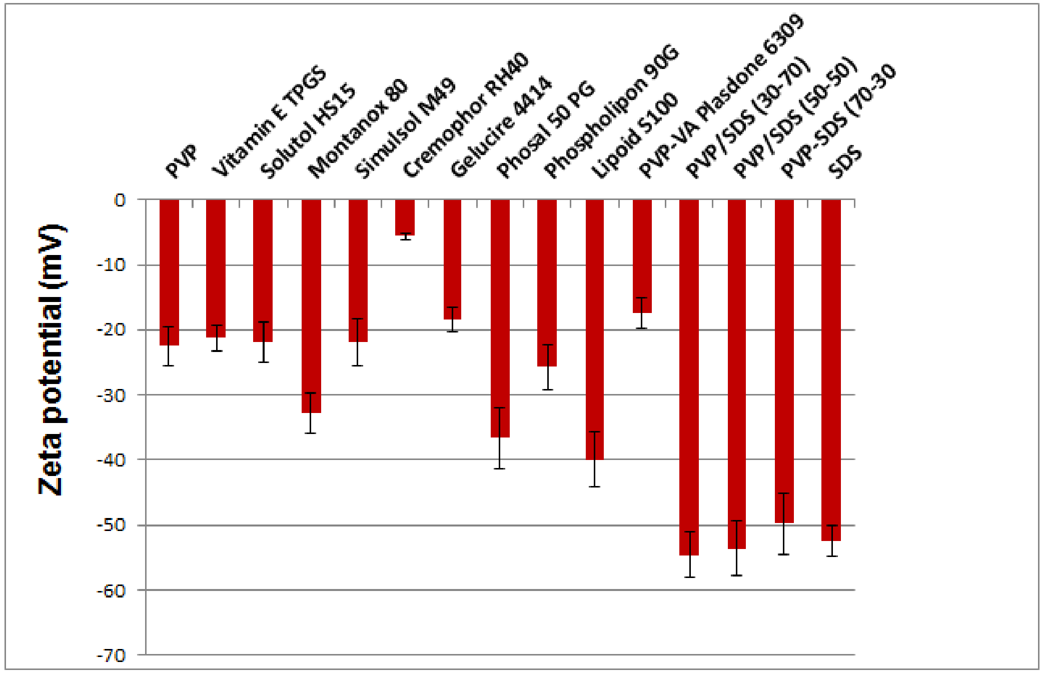
- More than 20 wetting/dispersant candidates (3%) + Hydrophobic API (20%) + WFI (87%) were assessed using visible observation, particle size measurement and stability after 2 weeks at room temperature
- full coverage of particles having approximately 80 nm mean diameter assuming a typical adsorption of 3 mg/m²

- HPC, PEG 8000, Montanov 68, Sodium polyacrylate, HPMC, Poloxamer 188 and Poloxamer 407 were discarded

Qualitative screening results: Zeta potential measurements (Step #1.2)

- **At this step the measurement of zeta potential of selected samples from step #1.1 were carried out. To ensure electrostatic repulsion, an absolute value greater than 15 mV was fixed as criterion.**

  - All the wetting/dispersant agents, except the Cremophor1 RH40, gave an acceptable zeta potential value
  - The charged species (SDS, PVP–SDS) lead to a high absolute value

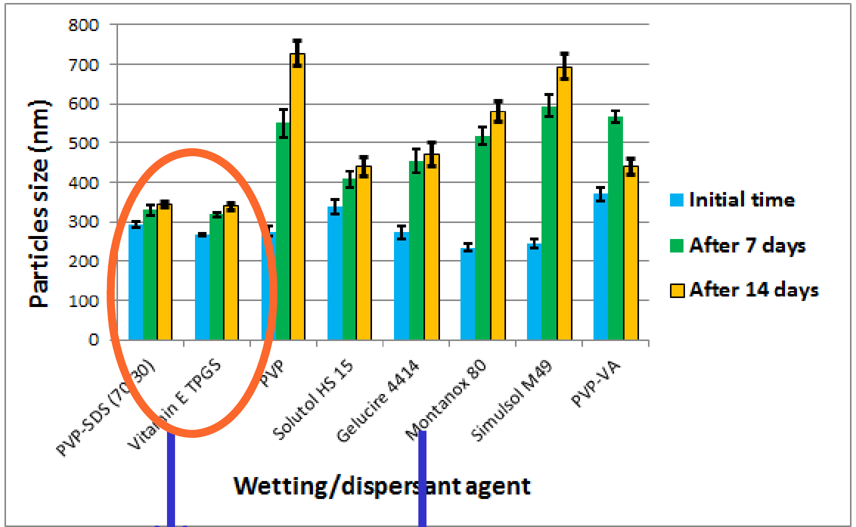


Qualitative screening results: Process ability assessement – Rheology (Step #1.3a)

- **At this step, the viscosity of unmilled suspension was measured as a function of shear rate as well as of thixotropy. The samples that gave viscosity greater than 10 mPa.s at shear rate of 1000 s^{-1} were excluded.**
  - **Essential to ensure faster milling kinetics as well as manufacturing-ability at industrial scale.**
  - **suspensions made of Phosal1 50 PG, Phospholipon1 90 and Lipoid1 S100 were excluded (viscosity higher than 10 mPa.s**

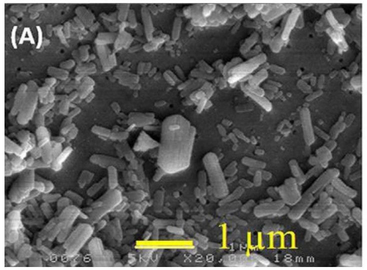
Formulation engineering – Lead generation – High shear milling.

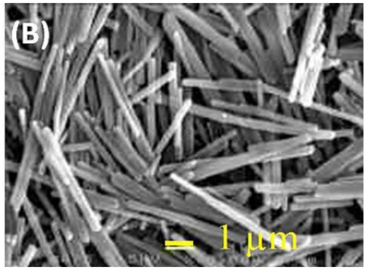
- **8 candidates were evaluated at high shear rate**



SDS/PVP at a ratio of 70/30 and Vitamin E TPGS provided the highest stabilization of the nanocrystalline formulations.

small, but irregular shaped particles





Needles shaped crystals outlining crystallization (Ostwald ripening)

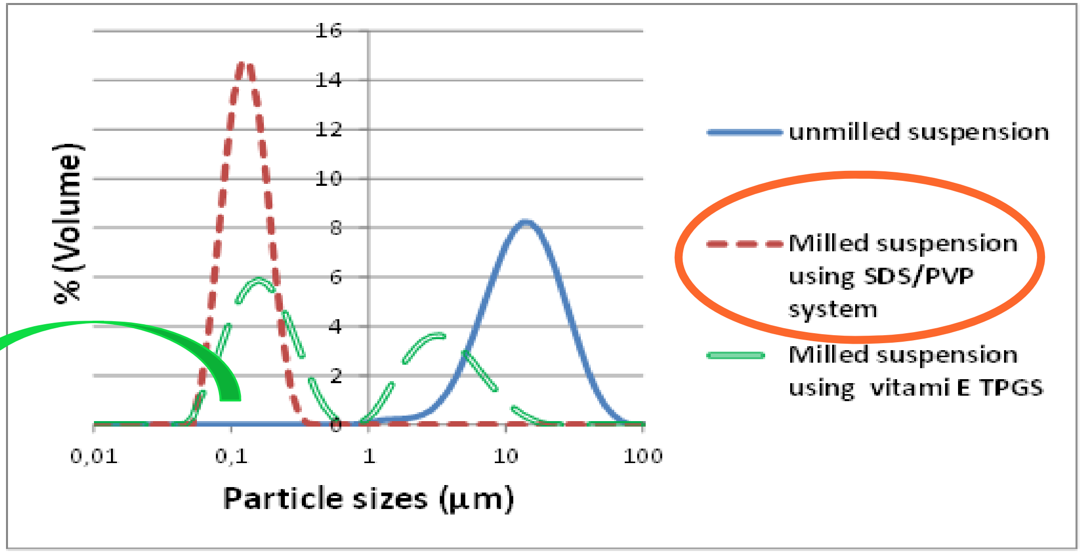
Formulation engineering – Lead generation – Long milling duration

- **High shear milling was performed during a long duration to assess the stabilization effectiveness**

13 hours at shear rate of 22000 s^{-1}

Overall shear strain of 1.0 10^9

Gel aspect

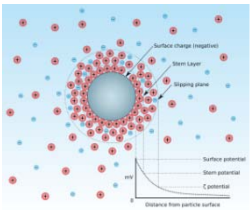


- SDS/PVP system leads to a suspension with particle size in the nanometric range having mono-modal distribution
- Vitamin E TPGS® led to a suspension with particle size in the micron range exhibiting bi-modal distribution. Aggregation can be due to extraction of stabilizer from particles by the applied stress during a long period and absence of electrostatic stabilization.

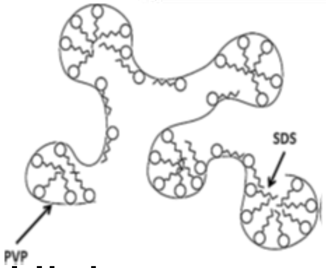
Formulation engineering –
Step by step approach (Part 2: Lead optimization)

Candidate Screening steps	Selection Criteria
Step # 2.1 : SDS-PVP ratio optimization • Surface tension measurement • zeta potential measurement	1) Low CMC value 2) High absolute zeta potential value
Step # 2.2: PVP-SDS amount optimization: • wetting evaluation • high shear milling evaluation, adsorption measurement	1) High wettability: maximum reduction in the surface energy of the powder 2) Generation of suspension with a high implicit specific surface area 3) Obtaining of high affinity adsorption isotherm
Step # 2.3: Stability evaluation	Absence of flocculation during 8 weeks of storage

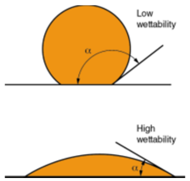
Part2: Lead optimization
Quantitative screening



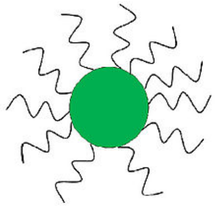
High Zeta potential



High synergy between PVP and SDS



High wettability



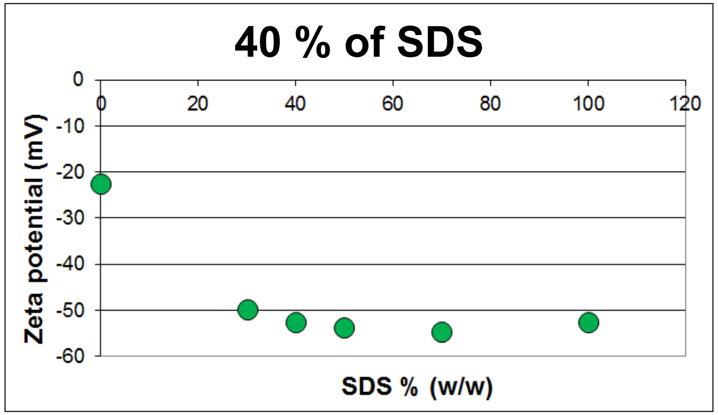
Strong adsorption

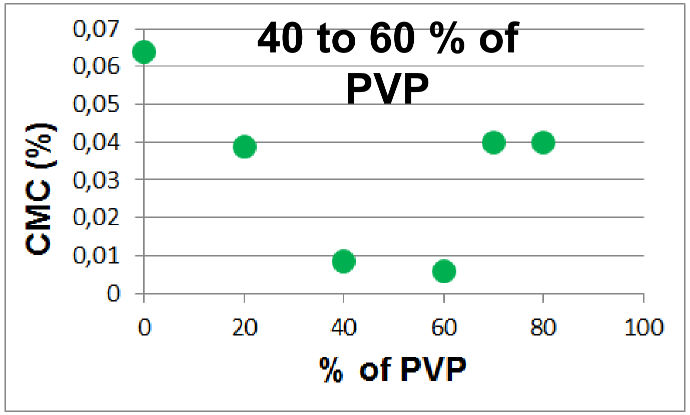
Milling evaluation

Stable and robust formulation

Formulation engineering – Lead optimization – Composition and concentration optimization of lead candidate

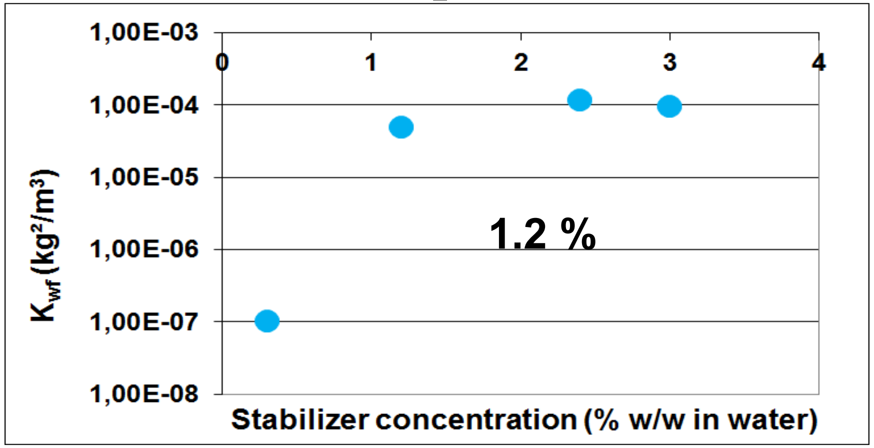
- **Composition optimization (zeta potential and PVP/SDS synergy)**

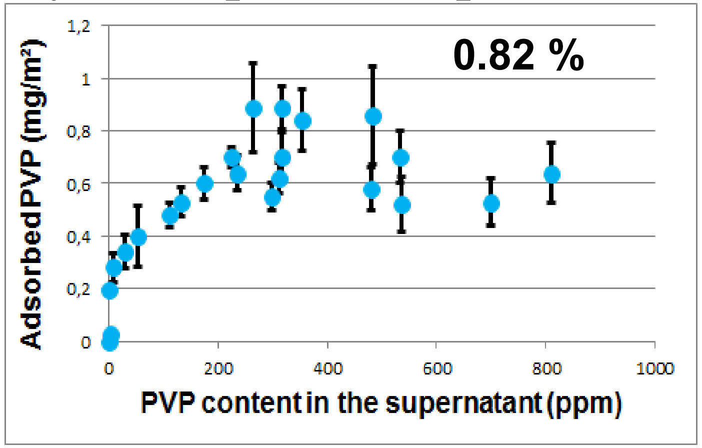




Optimal composition PVP (60 %) – SDS (40%)

- **Concentration optimization (wettability, adsorption and process-ability)**

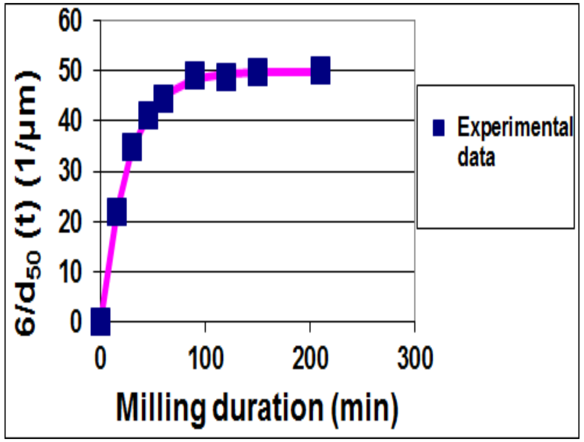




Process ability

Formulation engineering/ Lead optimization – Concentration optimization – High shear milling

- **Milling kinetic were conducted at different concentrations of stabilizer**



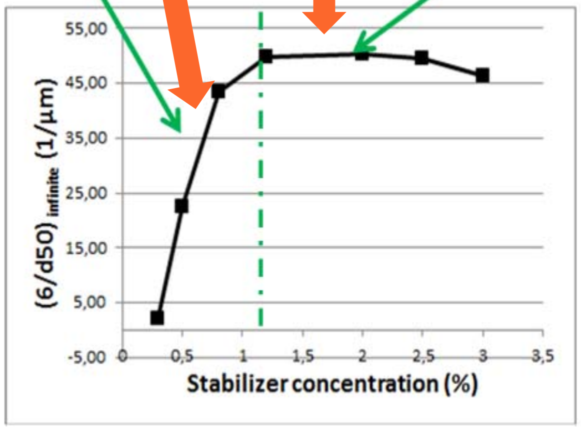
$$\frac{6}{d_{50}} = \left(\frac{6}{d_{50}}\right)_{\infty}\left(1 - e^{\frac{-t}{\tau}}\right)$$

Not suffisant stabilizer to cover the surfaces produced by the technology

Mechanical energy limitation to produce higher surfaces

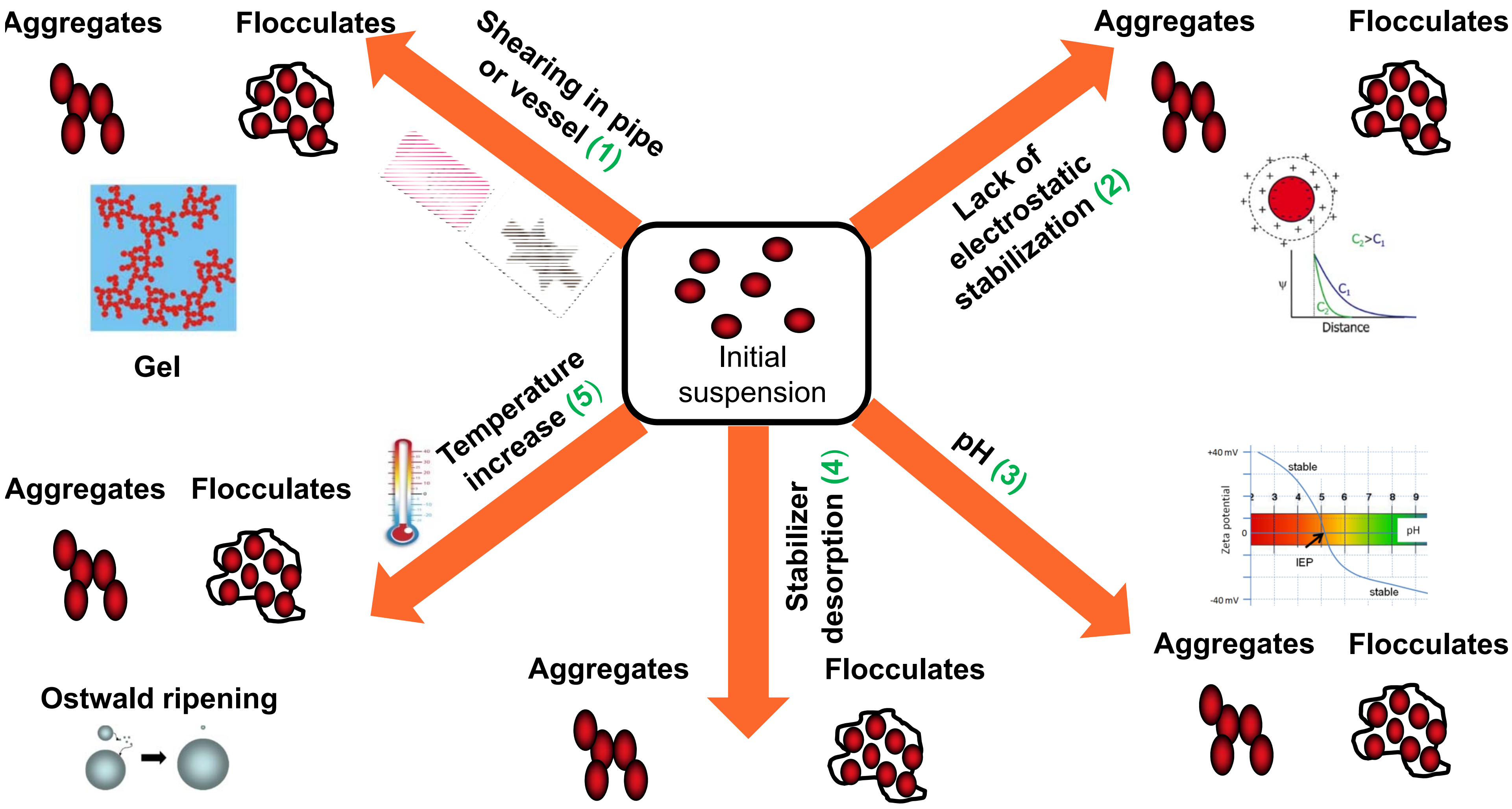
Poor domain

Rich domain



Optimal concentration =1.2 % of PVP – SDS at ratio of 60-40 %

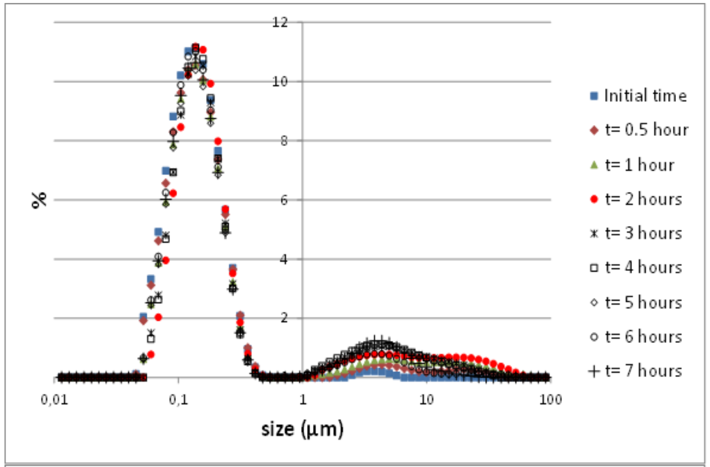
Formulation engineering – Robustness evaluation - risk assessment



Formulation engineering – Robustness evaluation - derisking approach: Test #1

Shearing (26000 s^{-1}) at native ionic strength (without salt addition)

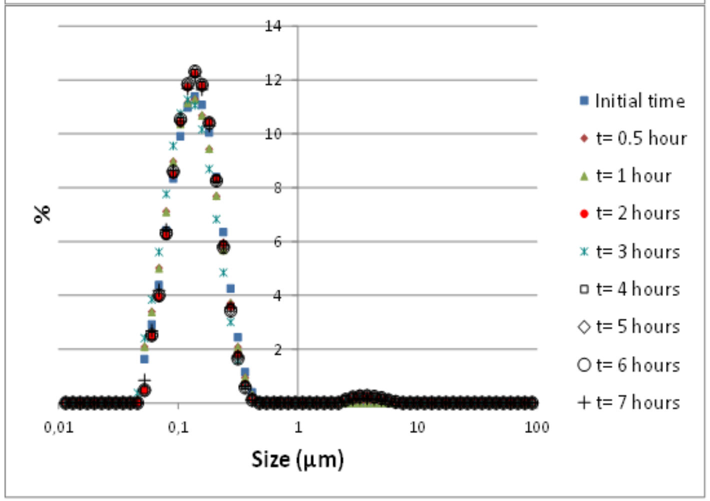
1



Increase of particle size without salt addition → Extraction of PVP chains by shearing due to the high solubility of PVP in water

Shearing (26000 s^{-1}) at 0.17 molar of Nacl (corresponding to 0.24 x CCC)

1



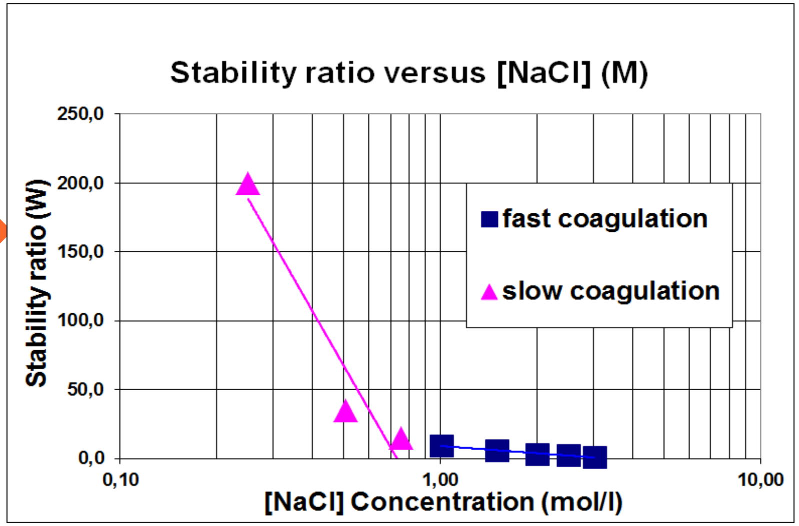
Adsorption of PVP becomes stronger and hence orthokinetic flocculation is reduced

⚠ Shear rate needs to be managed during downstream processing (threshold?)

Formulation engineering – Robustness evaluation - derisking approach: Tests # 2 & 3

Measurement of coagulation rate at different concentration of electrolyte

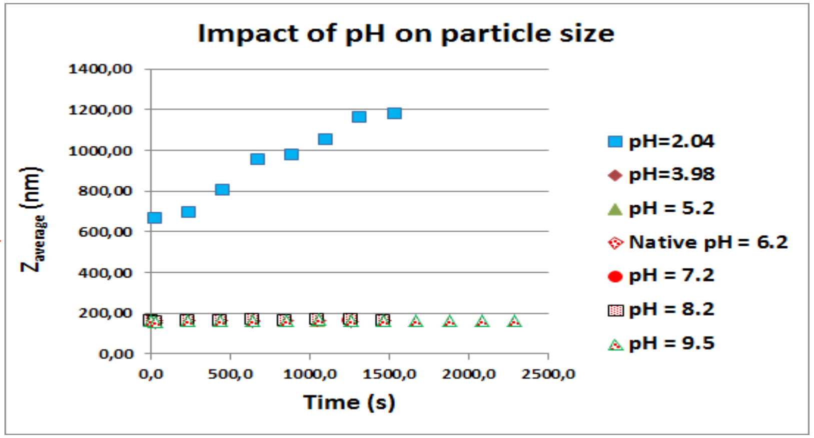
2



C.C.C ~ 0.7 of Nacl indicating high colloidal stability

Dilution of nano-suspension in acidic or basic solution covering a wide range of pH.

3

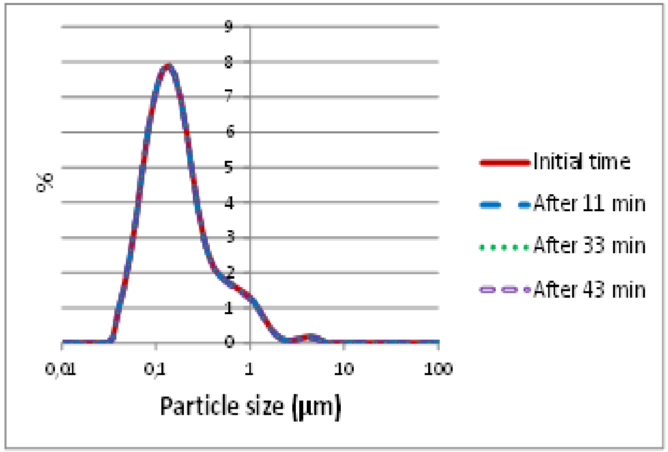


Particle size did not change over time when the pH was higher than 2

Formulation engineering – Robustness evaluation - derisking approach: Tests # 4 & 5

Dilution of nano-suspension in water (f=1000) under stirring conditions

4



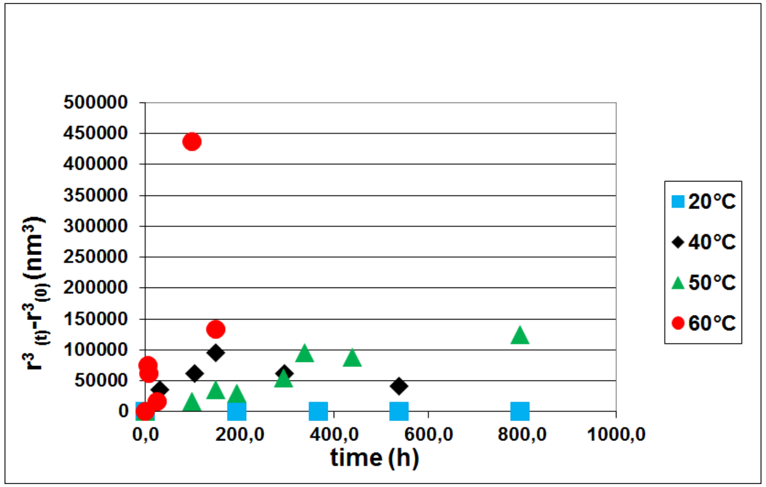
Unchanged particle size distribution indicating high colloidal stability

Measurement of particle size as function of storage time at different temperature

Crystal growth

5

$r^3_{(t)} - r^3_{(0)}$



As the temperature increases to 40°, 50° and 60 °C, significant increase of particle size with time is observed

Ostwald ripening

Heat protectant needs to be added if autoclaving is considered

Process engineering: Comparison of manufacturing technologies for production of nano-crystalline suspension: case study

Milling principles: HPH vs beads milling

- High pressure homogenization

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By courtesy from GEA Niro-Soavi

  - The homogenization valve converts the High Pressure pump to HPH
    - Particles collision
    - High kinetic energy
    - Cavitation (formation, growth, and implosive collapse of vapour bubbles in a liquid)

- Beads milling.
  - The high energy and shear forces generated as results of impaction of the grinding media with the API provide the energy input to break the API

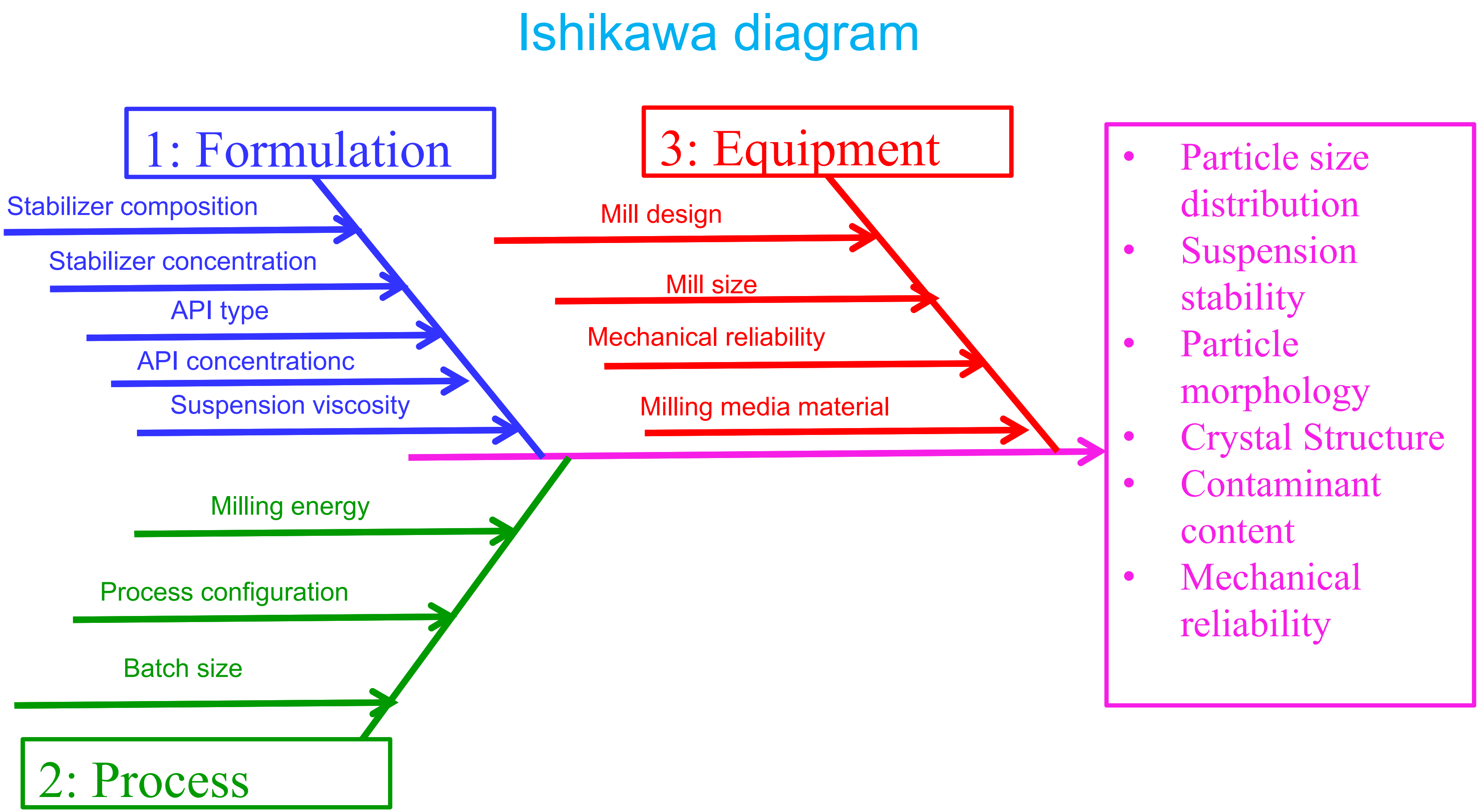
..\Thèse\NETZSCH_DeltaVita(R)15-300_Animation.wmv

By courtesy from Netzsch

Which technology is suitable for pharmaceutical application?

- The selection of suitable technology for the production of nano-crystalline suspension depends on a number of questions about the quality of produced nanosuspension by both technologies such as:
  - How does the technology impact the formation and stability of nanosuspensions, hence their overall performance?
  - How does the physical stability of the particles differ when using the same API in both technologies?
  - How are the particles size distribution and shape impacted by the technology used?
  - Which technology is easily scaled-up, providing little batch-to-batch variation?
  - How sensitive is the technology towards generation of residues of the milling media in the final product due to erosion?
  - How does the crystal structure or amorphization of the nanoparticles change when using both technologies
  - Are the technologies sufficiently reliable, robust and compliant with pharmaceutical regulations for the production of pharmaceutical suspension that can be used for different delivery systems?
- Systematic comparison of both technologies was carried out

Which technology is suitable for pharmaceutical application? Parameters to be investigated



Data analysis and methodology for HPH and beads milling comparison: Quantitative tools

- **Although 5% of all energy is used in size reduction, the energetic methodology still as relevant approach to study milling process.**
  - To achieve the milling in the nano range, high input of energy is required (surface tension of milling medium time new surface area generated)

$$E_s = \gamma \times \Delta SSA$$

  - Rittinger proposed that the energy required for particles size reduction was directly proportional to the area of new surface created as described by Eq.

$$E = C \times \left(\frac{1}{d_2} - \frac{1}{d_1}\right)$$

  - Where E is the required energy of milling, C is constant, d_1 is the starting and d_2 the final particle size

Data analysis and methodology for HPH and beads milling comparison: Quantitative tools

- In 1958, Tanaka suggested a first order equation for characterizing the kinetics of milling in batch mode

$$SSA_{(t)} = SSA_{(\infty)} \times (1 - e^{-k \times E_m})$$

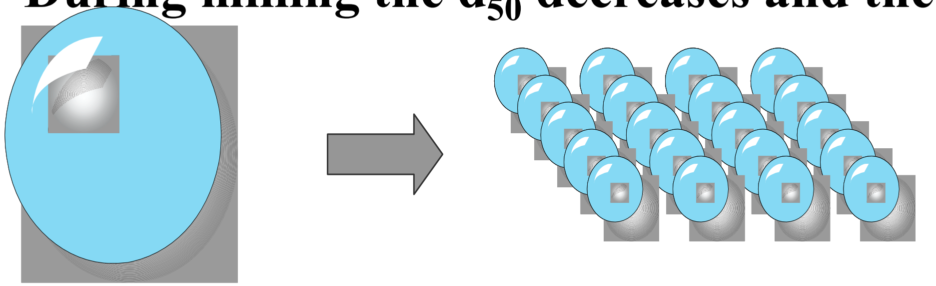
- Where, SSA(t) is specific surface area after time t, Em is the specific energy input, SSA(∞) the specific surface area at equilibrium and k a constant.
- Later in 1972 Chodakov improves the previous model by introducing the specific power as described by the equation.

$$SSA_{(t)} = SSA_{(\infty)} \times (1 - e^{-k' \times t}) \qquad k = \frac{k'}{P}$$

- The constant k' implies the significance of rate constant of new surface formation and P is the specific power of milling.

Data analysis and methodology for HPH and beads milling comparison: Quantitative tools

- **As the product is made of suspension, it is not easy to measure the specific surface area**
- **During milling the d_{50} decreases and therefore SSA increases**



$V = \pi d^3/6 \rightarrow m = \rho^* \pi d^3/6$

$S = \pi d^2$

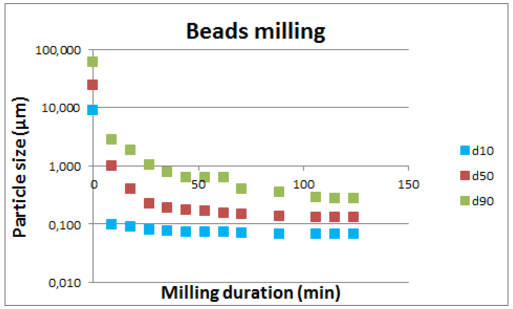
$SSA = S/m = 6/\rho^* d \sim 6/d_{50}$

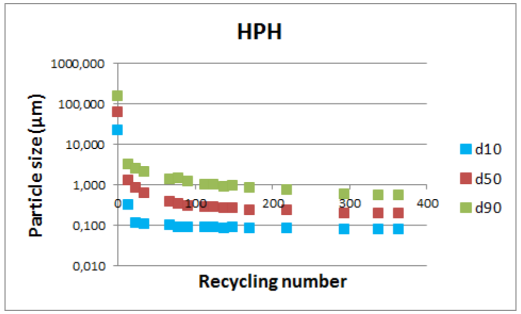
- **The created SSA = SSA $_{(milled)}$ – SSA $_{(unmilled)}$**
- **SSA $_{(milled)}$ >> SSA $_{(unmilled)}$ → Created SSA = SSA $_{(milled)}$ ~ $6/d_{50}$**

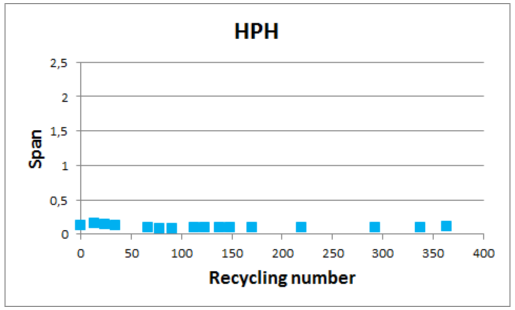
- Milling kinetic can be followed up by the increase of 6/d50

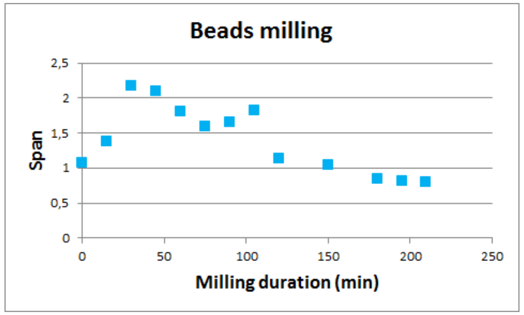
Data analysis and methodology for HPH and beads milling comparison: Qualitative tools (1)

- During milling the d_{90} and d_{50} decrease at the same speed whereas the d_{10} decreases much more slowly. The results suggest that d_{90} and d_{50} belong to the same category of milling behavior.
- Particles below 0.1 µm are not milled
- Span = $(d_{90}-d_{10})/2*d_{50})$ provides a limited information insofar as the amplitude of distributions is quite large. It not provides any information on the symmetry of the distributions



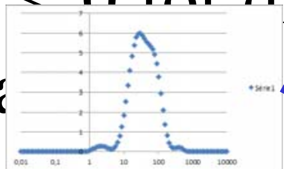


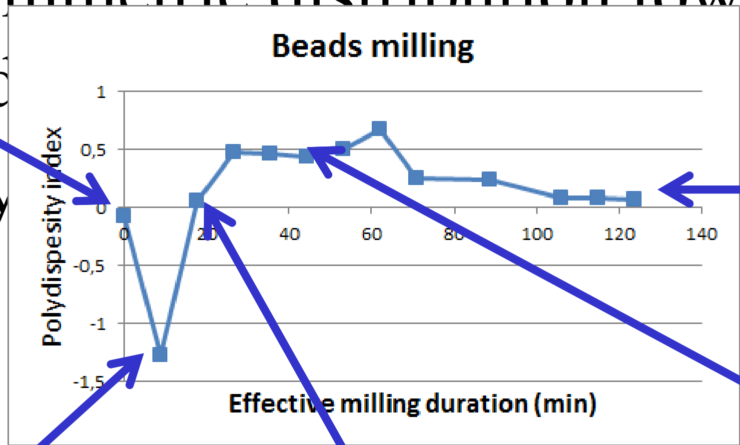


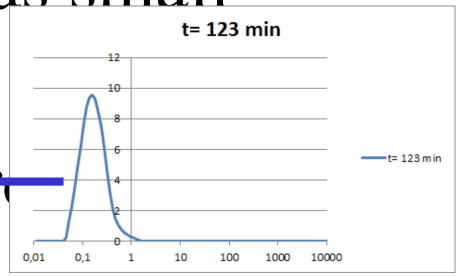


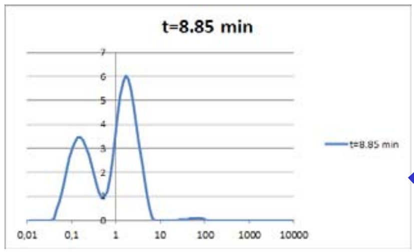
Data analysis and methodology for HPH and beads milling comparison: Qualitative tools (1)

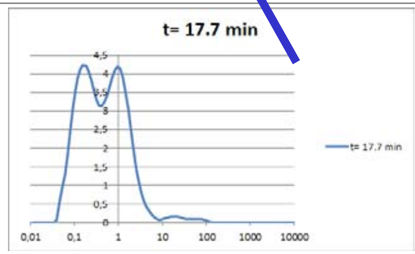
- Polydispersity index = $\ln(d_{10}*d_{90}/d^2_{50})$ is more appropriate to describe the symmetry of distribution.
  - PI = 0 for log-normal distribution
  - PI < 0 for dissymmetric distribution towards small di… 50/d…
  - PI > 0 for dissy… …vard… diameters

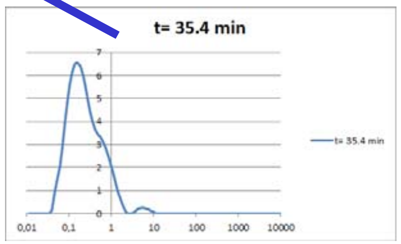












Data analysis and methodology for HPH and beads milling comparison: Qualitative tools (1)

- Polydispersity index = $\ln(d_{10} * d_{90} / d^2_{50})$ is more appropriate to describe the symmetry of distribution.
  - PI = 0 for log-normal distribution
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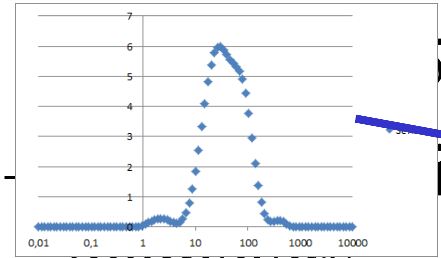
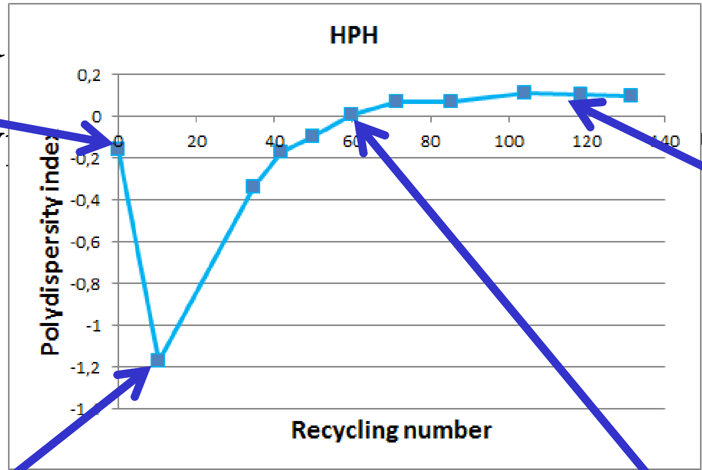
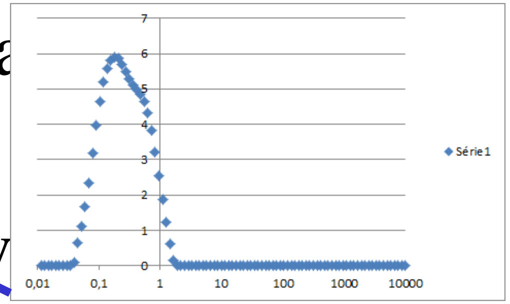
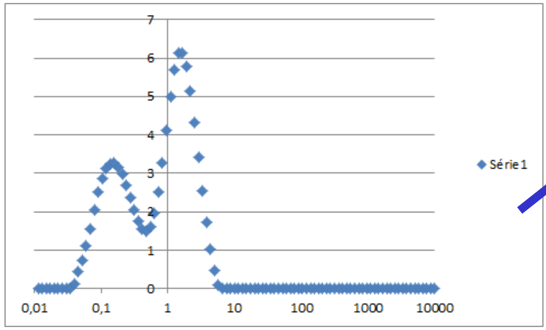
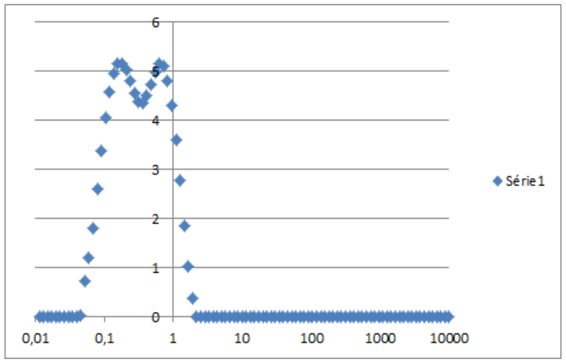




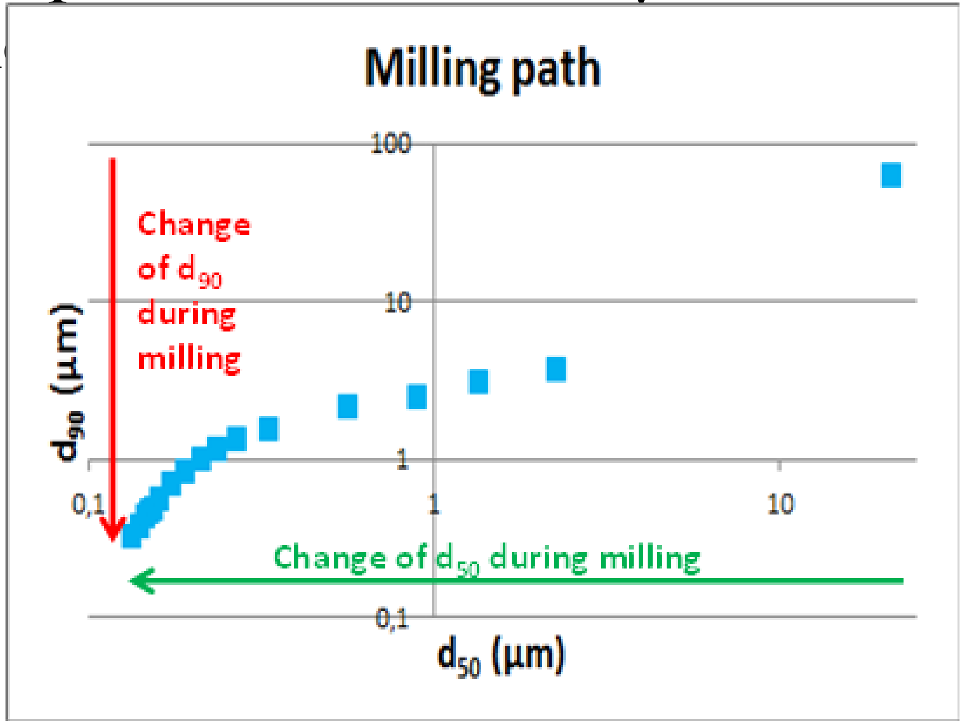




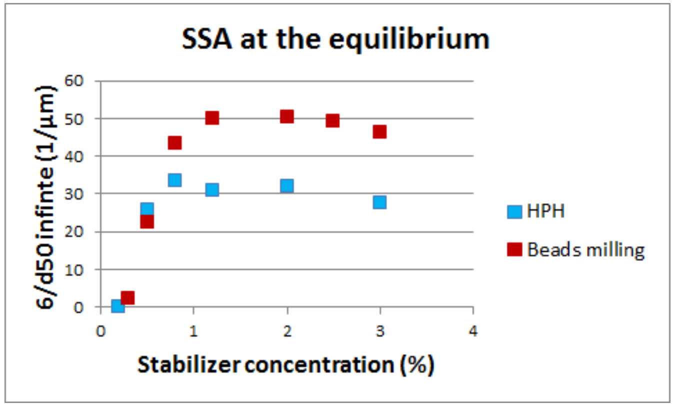


Data analysis and methodology for HPH and beads milling comparison: Qualitative tools (2)

- Particle size reduction Path
  - As d90 and d50 belong to the same category of milling behavior and particles below 0.1 µm are not milled
  - Proposal: m



Technological evaluation Comparison: Formulation impact: Stabilizer composition and it's content (1)

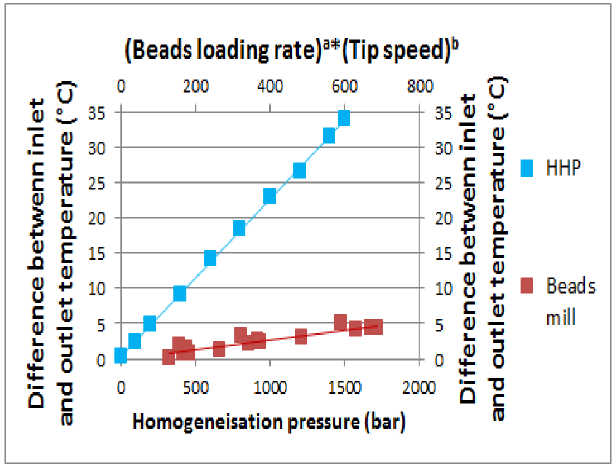


Beads mill Vs HPH

- For both technologies, one can observe that the impact of % of stabilizer reveals 2 regimes: Poor regime where the created new surface are driven by the stabilizer content and rich regime where the created new surfaces are driven by the technology
- In order to get a maximum surface reduction the suspension has to be formulated in the rich Regime

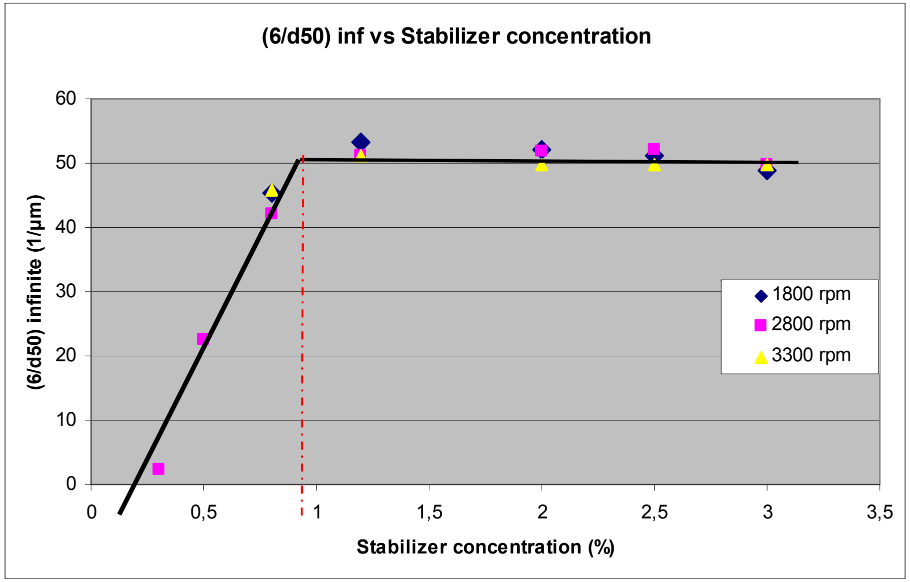
Technological evaluation Comparison: Formulation impact: Stabilizer composition and it's content (1)

- The HPH leads lower created new surfaces. This may be due to
  - The fact that product is submitted to high temperature amplitude which could leads to Oswald ripening or
  - low plateau of adsorption isotherm at 45°C
  - difference in terms of stress level between HPH and bead mill

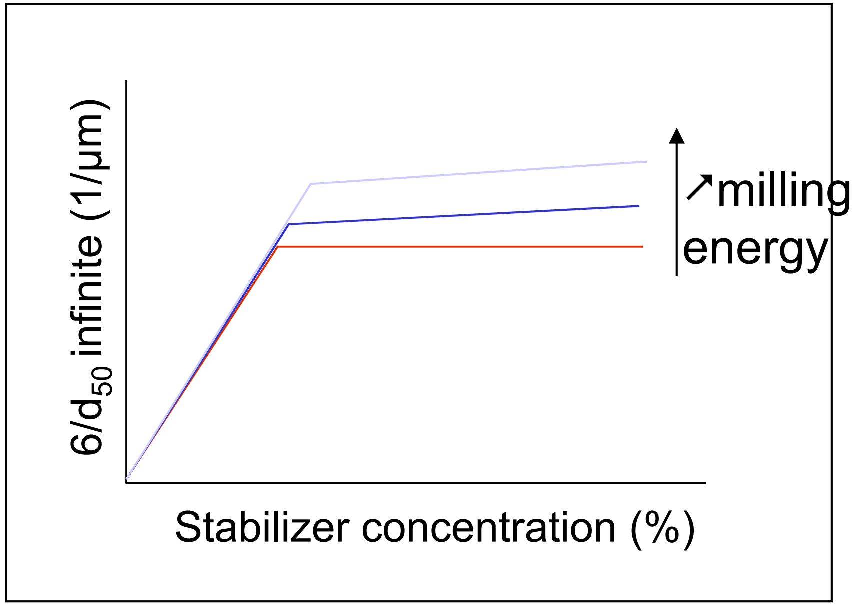


Technological evaluation Comparison: Formulation impact: Stabilizer composition and it's content (Beads milling)

Nanosuspension

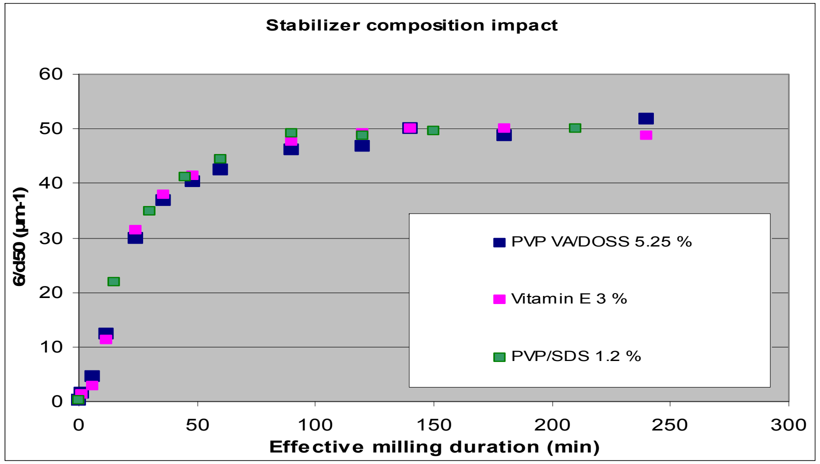


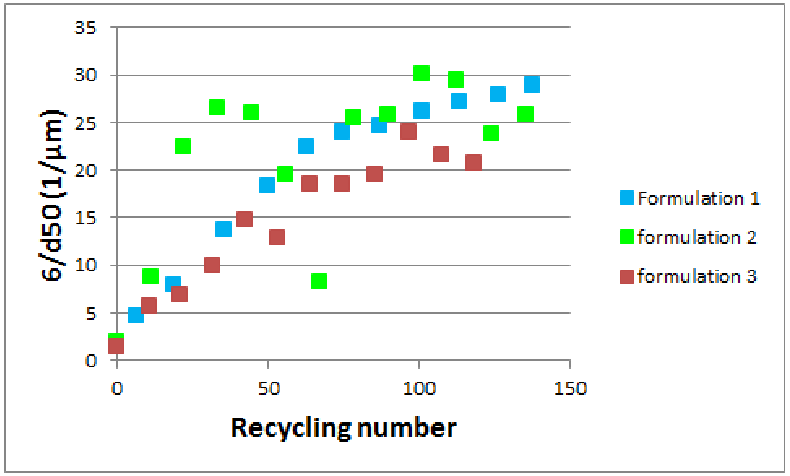
Emulsion (from literature: $((6/d50)_{\infty} \sim P^{0.6\ \text{to}\ 0.9})$



- No impact of milling hydrodynamic on nanocrystals size reduction in rich domain
- **Impact of milling hydrodynamic on emulsion size reduction in rich domain**

Technological evaluation Comparison: Formulation impact: Stabilizer composition and it's content





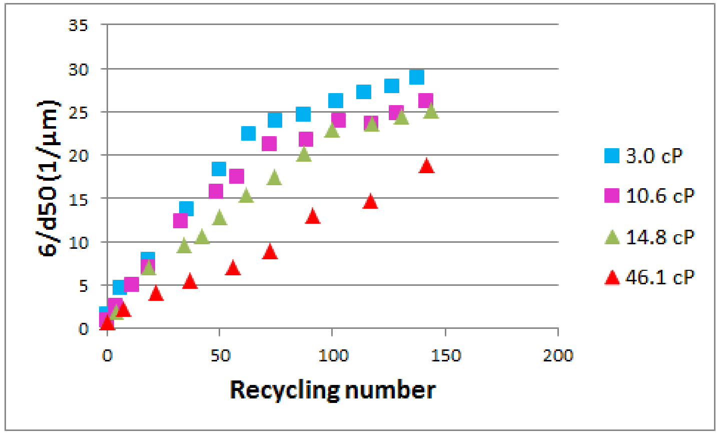
Beads mill

Même echelle

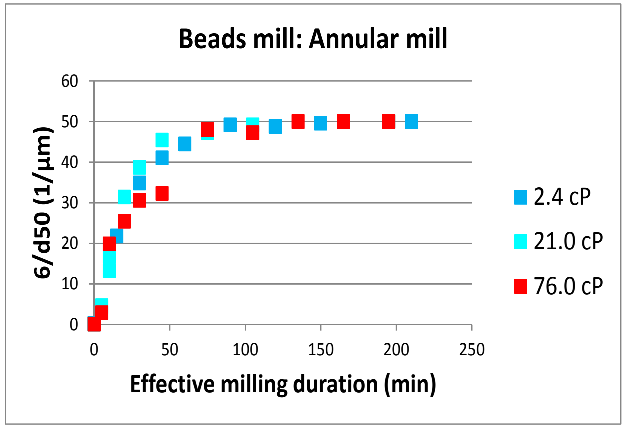
HPH

- As far as the formulation is stable, whatever the used formulation there is no significant impact on the equilibrium specific surface area

Technological evaluation Comparison:Formulation impact: Viscosity



HPH



Beads mill

L.Peltonen *et al*. reported that the high viscosity enhances the stability of the end product but High viscosity of milling vehicle may require longer processing times (*)

- For the HPH, higher is the viscosity, slower is the milling kinetic
  - power density~viscosity$^{-0,332}$
- For the beads milling , Annular mill and pin-counter-pin mill doesn't react similarly to rheology impact (still under

(*) Leena Peltonen, J.H., 2010. Pharmaceutical nanocrystals by nanomilling: critical process parameters, particle fracturing and stabilization methods. Journal of Pharmacy and Pharmacology 62, 1569–1579

Technological evaluation Comparison Formulation impact: API considered

–API A

MW = 497.4 g/mol

T_m = 156.7°C

Density = 1.419 g/cm^3

LogP = 6.9

Solubility:

- **API B**

MW = 456.4 g/mol

Tm = 241.6°C

LogP = 4.75

- **API C**

MW = 401.4 g/mol

Tm = 183°C

LogP: 4.1

Solubility: 7.1 µg/ml

- **API D**

MW = 411.85 g/mol

Tm = 240°C

Density = 1.48 g/cm3

LogP = 2.9

- **API E**

MW = 255.3 g/mol

Tm = 166°C

Density = 1.328 g/cm3

LogP = 2.9

- **API F**

MW = 408.5 g/mol

Tm = 183.7°C

LogP > 5.7

- **API G**

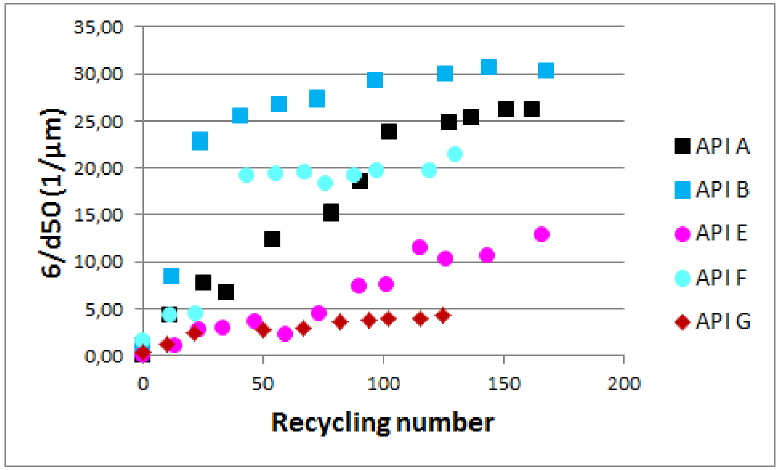
MW = 405 g/mol

Tm= 92.7°C

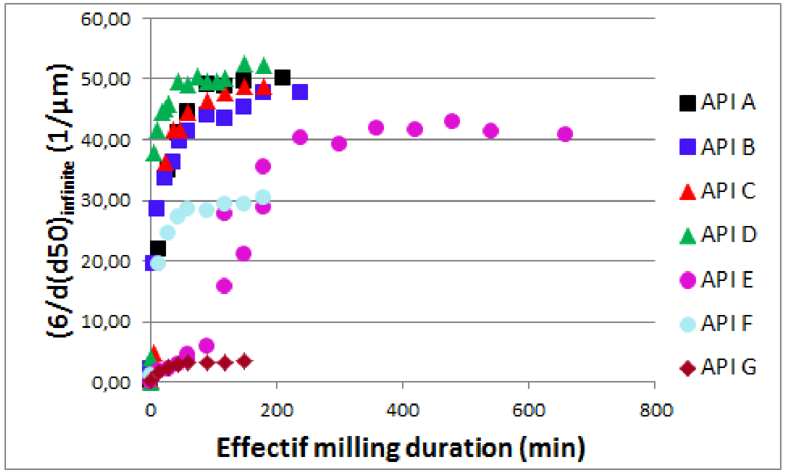
Density = 1.255 g/cm3

LogP = 2.5

Technological evaluation Comparison
Formulation impact: API considered



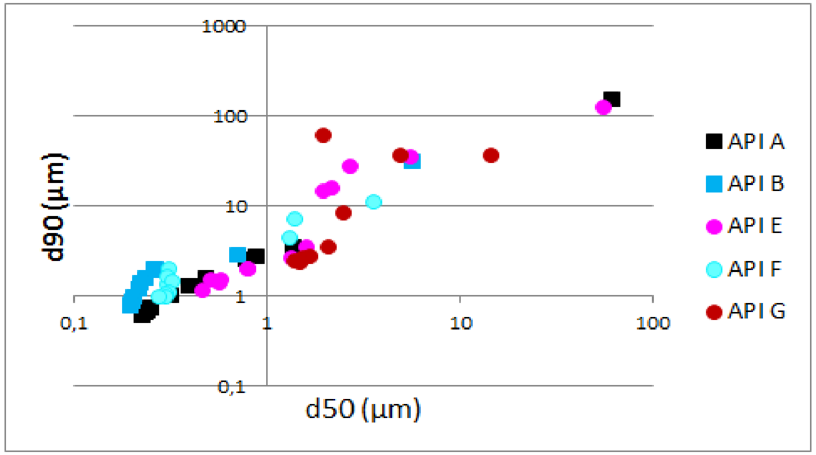
HPH



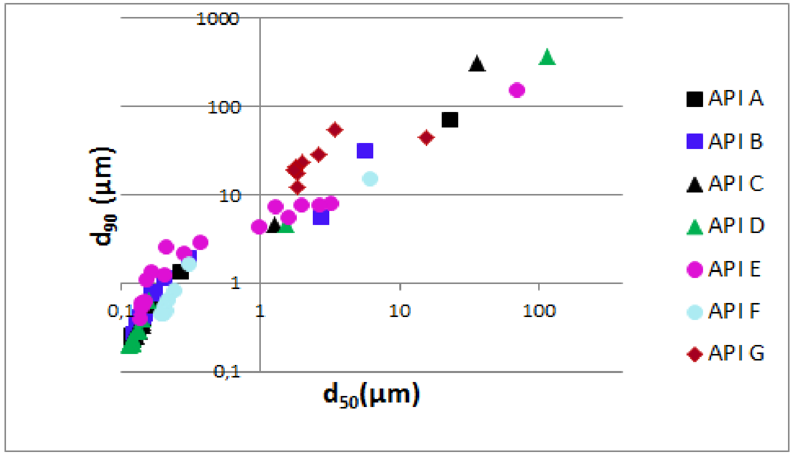
Beads mill

- For the both technologies the milling kinetic is dramatically impacted by the API type
- Provided the formulation is stable, the milling kinetic profile is the same whatever the technology used
- Some API are difficult to mill or cannot be milled. These API will require alternative approaches (Bottom up, emulsion, liposomes)
- The milling ability is not correlated with API characteristics (no obvious trend)

Technological evaluation Comparison
Formulation impact: API considered



HPH

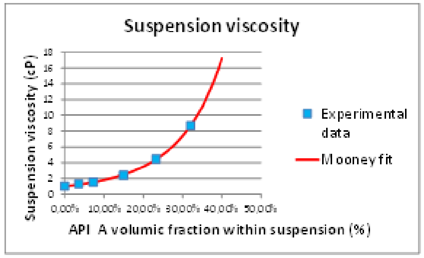


Beads mill

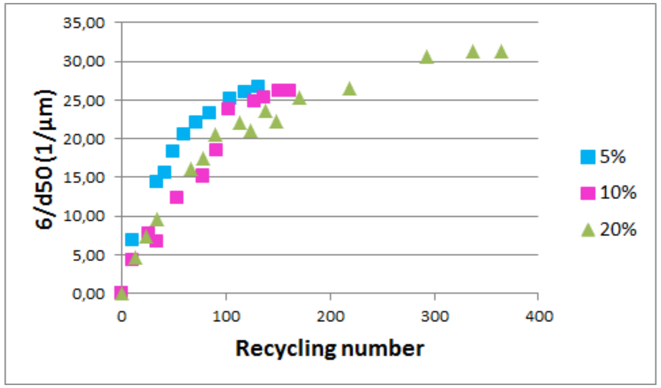
- The plot of d90 versus d50 shows that the profile of d90 versus d50 is API dependent

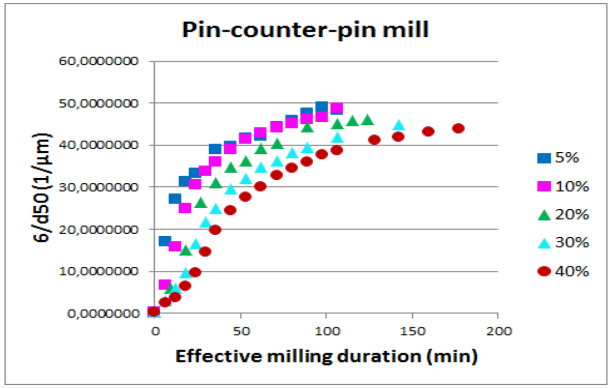
Different process
signature or different
milling mechanism

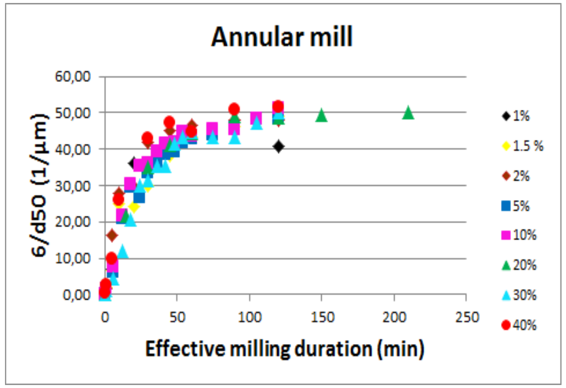
Technological evaluation Comparison Formulation impact: API concentration



- The higher is the API loading, the higher is the viscosity







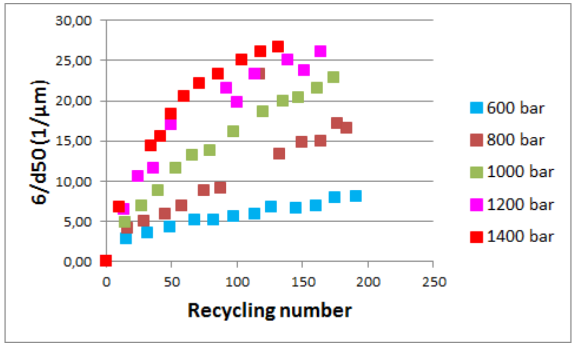
HPH

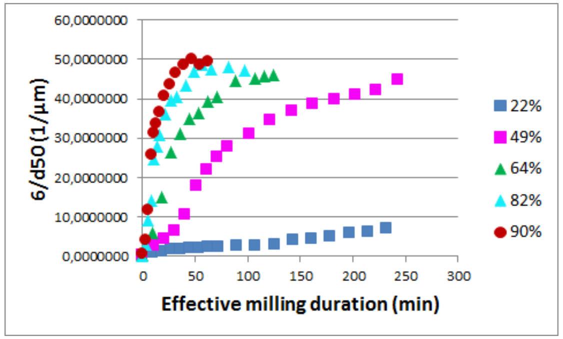
Beads mill

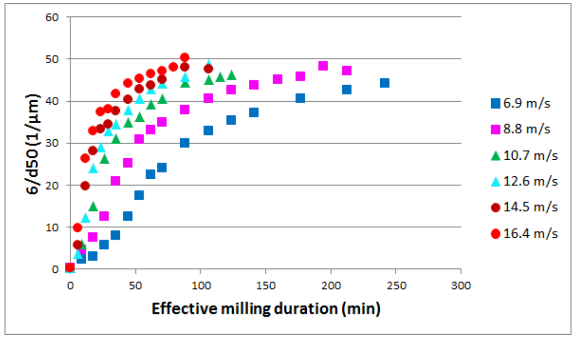
- For HPH and Annular beads mill the API concentration has not any significant impact on the milling kinetic. However, for pin counter pin mill, one can observe that the lower is the concentration, the faster is the milling kinetic: matter of viscosity

Technological evaluation Comparison
Operating parameters impact: Milling Energy

$Pw = P^* Q$ $\qquad$ $Pw \sim (N^x \phi^y)/t$







HPH

Beads mill

- For both technologies the increase of milling energy (Pressure for HPH, Rotation speed and/or beads filling ratio for beads mill) leads to a faster milling kinetic

HPH

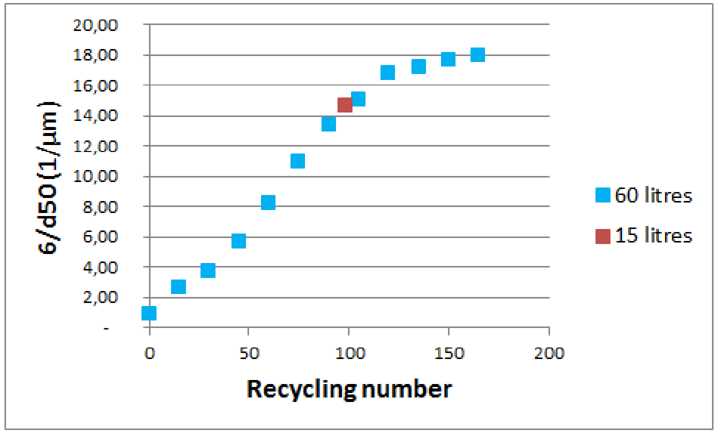
Time to reach 63 % of equilibrium SSA varies as (Pressure)$^{-2.6}$

Beads mill

Time to reach 63 % of equilibrium SSA varies as (tip speed)$^{-1.95}$ and as (Beads loading rate)$^{-3,9}$

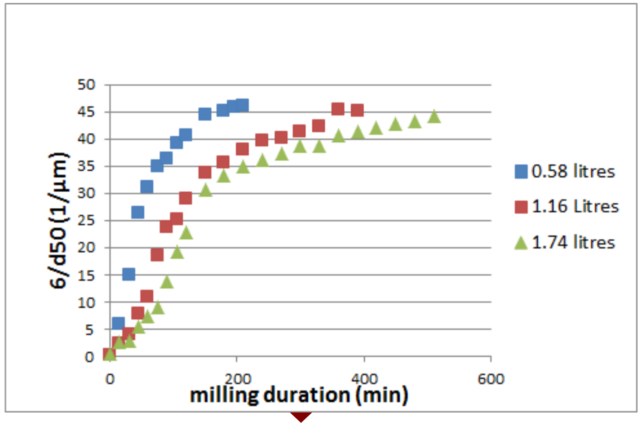
Technological evaluation Comparison
Operating parameters impact: Batch size impact

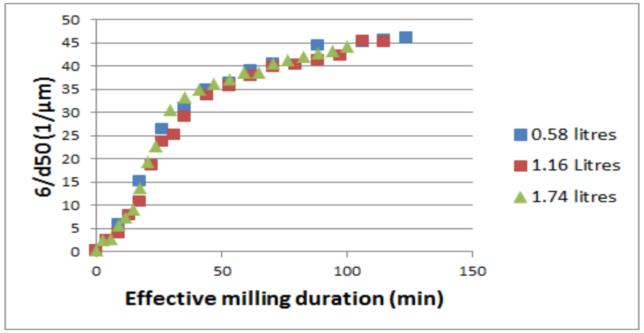
HPH



$$RN = Duration \times \frac{HPH \ \ flow\,rate}{Batch \ size}$$

Beads milling

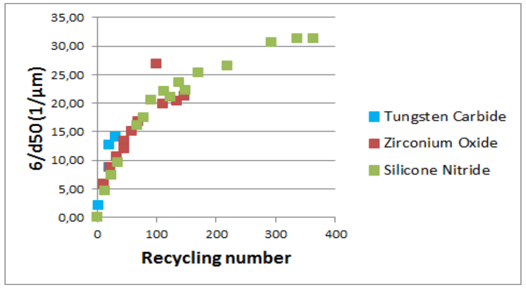


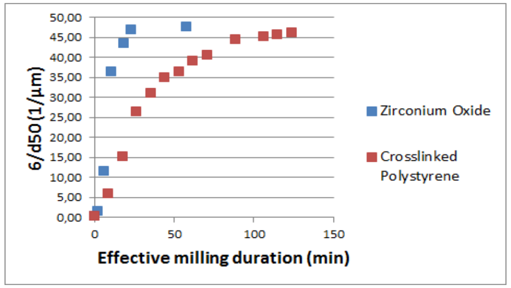


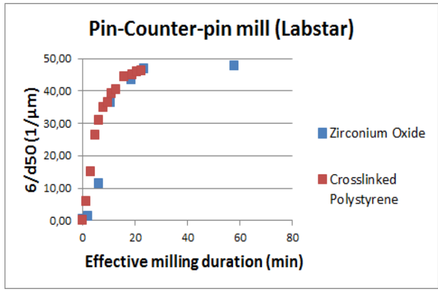
- Effective milling duration is the key parameter

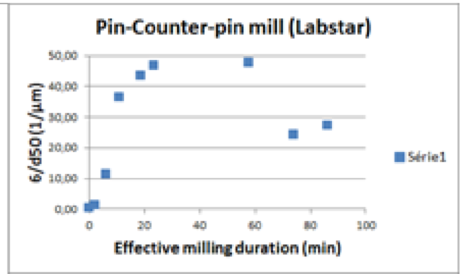
$$Effective \ milling \ duration = Duration \times \frac{Mill\,void \ volume}{Batch\,Volume}$$

Technological evaluation Comparison
Milling equipment impact: media material







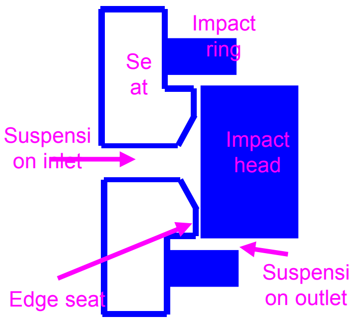


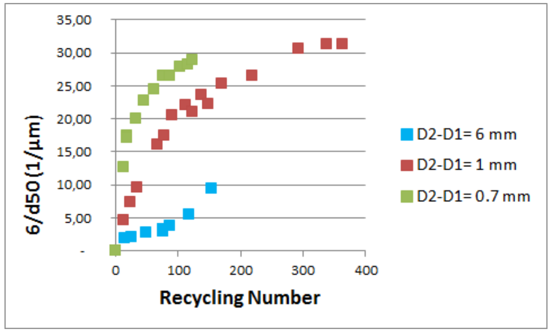
HPH

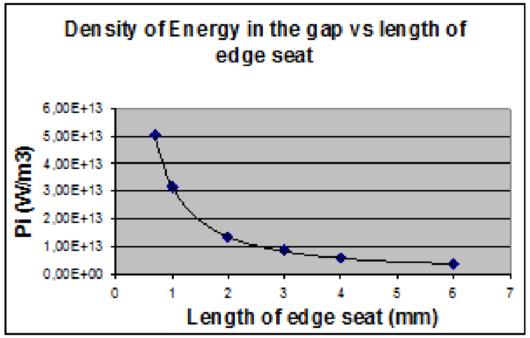
Beads mill

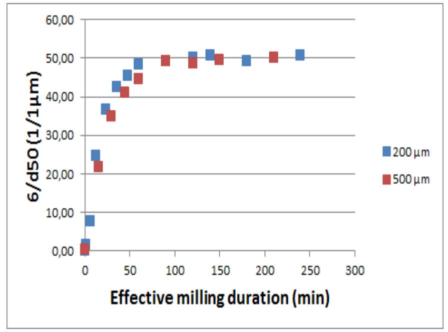
- **For HPH case, no significant impact of valve material is observed at studied scale.**
- **For Beads milling case, The milling kinetic is impacted by the density of the beads material.** $SI \propto SI_{gm} = d_{gm}^3 \rho_{gm} v_t^2$
  - The milling kinetic Polystyrene when it is corrected by beads density ratio gives the similar kinetic as Zirconium oxide
  - Using Zirconium oxide the increase of milling time leads to decrease of the specific surface area “negative grinding phenomenon” described by [Jimbo et al. 1990]. This phenomenon is in a very close relation to the aggregation and agglomeration. This phenomenon was not observed when Cross linked polystyrene bead were used

Technological evaluation Comparison
Milling equipment impact: media size









HPH

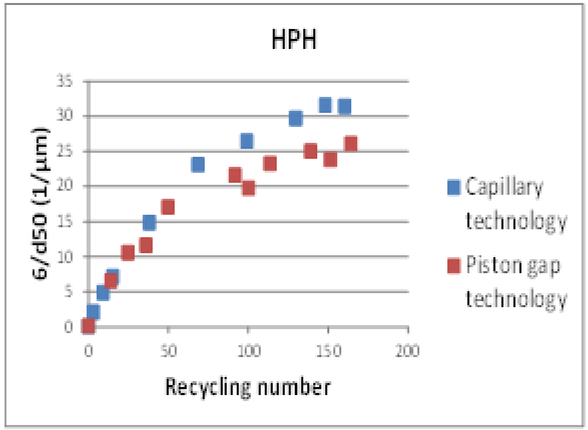
Beads mill

- For HPH case, the valve geometrical configuration impacts dramatically the milling kinetic. In fact, the lower is the length of edge seat; the faster is the milling kinetic. This could be explained by the increase of density of the energy (HPH power/ Gap volume) in the gap between the seat and the impact head.
- For the beads mill case, using PS beads, the milling kinetic is not significantly impacted by the beads size

Technological evaluation Comparison
Milling equipment impact: Mill configuration

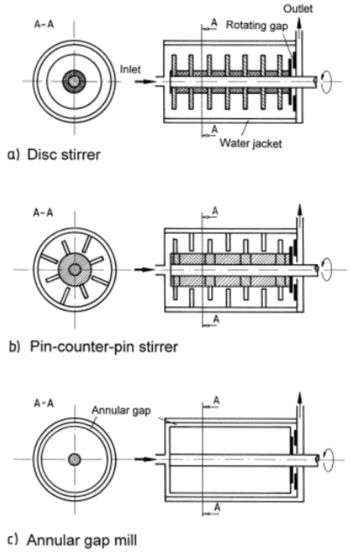
HPH

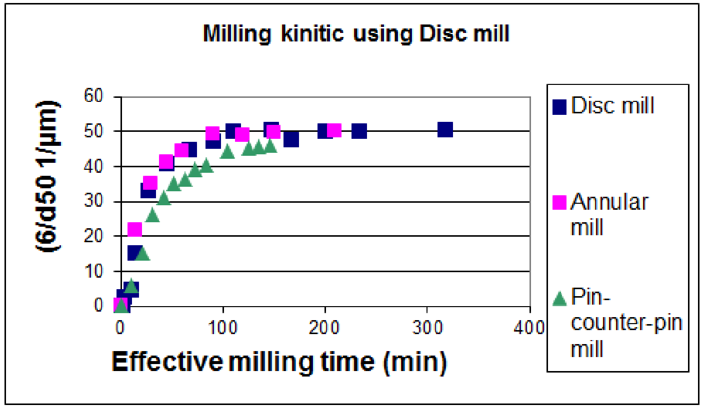
Piston gap vs Microfluidics)



No significant impact on the milling kinetic for the considered API

Beads milling





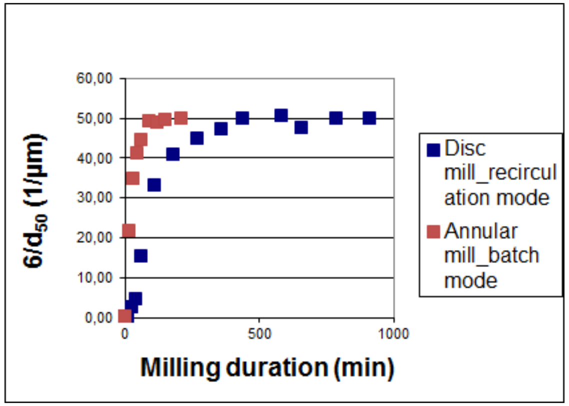
Whatever the technology used, there is no significant impact on the milling kinetic for the considered API

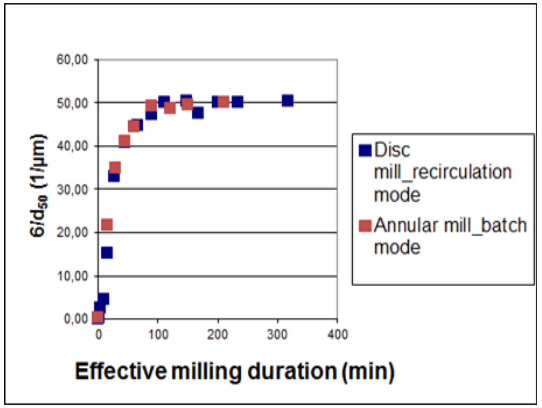
Technological evaluation Comparison Process configuration

HPH

Not performed
According internal experiments:
1 discrete pass = 3 recycling

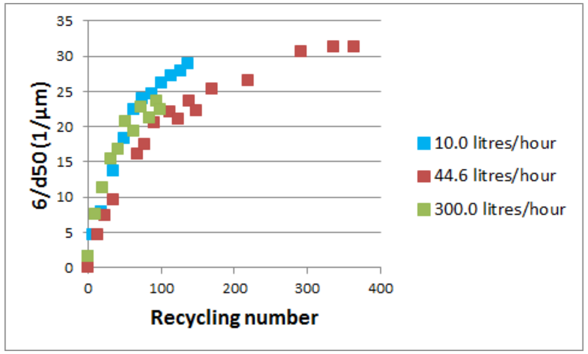
Beads milling





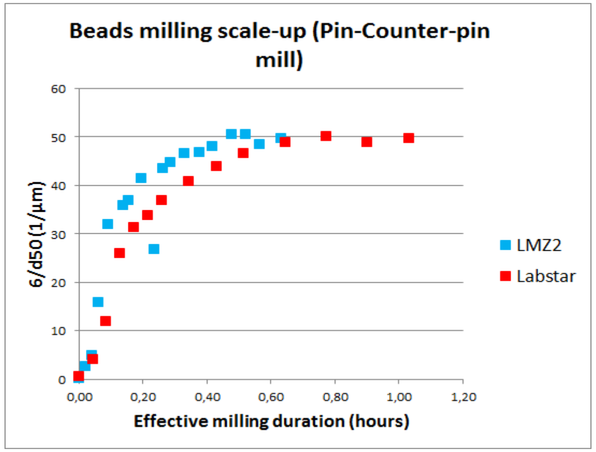
- If the milling kinetics are plotted versus the real milling time the batch mode is faster than the recirculation mode.
- In the opposite case if they are plotted versus effective milling time the batch mode and recirculation mode are equivalent

Technological evaluation Comparison
Milling equipment impact: Scale



HPH

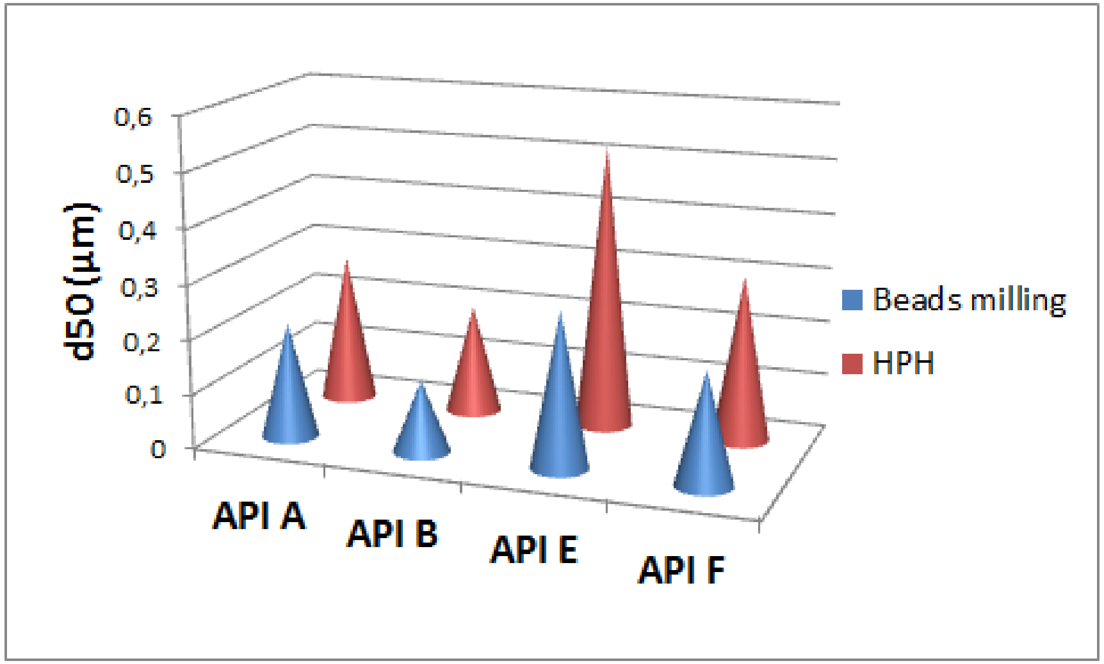
Recycling number is the invariant parameter



Beads mill

Keeping the same milling power density

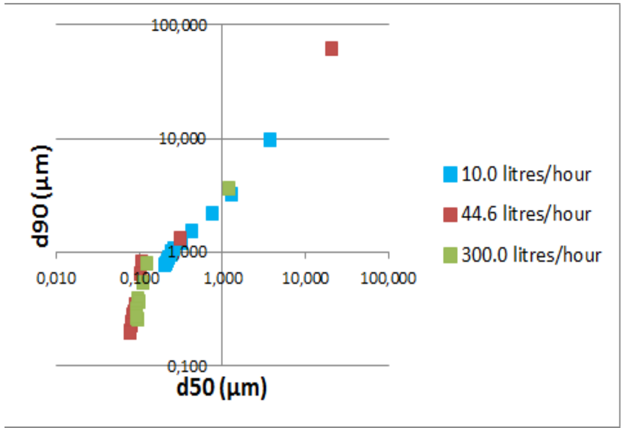
Technological Comparison
Suspension physical quality/Particles size/d50

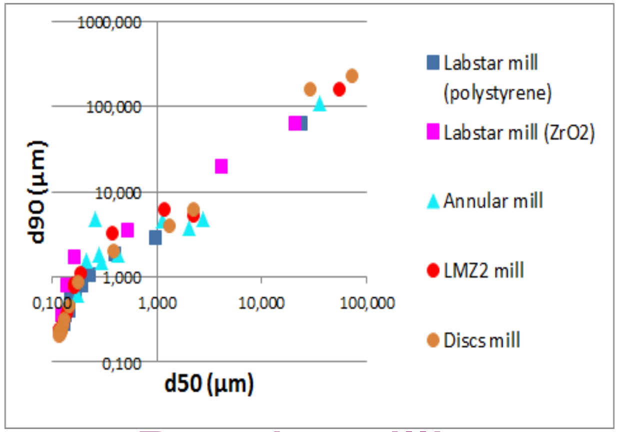


- HPH using 120 as recycling number
- Beads milling using 150 min as effective milling time

- The beads milling is more powerful than HPH. It leads to a d50 lower than obtained with HPH

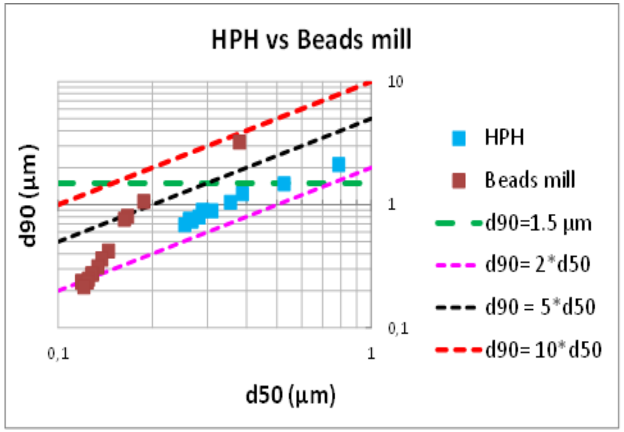
Technological evaluation Comparison
Suspension physical quality/Particles size distribution

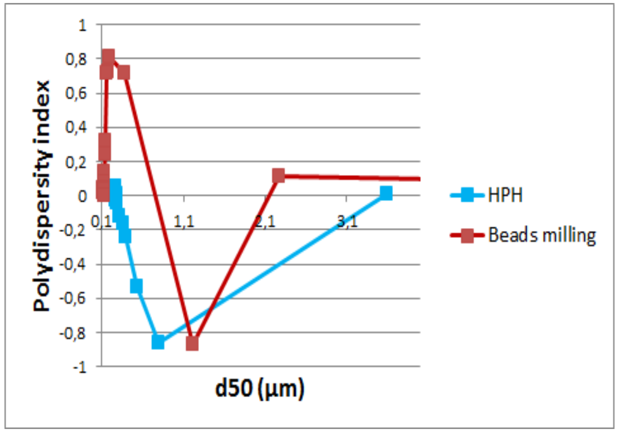




HPH

Beads milling

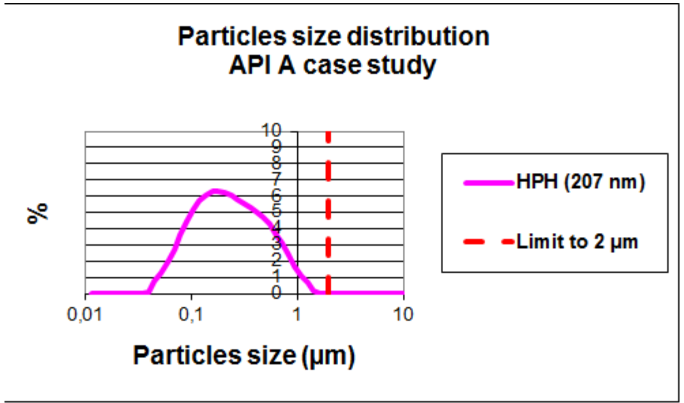


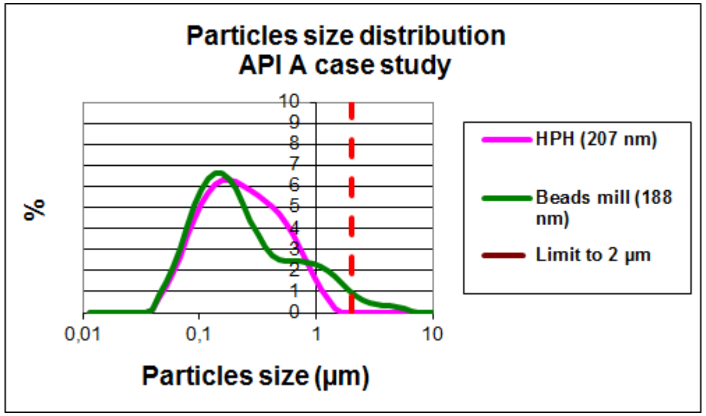


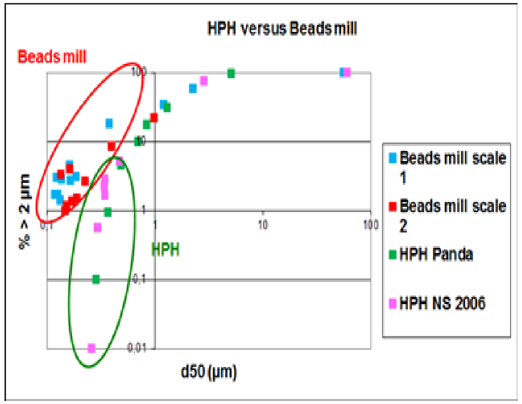
Comparison

- For the both technologies, whatever the process parameters and whatever the used scale, the relationship of d90 vs d50 is described by a unique master curve (technology signature) for given API
- The HPH leads to a narrower particle size distributions (lower d90 for a same d50)

Technological evaluation Comparison
Suspension physical quality/Particles size

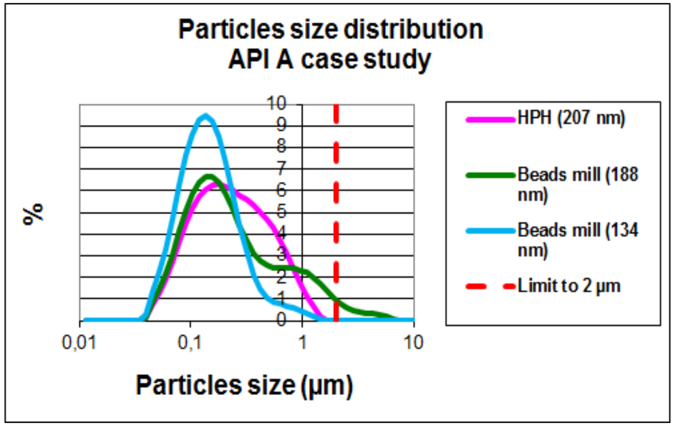






- The HPH leads to particles size distribution with all particles lower than 2 µm

- For producing approx the same d50 (200 nm, The HPH leads to a narrow PSD



- The beads milling is more powerful than the HPH.

Technological evaluation Comparison Physical quality/crystalline structure (Freeze dried material)

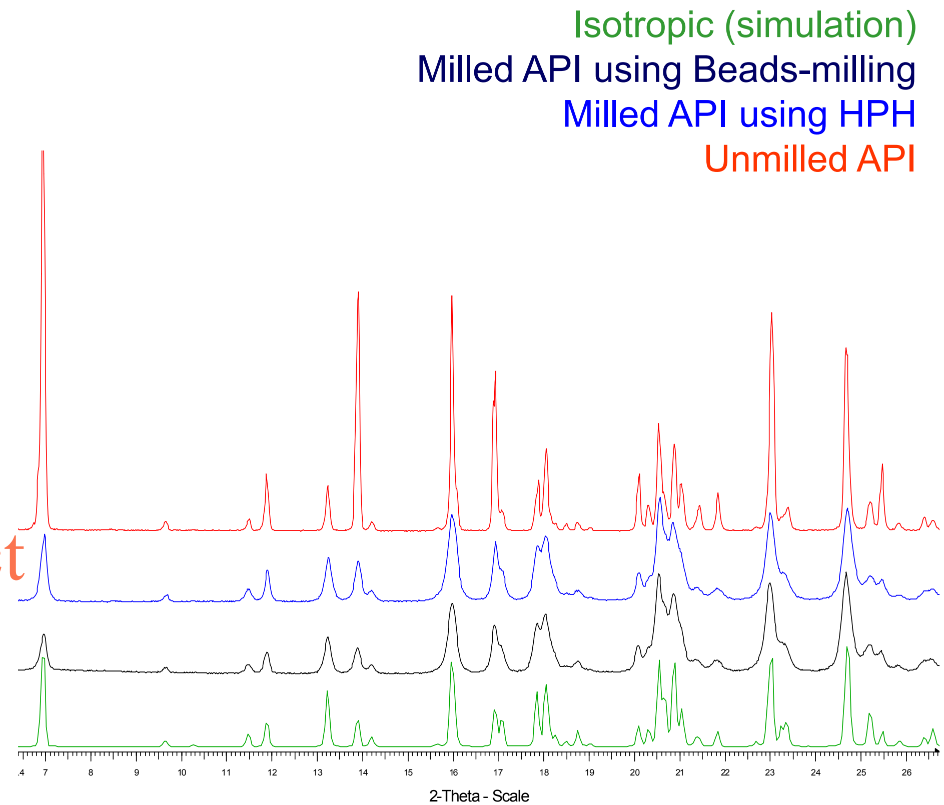
- **X-ray powder diffraction:**

Crystalline phase identification

Amorphization (non quantitative)

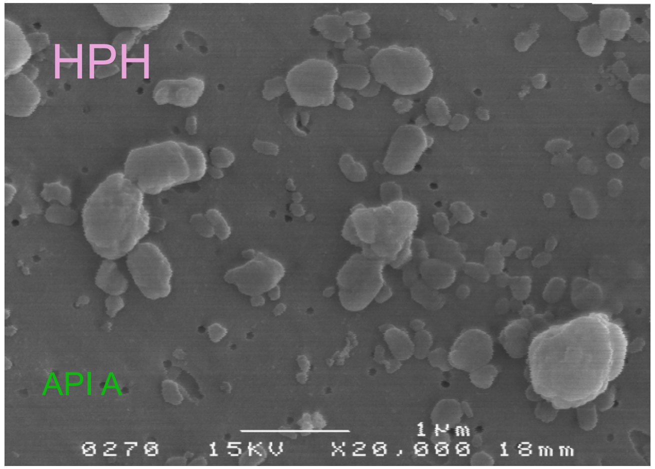
Preferred orientation effect ⇨ anisotropicity

Peak broadening ⇨ Crystallite size



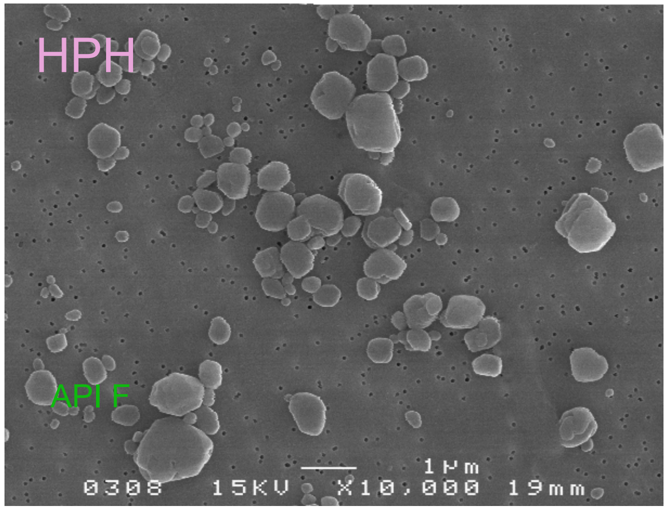
XRPD: From XRPD point of view, no significant differences among both nano-milling technologies

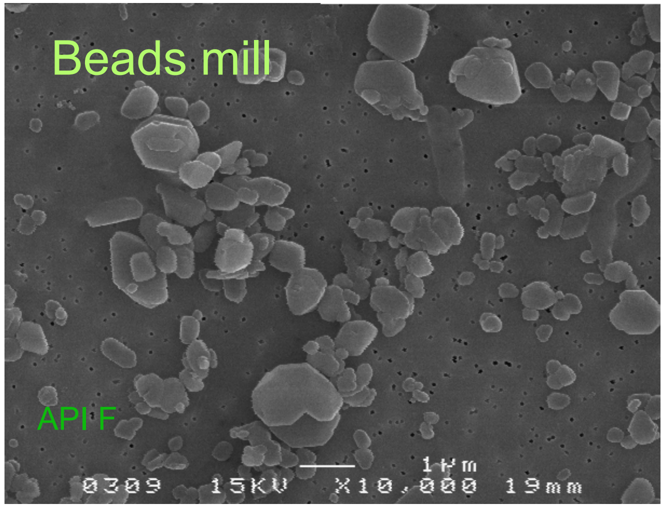
Technological evaluation Comparison
Physical quality/crystal morphology (suspension)



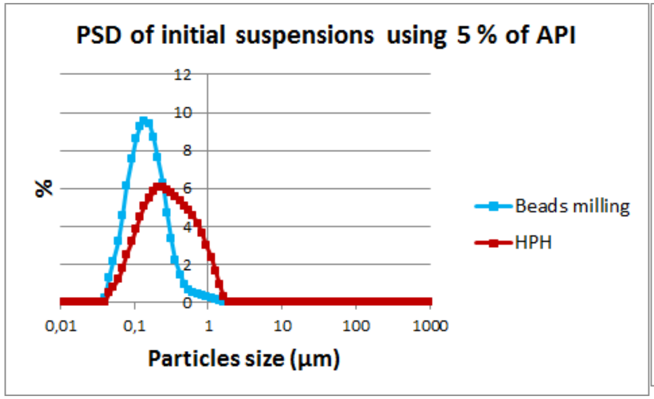
According to the technology used, the morphology can be different (API dependent)

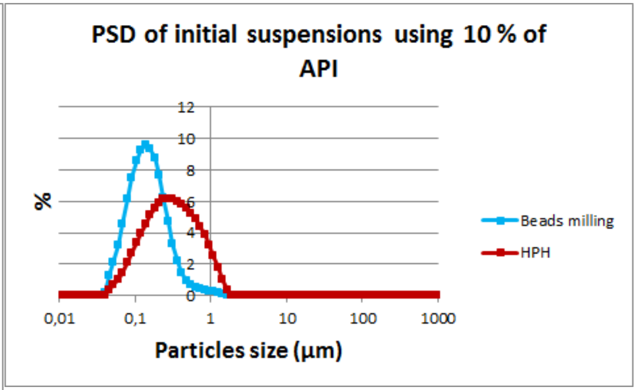
Different 3D distribution of molecular interactions within the crystal

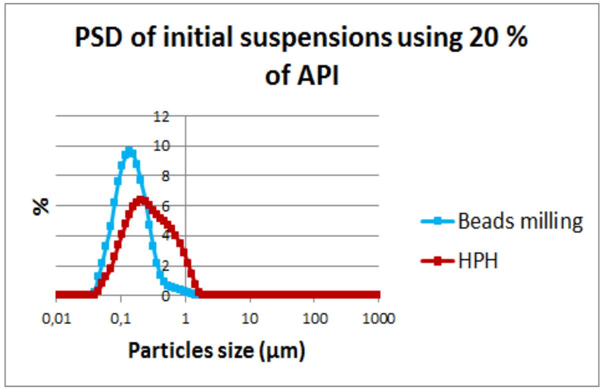




Technological evaluation Comparison
Suspensions stability as function of concentration



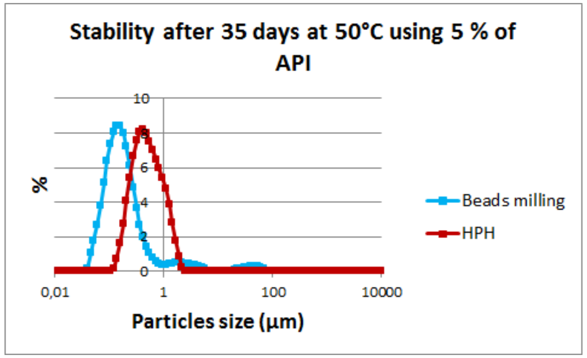


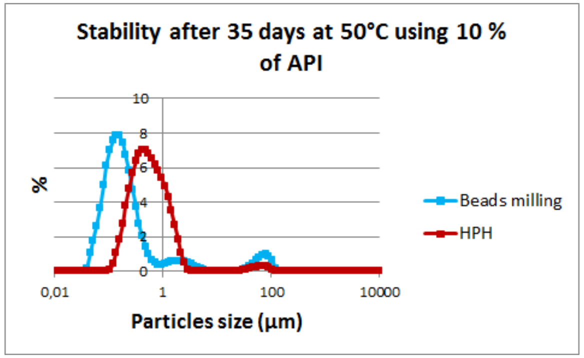


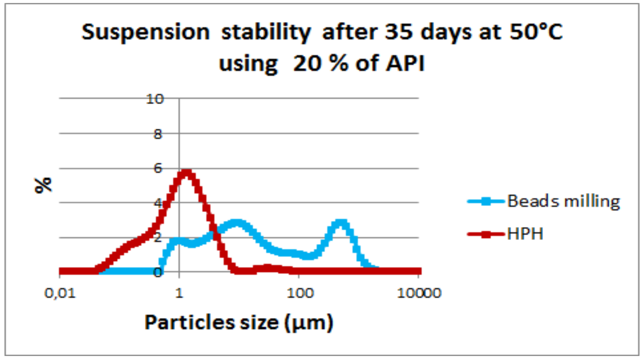
- For both technologies the initial particles size distribution is not impacted by the API concentration

- Whatever the API concentration the beads milling leads to a smaller particles size

Technological evaluation Comparison
Suspensions stability as function of concentration







- **Destabilizing effects become more appreciable at higher solids concentrations**
  - **The particle repulsion necessary to provide stability increases with increasing solids concentrations due to increase of collision frequency with particles concentration**
- **The increase of the particles size starts may be with Ostwald ripening when particles size is below <1 µm (kelvin) due probably to low plateau of adsorption at high temperature leading a free surfactant with solubilized API**
- **When the particles become larger than 1 µm than flocculation occurs**
- **The phenomenon is more significant for the beads milling may be due to morphology or size impact**

Technological evaluation Comparison
Equipment robustness/Beads milling: Erosion

- Quantification done by using ICP method

Sample (ppm)	Al	Cr	Fe	Ni	Si	Ti	W	Y	Zr
Lot0509021513 reference unmilled material	< 3	< 3	< 3	< 3	25	< 3	< 3	< 3	< 3
Beads mill NM2 using cross-linked polystyrene beads	< 3	< 3	< 3	< 3	20	< 3	< 3	< 3	< 3
Beads mill Labstar using $Zr0_2$ beads	25	25	40	5	35	5	100	45	500

- Beads milling: When using polystyrene beads no contamination from the stainless steel part is observed. T
- The erosion of polystyrene beads is quantified by filtration and weighing: Less than 5 ppm

Technological evaluation Comparison Equipment robustness/HPH: Erosion

Quantification done by using ICP method

Samples	Al mg/kg	Cr mg/kg	Fe mg/kg	Ni mg/kg	Si mg/kg	Ti mg/kg	W mg/kg	Y mg/kg	Zr mg/kg
unmilled material	< 3	< 3	< 3	< 3	25	< 3	< 3	< 3	< 3
Milled using HPH ZrO2	< 3	< 3	< 3	< 3	30	< 3	< 3	< 3	< 3
Milled using HPH TC+ NiTi	< 3	< 3	< 3	< 3	25	< 3	< 3 approx 2	< 3	< 3

No contamination

Technological evaluation Comparison
Equipment robustness: Beads milling
Reliability: Risk assessment

– Equipment robustness

Possible issues	Main causes	Corrective action to be done during manufacturing	Impact	
			Safety	Quality
Selector clogging	Big particles size Suspension settling	Mill opening Beads discharging Selector replacement	Toxic suspension handling	Sterility breaking off
Electrical over intensity	Caking due to the formulation issue	Mill opening Beads discharging Mill cleaning	Toxic suspension handling	Sterility breaking off
Damaging of mechanical seal	Defective gasket or bad lubrication	Mill opening Beads discharging Sealing system replacement	Toxic suspension handling	Suspension dilution Sterility breaking off
Leakage of sealing liquid system in the process	Tightness of the system	No possible corrective action. The leakage is inherent to the technology	N.A	Suspension dilution: 28 g/h

Technological evaluation Comparison
Equipment robustness: HPH
Reliability: Risk assessment

Possible issues	Main causes	Corrective action to be done during manufacturing	Impact	
			Safety	Quality
Leakage from O-ring	Extrusion due to the high pressure and low Material mechanical resistance	HPH opening O-ring replacement	toxic suspension handling	Product loss and quality impact in case of aseptic process
Damaging of mechanical seal	Defective gasket due to a low Material mechanical resistance or bad lubrication	HPH opening Sealing system replacement	toxic suspension handling	Product loss and quality impact in case of aseptic process

Technological evaluation comparison Conclusion related to the nano-particles application

	HPH	Beads mill
Milling performances	d50 in the range of 200- 500 nm d90 < 1.5 µm All particles < 2 µm	d50 in the range of 100- 500 nm d90 < 1.5 µm All particles < 2 µm
Production experience	No experience so far with nanoparticles but daily used for parenteral emulsions	Used for Oral administration
GMP compliances	Time being, no critical issue is observed	Time being, no critical issue is observed
Robustness and reliability	The equipment works at 90 % of its maximum power Negligible contamination	Could be operated only at 30 % of its maximum power

Beads milling: Value chain

Planetary mill

10
ml
Vial

(Use volume: 4 to 15 ml)

•Support to candidate selection and formulation screening

Pin mill

(Use volune: 50 ml)

•Process development, PK and tox batches

Pin mill

(Use volume: 0.5 to 3 litres)

•Process development fine tuning and robustness

•GLP tox Batch

Pin mill

(Use volume: 3 to 60 litres)

•Technical and GMP batches

HPH: Value Chain

- **From support to candidate selection up to Phase III clinical supplies**

Planetary mill

10 ml Vial

Total API needs: 2-3 g

(Use volume: 4 to 15 ml)

•Support to candidate selection and formulation screening

HPH: 10 l/h

Total API needs:

60-120 g

(Use volume: 50 to 250 ml)

•Process development, PK and tox batches

•Choice of sterilization process

•Aptness to freeze-drying

HPH: 35 l/h

Total API needs: 0.2-5 kg

(1-5 L)

•Process development fine tuning and GLP tox Batch

•Stability of sterilized/freeze-dried material

HPH: 300 l/h

Total API needs: 0.5-3 kg

(10 to 60 L)

•Technical and GMP batches

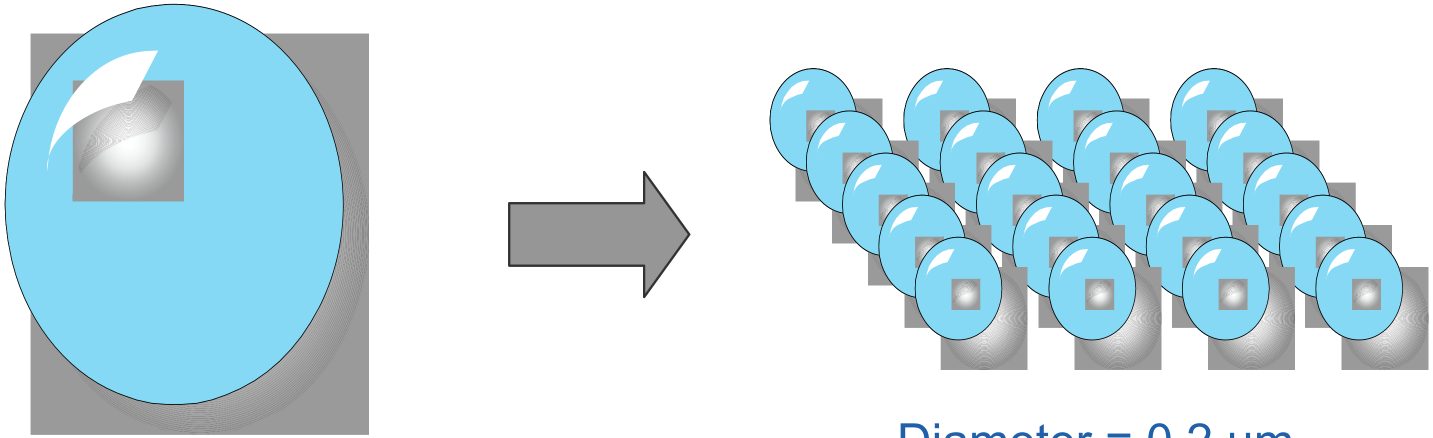
PK vs solution

PK/biodistribution vs scale

Thank you

Expected added value of Nano-crystals

- Reduction of the particles size leads to an increase of specific surface area



Diameter = 50 µm

1 sphere

Surface = 7850 µm²

Diameter = 0.2 µm

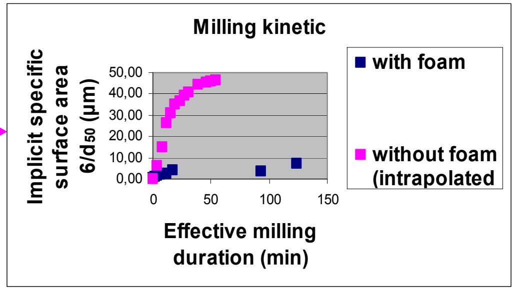
$1.56\ 10^7$ spheres

Surface = $1.96\ 10^6$ µm²

Factor = 250

Technological evaluation Comparison

Foaming issue

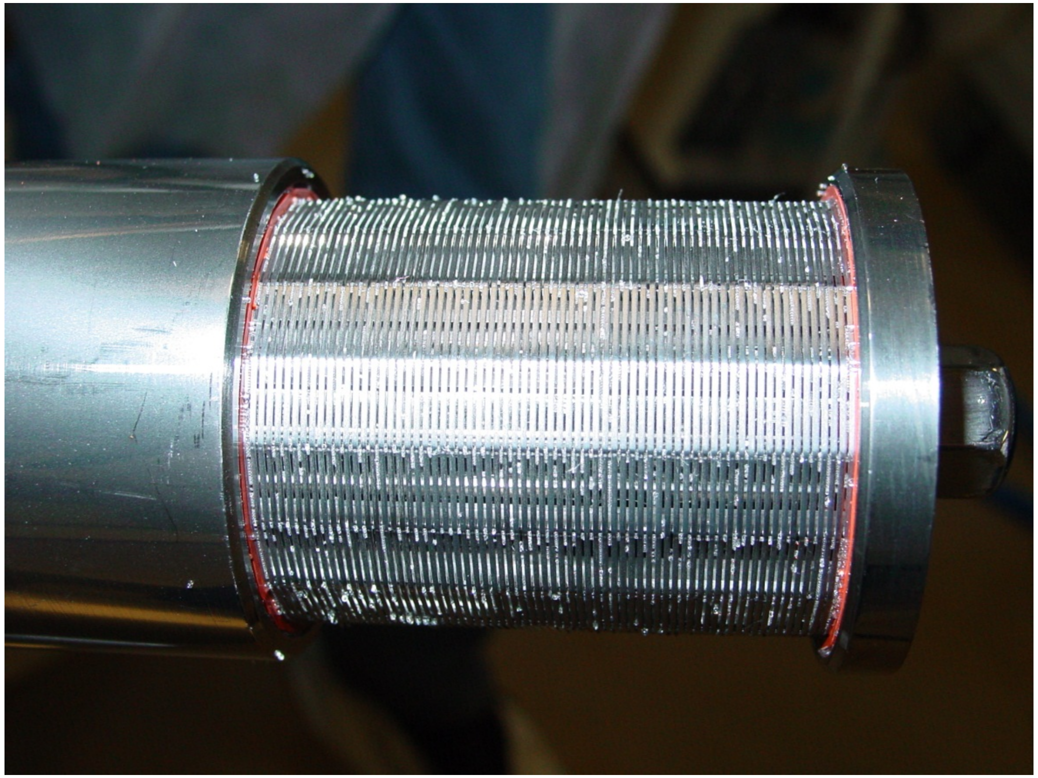


To be considered earlier in the development

Technological evaluation Comparison

Equipment robustness: Beads milling

Reliability: Risk assessment



Technological evaluation Comparison

Equipment robustness: HPH

Reliability: Risk assessment

Technological evaluation Comparison

Equipment robustness: HPH

Reliability: Risk assessment

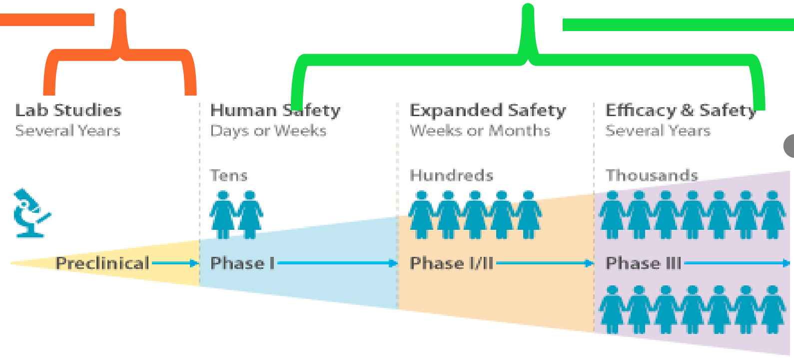
Modeling

Agenda (presentation overview)

- Context & objectives
- Introduction
- Formulation engineering
- Process engineering
- **Bead milling process modelling**
- Conclusions

Bead milling process modelling – Introduction

- During product design, the milling process needs to be developed at early stage using miniaturized equipment that can use a small amount of API.

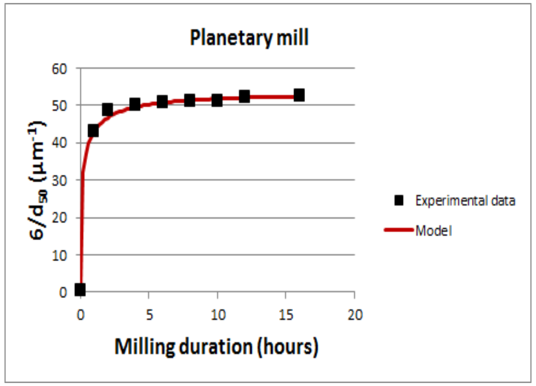


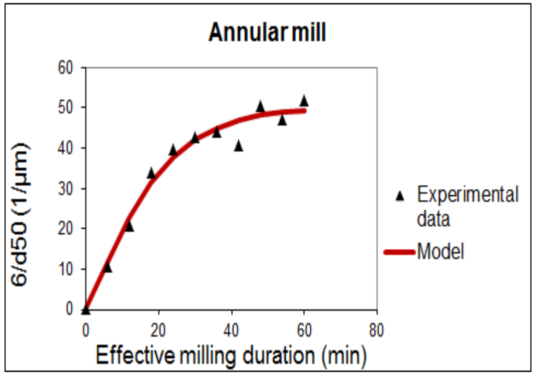
- During scale-up activities, it is important to reproduce the physical quality of suspension tested in phase 1 (proof of concept)

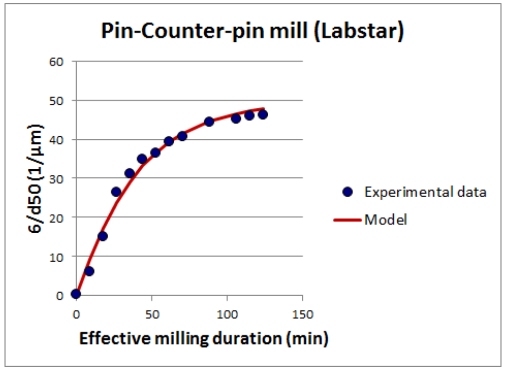
- A typical milling tool box that can be used from support to candidate selection up to Phase III clinical supplies can involves different mill configurations
  - Similar physical quality of milled suspension needs to be guaranteed all along product development in order to keep biopharmaceutical attributes identical.
- Modelling of milling process is required for
  - Prediction and simulation of milling process
  - Process transfer from one mill to another within the milling tool box (scale-down / scale-up) despite the difference in terms of technological configuration

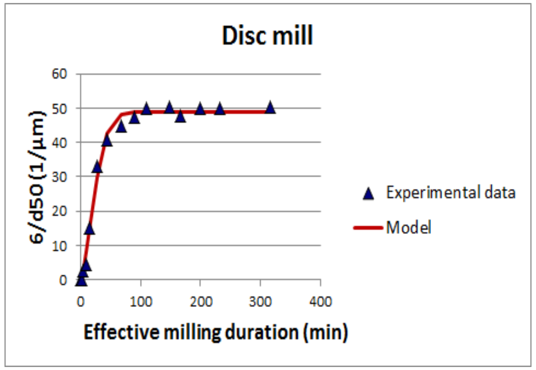
Bead milling process modelling –Does Avrami's equation fit all milling kinetics from milling tool box?

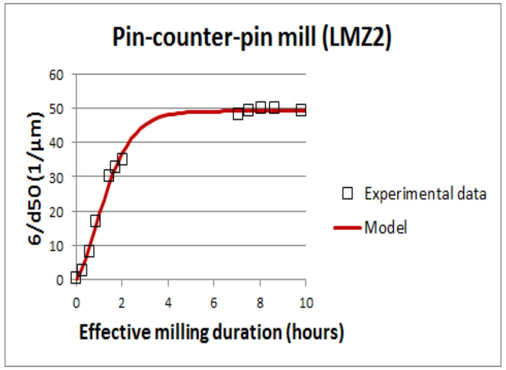
$$\left(\frac{6}{d_{50}}\right)_{(t)} = \left(\frac{6}{d_{50}}\right)_{(\infty)} \times (1 - e^{-(\frac{t}{\tau})^{n^*}})$$





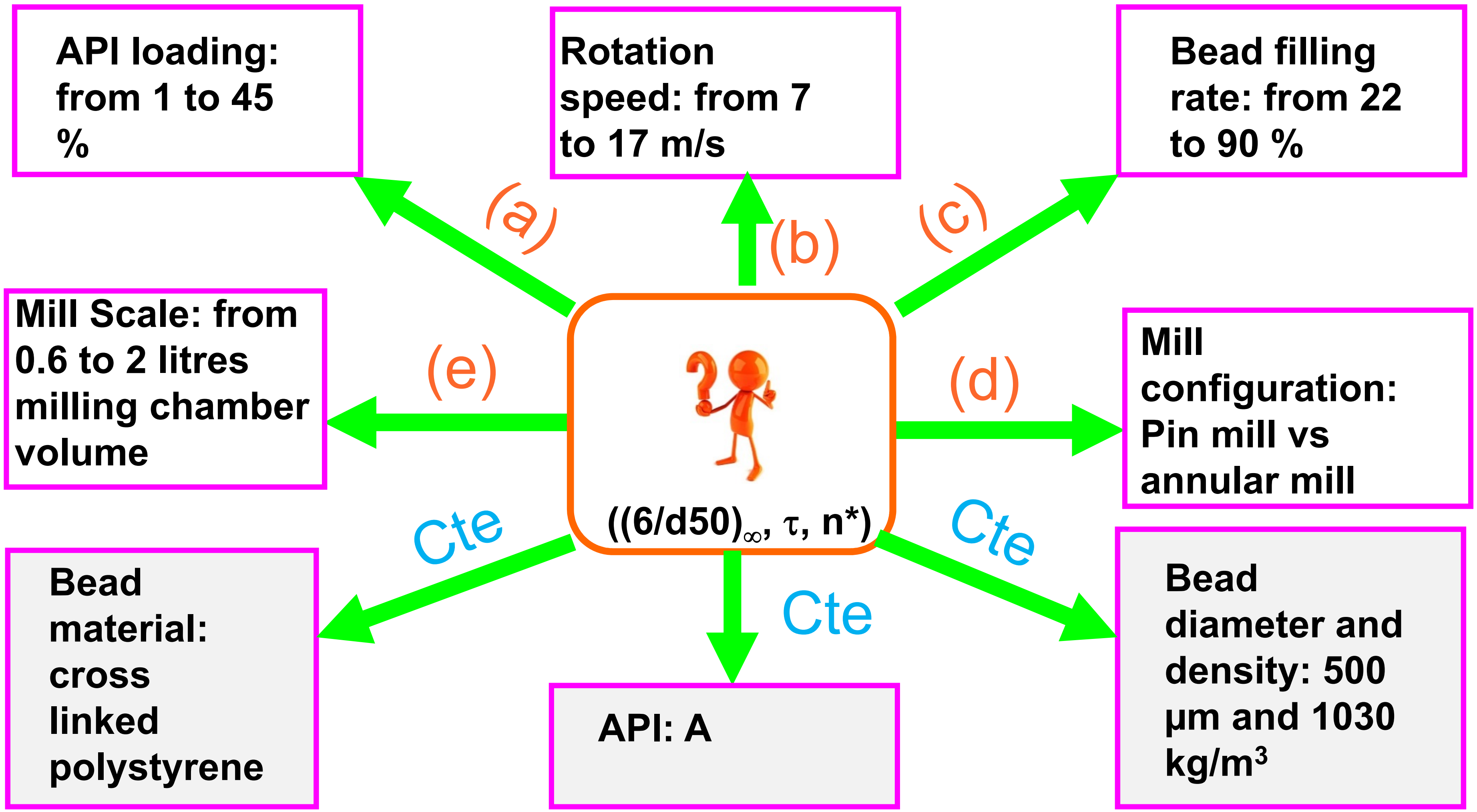




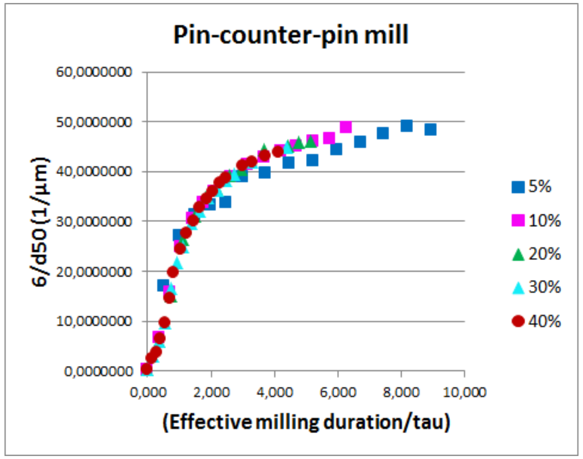


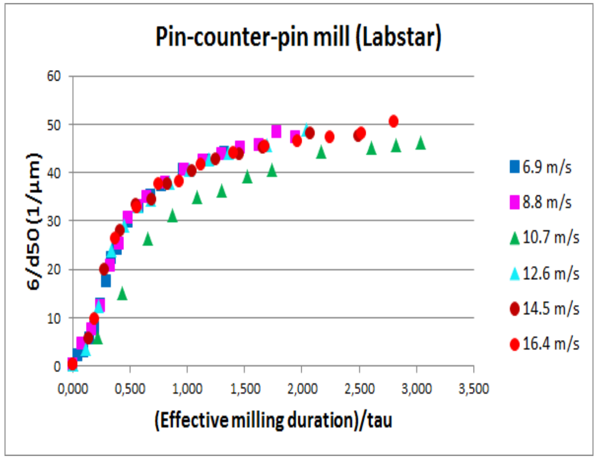
- The model is able to well fit all milling kinetic of different mills
- The 6/d50 at the equilibrium of all mills is about 50 μm^{-1}~ 120 nm

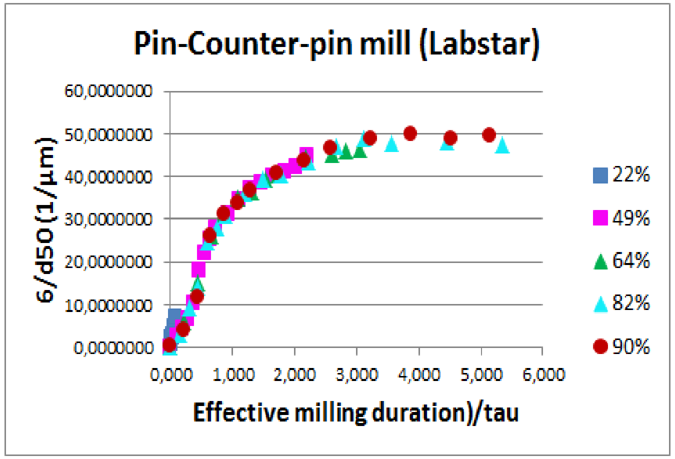
Bead milling process modelling – Considered process parameters



Bead milling process modelling – Impact of process parametrs on $(6/d_{50})_\infty$







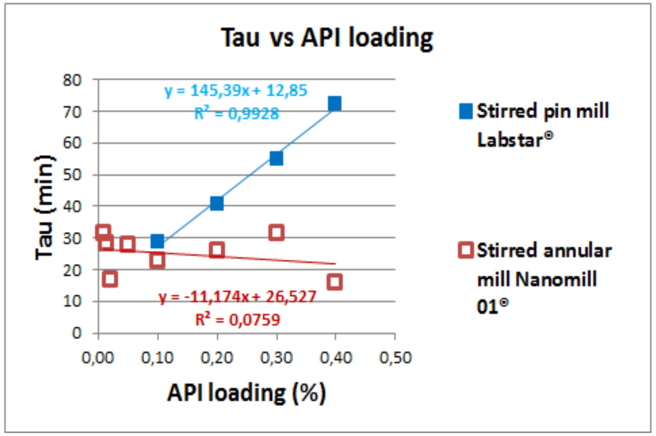
API loading Rotation speed Bead filling rate

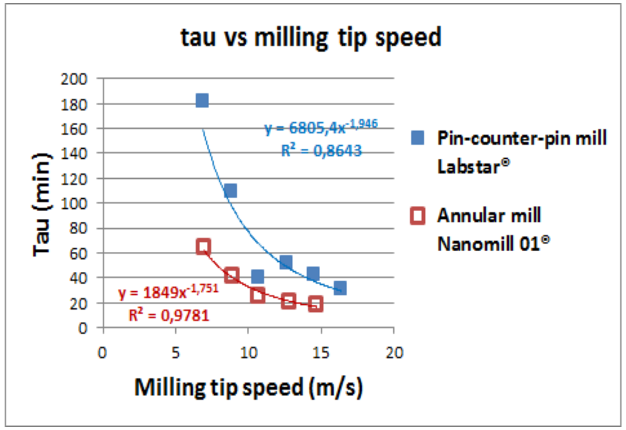
- When $(6/d_{50})$ is plotted as function of (milling duration/τ), the overall kinetics can be derived from one master curve having $(6/d50)_\infty$ around 50 μm^{-1}.

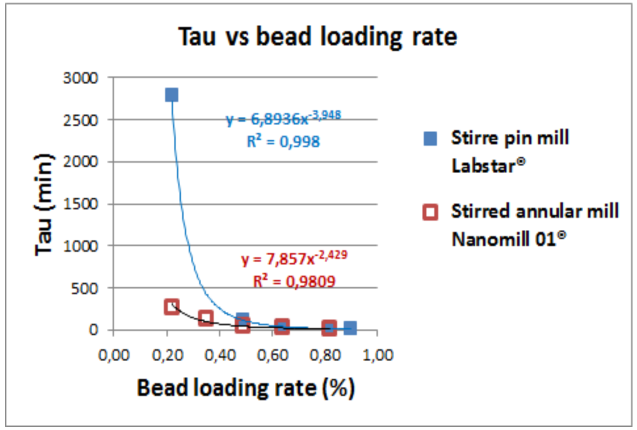
- The master curve suggests that the milling kinetic follows a first order law

➡ Master curve is obtained at $(t/\tau)^{n^*}$ in our case study n*=1

Bead milling process modelling – Impact of process parametrs on characteristic time (Tau)







API loading (C)

Rotation speed

Bead filling rate (ϕ)

	Annular mill	Stirred pin mill
API loading	No impact	$\tau = 145 \times C + 13$
Rotation speed	$\tau = 1850 \times V^{-1.75}$	$\tau = 6805 \times V^{-1.95}$
Bead loading rate	$\tau = 8 \times \emptyset^{-2.5}$	$\tau = 7 \times \emptyset^{-3.9}$

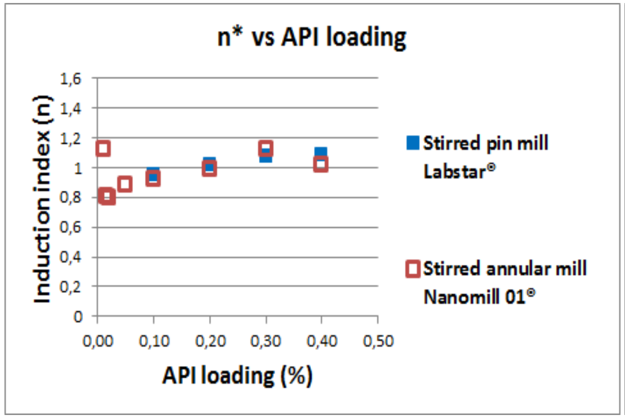
Impact of viscosity

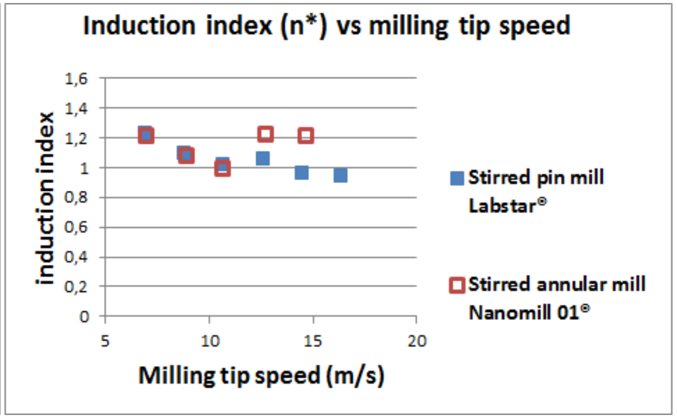
Impact of kinetic energy

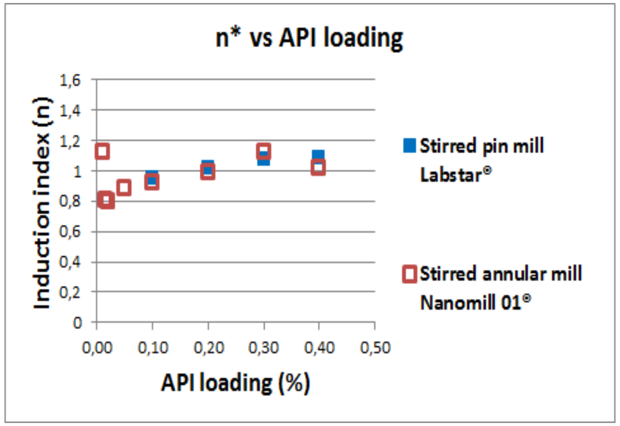
Impact of stress number

Both mills behave differently (geometry impact?)

Bead milling process modelling – Impact of process parametrs on induction index(n*)



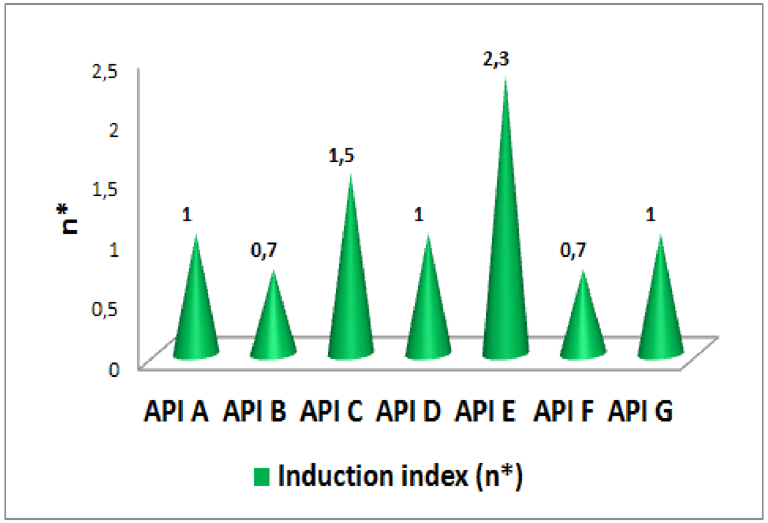




API loading (C)

Rotation speed (V)

Bead filling rate (ϕ)



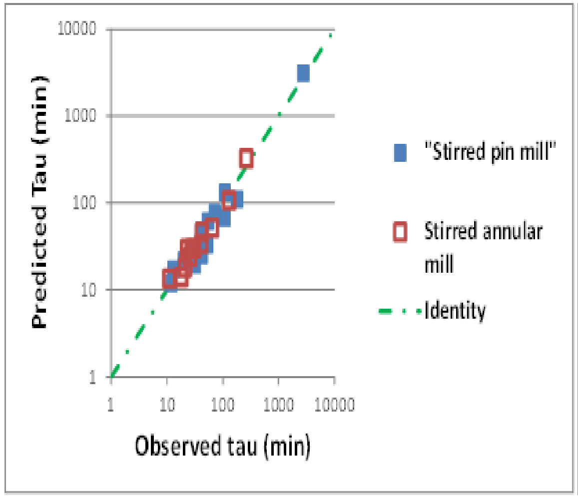
n* is highly API dependent
Can give an indication on API hardness

Bead milling process modelling – Design of global model

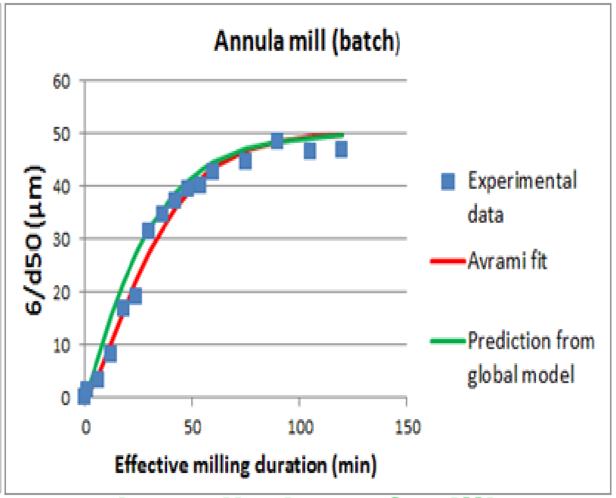
- $(6/d_{50})_\infty$ and n* are constant (50 μm^{-1} and 1 respectively)
- Only tau is process parameters dependent

$$\tau_a = k_1 \times V^x \times \phi^y \qquad \tau_p = (k_2 \times V^{x'} \times \phi^{y'}) \times (k_3 \times C + k_3)$$

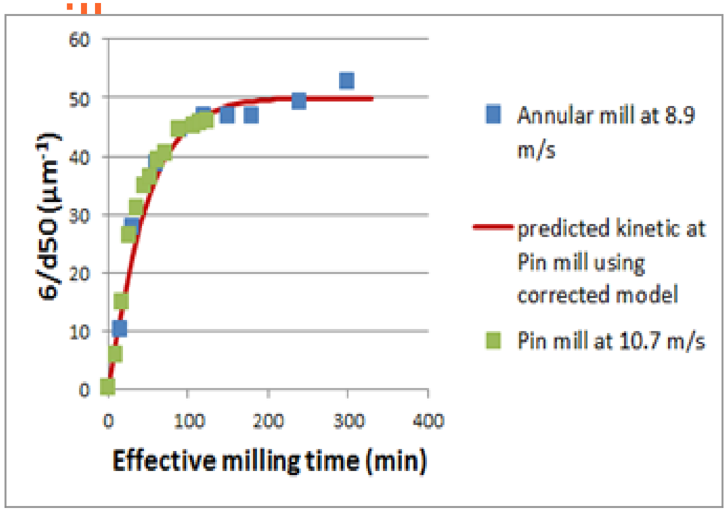
Annular mill — Stirred pin



Good prediction of observed values



good prediction of milling kinetic with parametrs not used during model design



Easy transfer from mill to another by using a correction factor

Bead milling process modelling – Design of global model

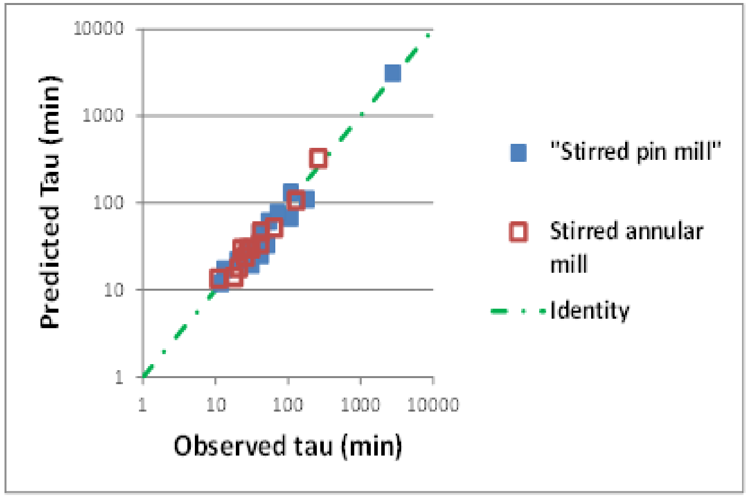
- $(6/d_{50})_\infty$ and n* are constant (50 µm^{-1} and 1 respectively)
- Only tau is process parameters dependent

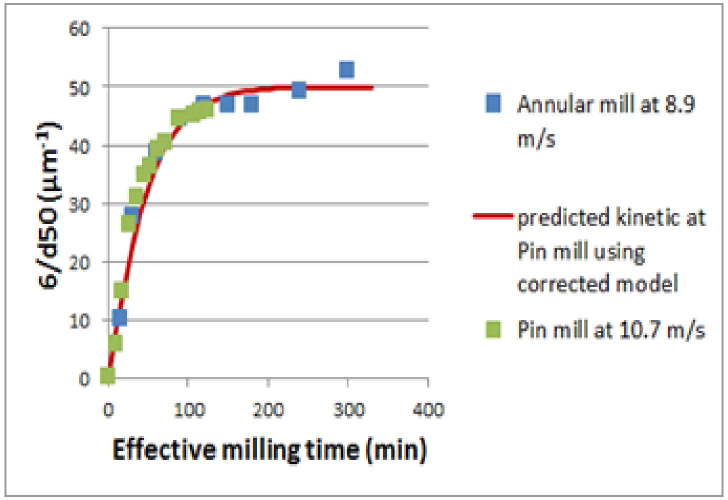
$$\tau_a = k_1 \times V^x \times \phi^y$$

$$\tau_p = (k_2 \times V^{x'} \times \phi^{y'}) \times (k_3 \times C + k_3)$$

Annular mill

Stirred pin mill





Good prediction of observed values

Easy transfer from mill to another by using a correction factor

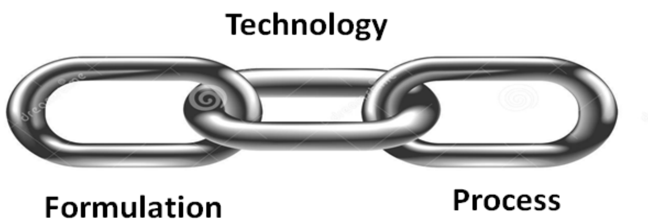
$N_{SP} = k \times N_A$

Outline of the presentation

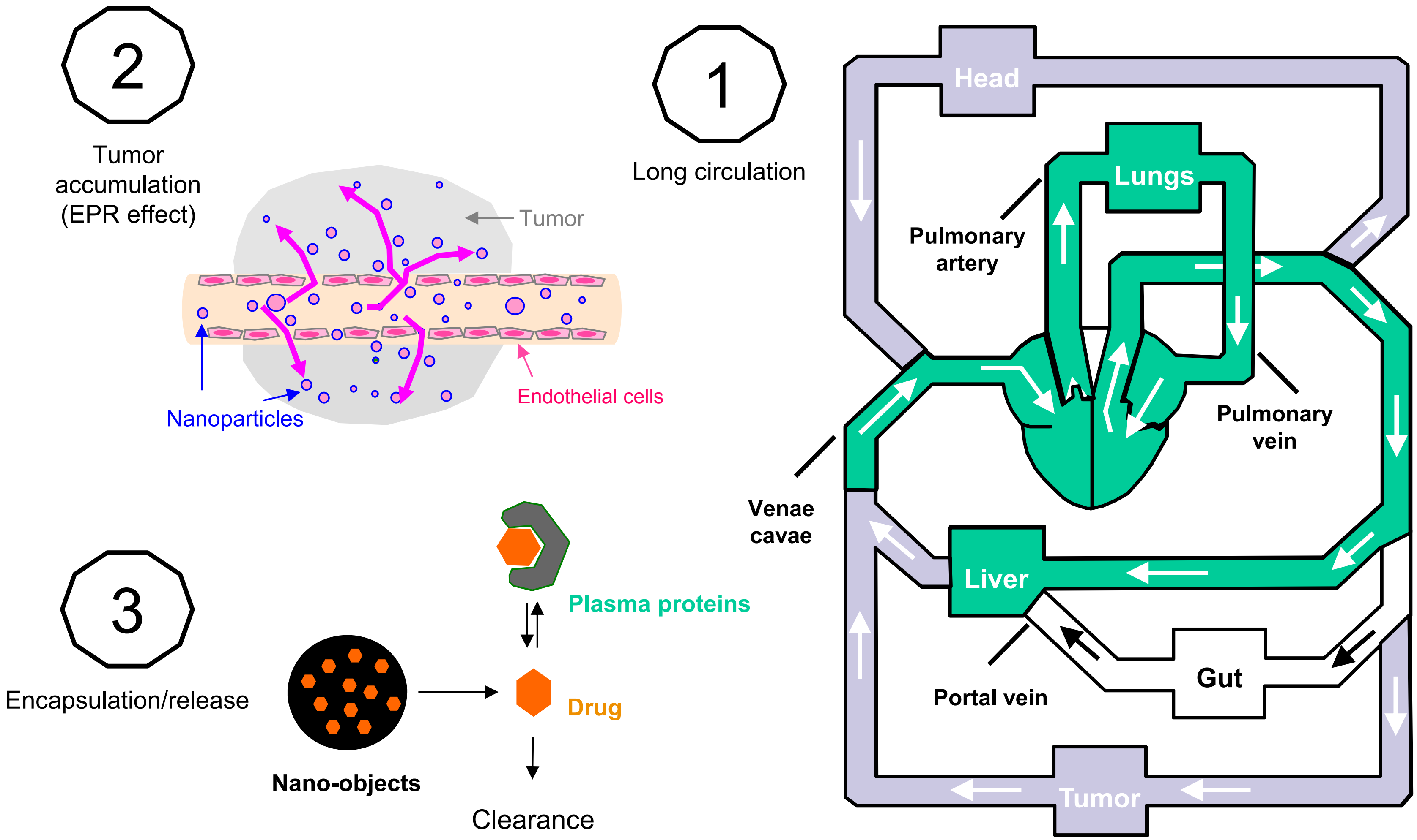
- Context & objectives
- Introduction
- Formulation engineering
- Process engineering
- Bead milling process modelling
- **Conclusions**

Conclusions

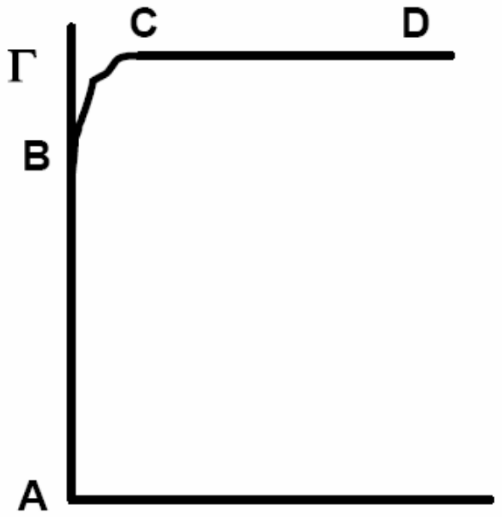
- **Formulation engineering**
  - **The developed approach can be considered as useful methodology to be applied in the frame fast to clinical evaluation of any API by achieving both time effectiveness and scientific rationale.**
- **Process engineering**
  - **As far as the formulation is sufficiently robust and stable both technologies are suitable for the production of nano-crystalline suspension**
  - **The bead mill is more powerful than the HPH. However, at the same average diameter (d50) of produced nano-suspension, the HPH leads to a tighten particle size distribution**
- **Bead milling process modelling**
  - **Using an adapted empirical Avrami's equation we were able to obtain a good fit for the experimental data whatever used technology, API and process parameters.**
  - **The global model can be used to help any process transfer from 1 mill to another**



Why too small (5/8): Long circulation and tumor accumulation



Adsorption Isotherms



- Conditions for efficient steric stabilization
  - (1) large Γ (point C in the Figure)
  - (2) large δ (layer thickness)
  - (3) large Ps (adsorption energy)
  - (4) $\chi < 0.5$ (good solvent for the polymer chain)
  - (5) low c (free polymer concentration)
  - note: (3) may conflict with (4) for homopolymers;
  - this conflict is absent for graft- and block copolymers