Microfluidics

Creating Nanoparticles with Microfluidizer® High-Shear Fluid Technology

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IDEX
MATERIAL PROCESSING

Microfluidics at a Glance

- Microfluidics has been located just outside of Boston, MA for **32 years** serving over **2000** customers worldwide. We have sold **~4,000** processors with localized sales and support in **47 countries.**

- **Microfluidizer®** high shear fluid processors can produce nanomaterials with a wide variety of multiphase applications. We have vast experience with process development of many different types of applications/formulations. We pride ourselves in our ability to help our customers get the most out of their materials.

- Microfluidizer Processors are used for R+D and manufacturing of **active pharmaceutical ingredients, vaccines, inkjet inks, coatings, nutraceuticals and cosmetics.**

17 of the top 20 pharma companies
8 of the top 10 biotech companies
4 of the top 5 chemical companies

...innovate with Microfluidics technology

What Microfluidics Does Best

- Nanoemulsions
- Cell disruption
- Protein recovery
- Uniform particle size reduction
- Nano/microencapsulation
- Nanodispersions
- Deagglomeration

"The overall satisfaction which we experienced with our laboratory model Microfluidizer processor eliminated the need to consider other equipment when it was time to scale up to production capabilities."
- Amylin Pharmaceuticals

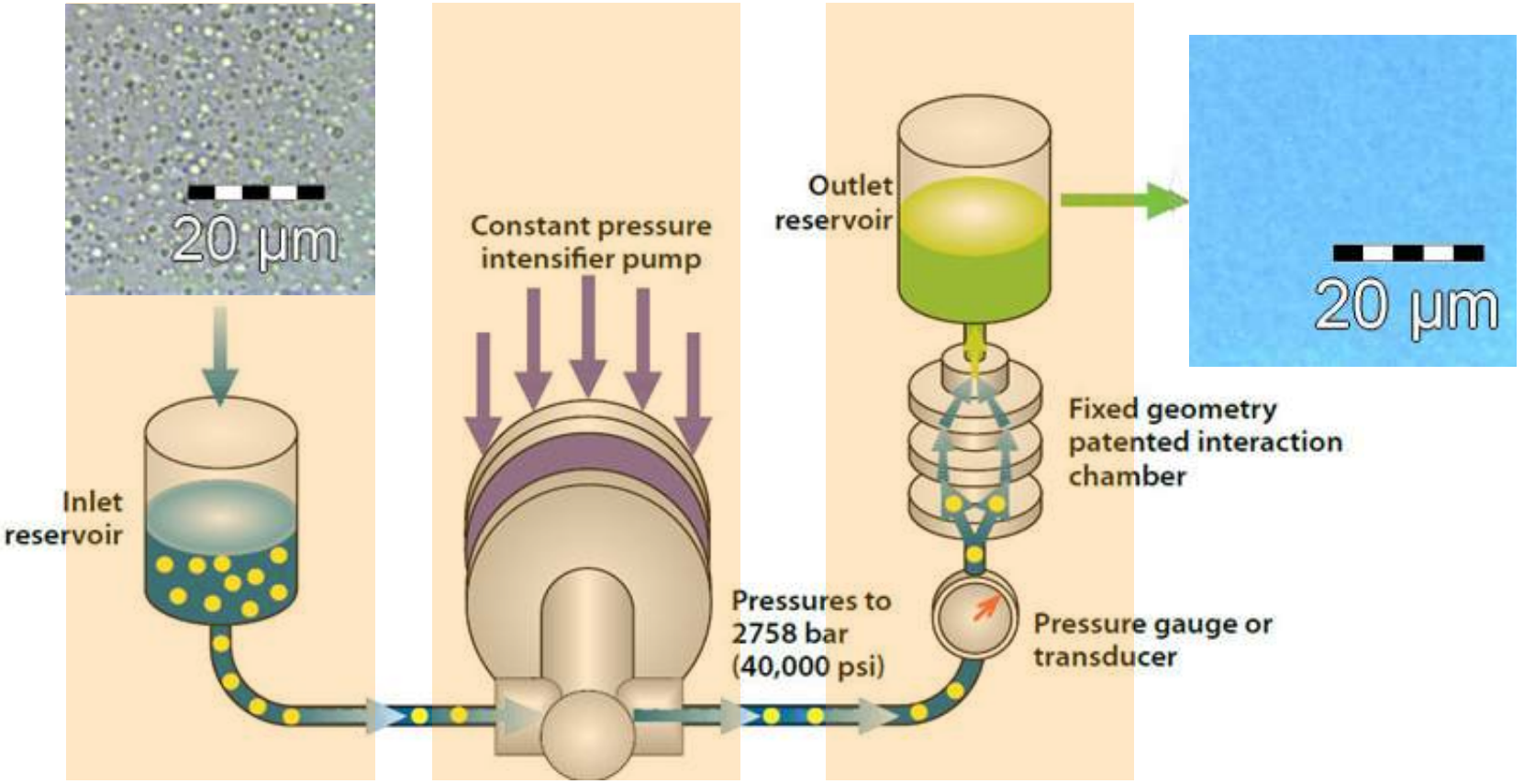
M-110P "plug n' play" benchtop lab model

M-110EH-30 pilot scale machine

M-700 series production machine

Fixed-geometry interaction chambers

Microfluidizer® Processors & Interaction Chamber (IXC)



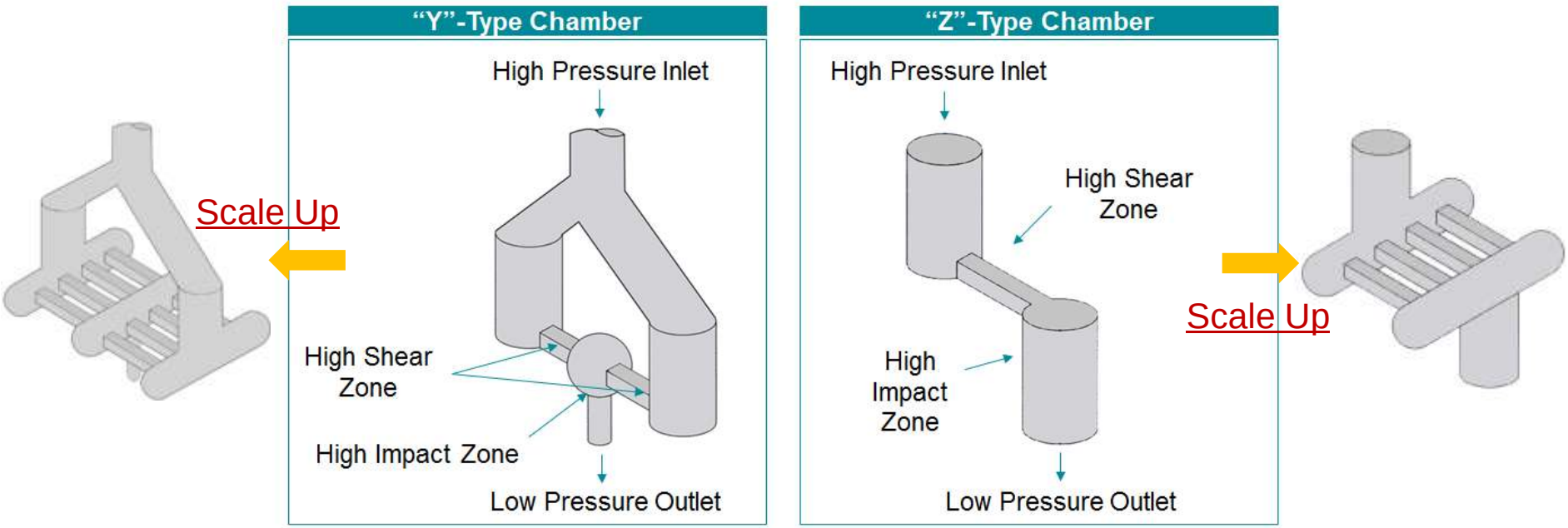
- Continuous processing
- Accommodates materials with high solid content
- Can accommodate high viscosities
- Can work in a wide range of temperatures

- High pressure used to deliver product into the interaction chamber
- Constant pressure pumping system

- Microchannels range from 50-500 microns
- Unsurpassed shear and impact forces
- Temperature regulated by a heat exchanger
- Lack of moving parts maximizes uptime
- Repeatable and scalable results

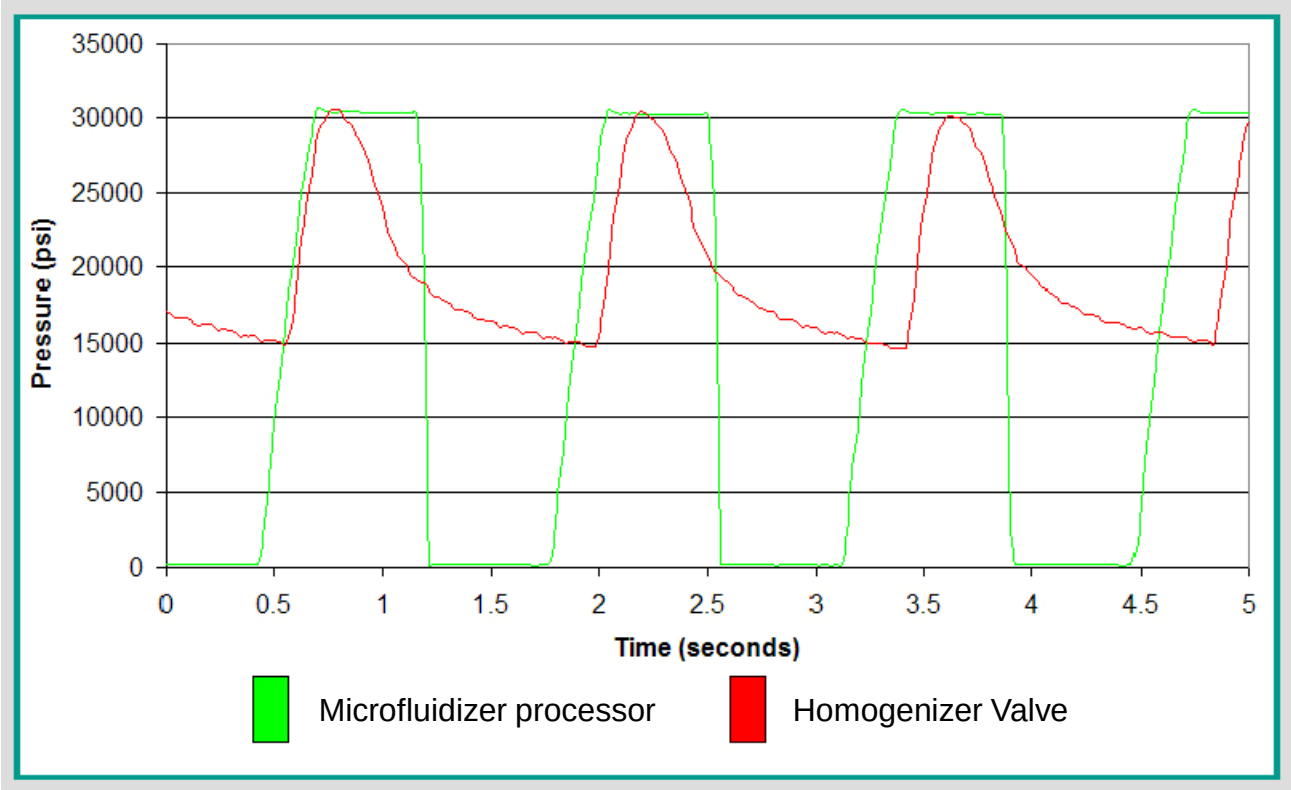
Microfluidizer® Processors & Interaction Chamber (IXC)

- **Exceptional performance –** Channel velocities over 400 m/s and generate shear rates up to 10^8 s^{-1}
- **Consistent processing –** Fixed geometry with no moving parts and guaranteed scalability
- **Long-wearing –** Made from diamond or ceramic materials
- **Ease of maintenance –** Clean-in-place and steam-in-place
- **Many options available** – Variable shape and size

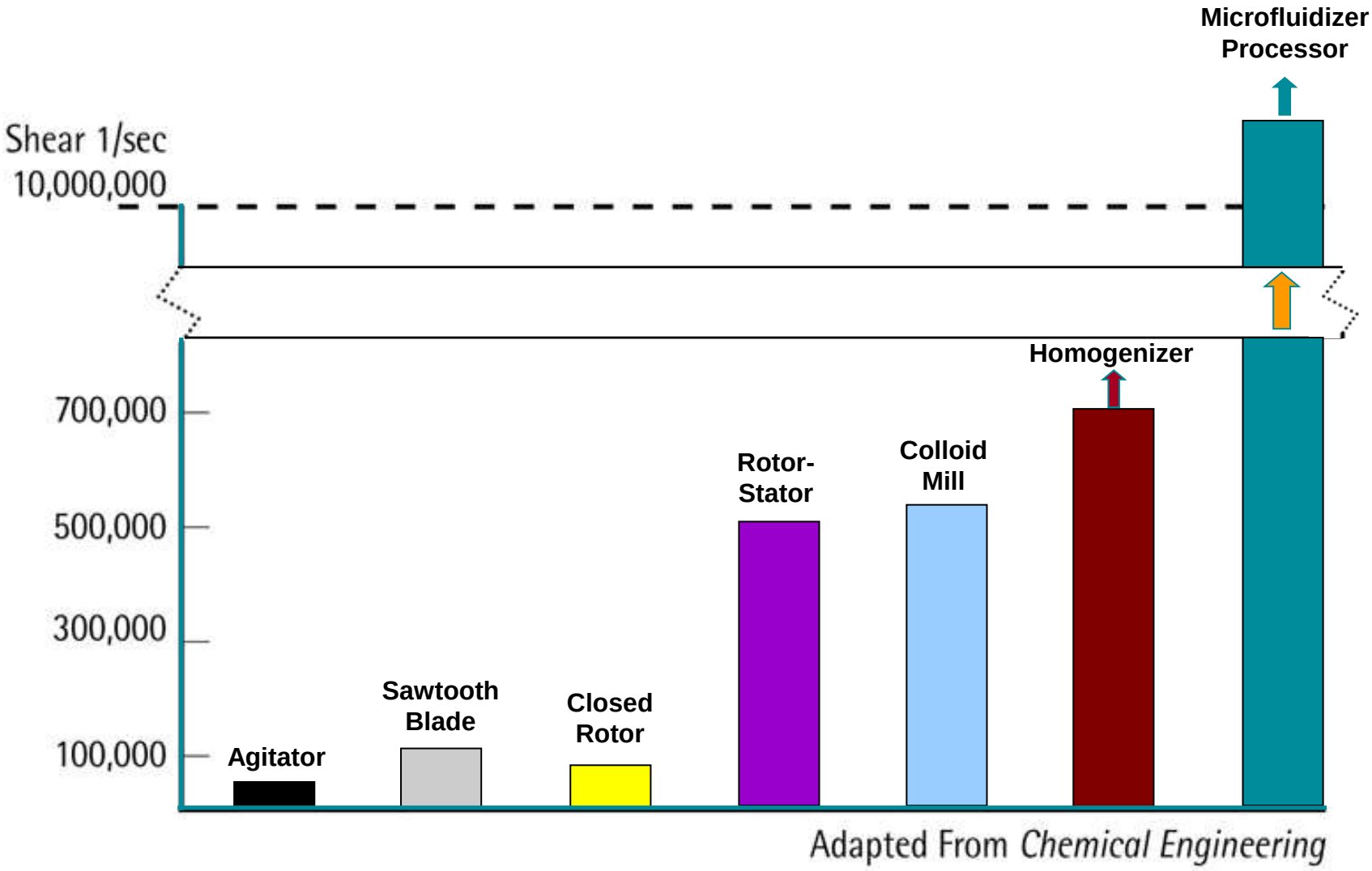


Constant Pressure and Constant Volume

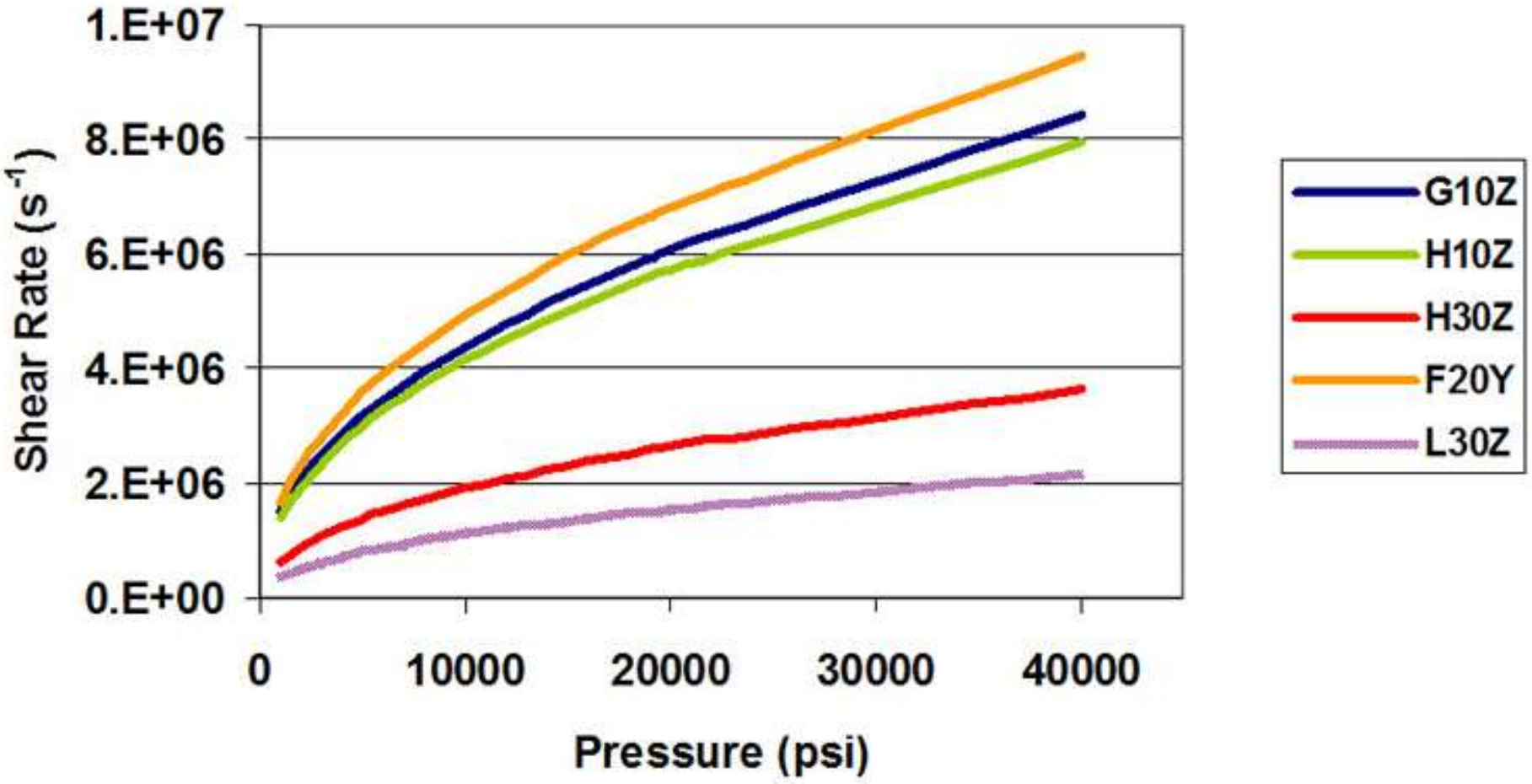
- Virtually all of the product is passed through the Microfluidizer processor at the target pressure.
- Only a small portion of the product is passed through the high pressure homogenizer at the target pressure.



Shear Rates for Various Technologies



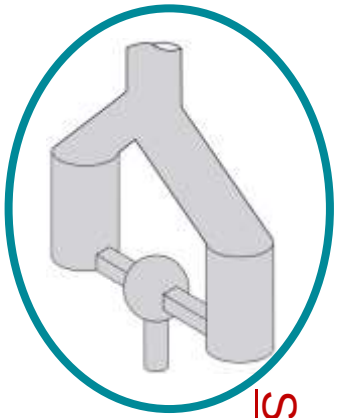
Nominal Shear Rates as a Function of Pressure & Chamber



Data shown for internal diameters vary from 75 μm – 300 μm

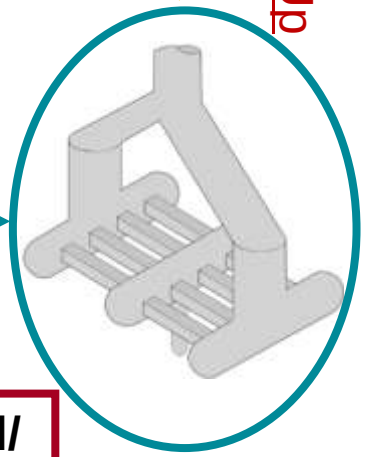
Benefits of Microfluidizer® Processors

- **How Microfluidics Technology is Unique**
  - > Constant Pressure Processing
  - > High Potential Processing Pressures
  - > Fixed Geometry Interaction Chambers
  - > Multi-Slotted Interaction Chambers
- **Resulting Benefits**
  - > Very small particle size potential
  - > Very consistent processing resulting in very narrow particle size distributions
  - > Guaranteed scale-up from lab scale to production scale
- **cGMP Compliance and CIP/SIP Capabilities**



LV1
1 mL hold up volume

Scale Up



M7250-20 Pharmaceutical/ Constant Pressure/SIP
Can process 8 LPM at 1300 bar

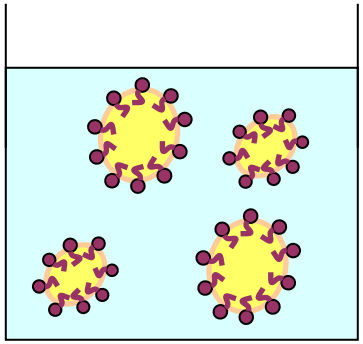
Nanoemulsion

Nanoemulsion

- Nano-emulsions are formed when two or more immiscible liquids are mixed and one phase is finely dispersed in the other (s)
  - Oil-in-water
  - Water-in-oil
  - Double emulsions (O/W/O, W/O/W)

- Surfactants stabilize the emulsions by decreasing the surface energy between the immiscible liquids; they can be ionic, polymeric, proteins or solid particles.

- Oil-in-water nanoemulsions are used for delivering water insoluble active pharmaceutical ingredients (APIs).

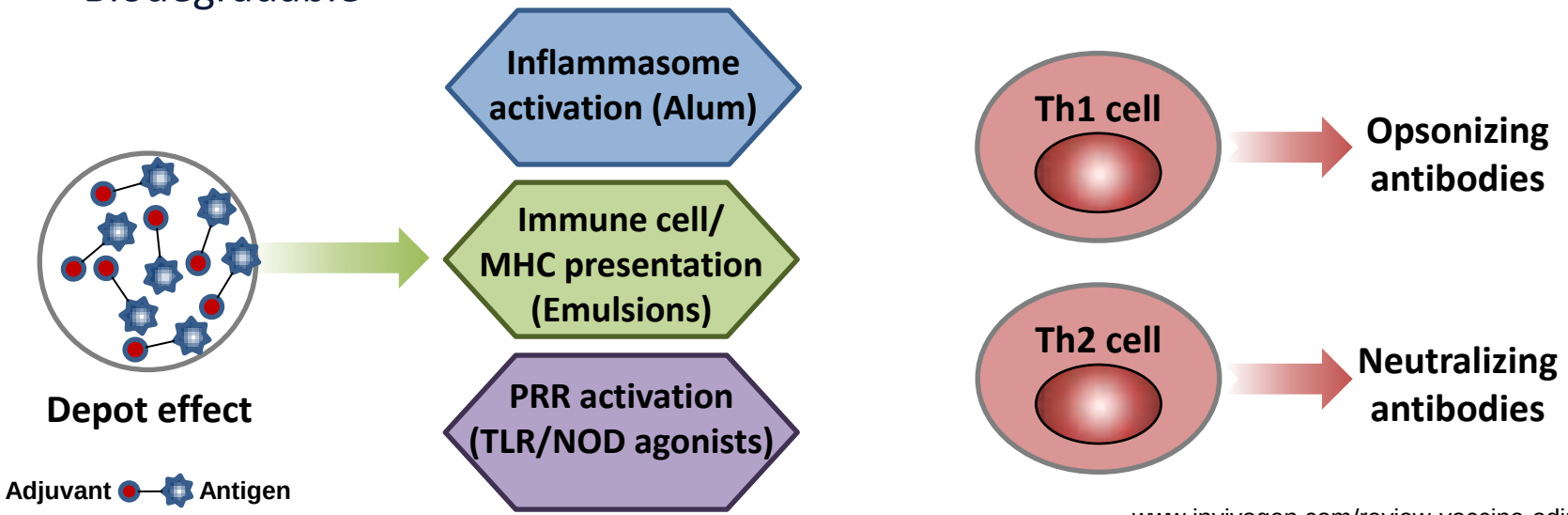


Discrete phase surrounded by surfactant

Continuous phase

Nanoemulsions as Vaccine Adjuvants

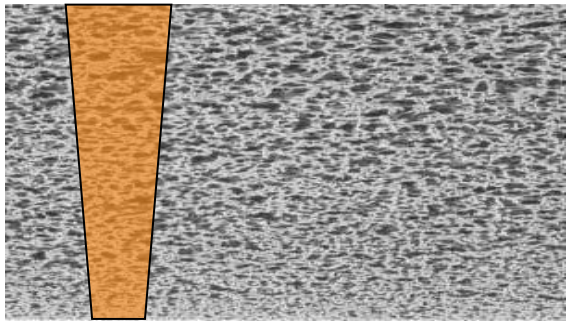
- Vaccines are biological preparations used to improve immunity to a particular disease
- Vaccine adjuvants are materials that enhance the efficacy of vaccines
- Most new vaccine adjuvants are emulsions or liposomes
  - Effective: Enhance both cellular (Th1), humoral (Th2) and major histocompatibility complexes (MHC) responses
  - Well tolerable
  - Biodegradable



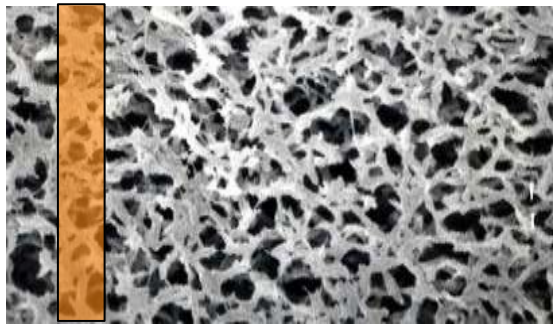
www.invivogen.com/review-vaccine-adjuvants

Nanoemulsion

- Nanoemulsion production challenges:
  - Stability
    - The presence of particles over 1 micron may destabilize the emulsion through Ostwald-ripening
  - Sterilization
    - Removal of bacteria by filtering though a 0.22 micron rated filter
    - Preferred sterilization method in vaccine adjuvants, cancer drugs (injectables)
    - Most particles should be below 0.22 microns so they do not plug the filter



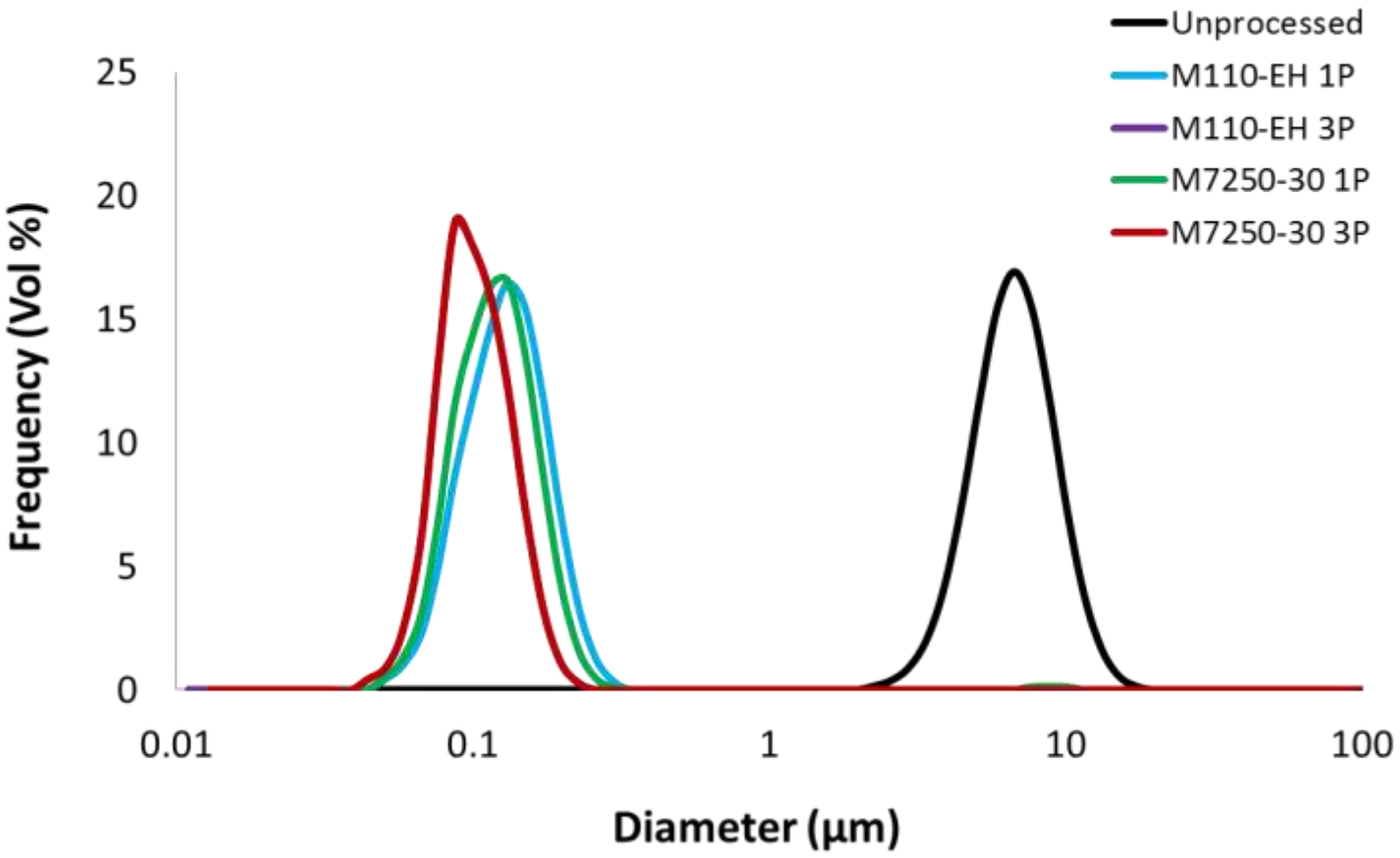
Asymmetric



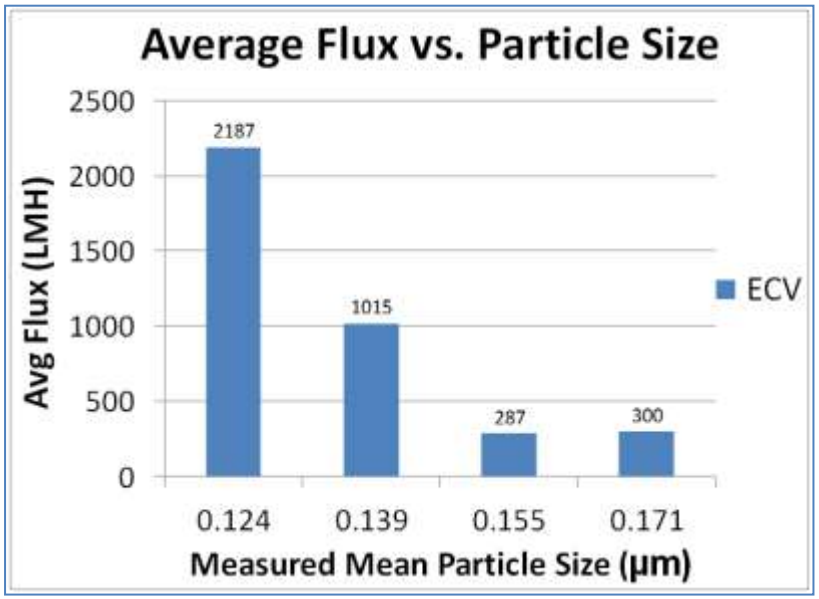
Symmetric

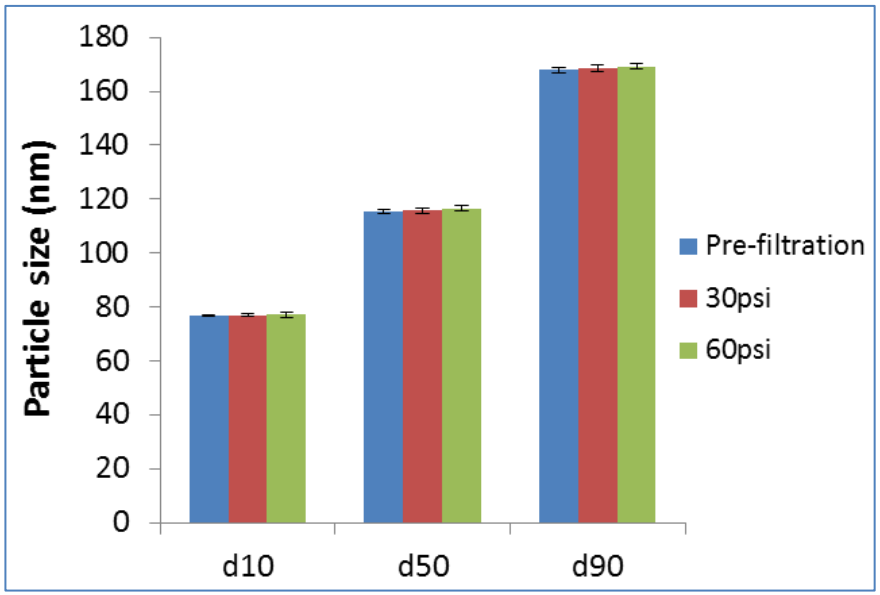
Nanoemulsion – Vaccine Adjuvant Nanoemulsion

- Nanoemulsions are promising new vaccine adjuvants
- Squalane Emulsion



Nanoemulsion – Vaccine Adjuvant Nanoemulsion



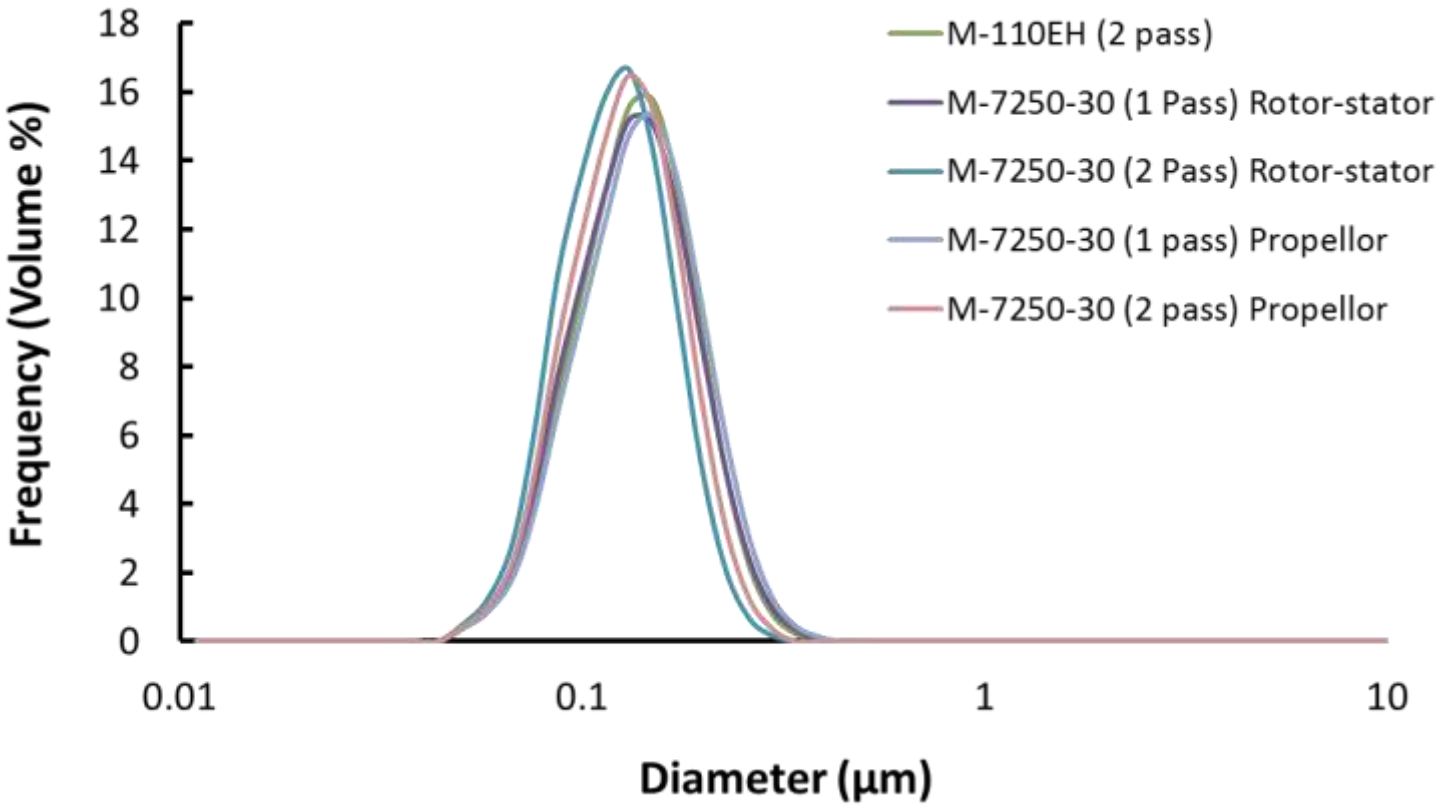


Collaboration with Pall Life Sciences

PALL Pall Corporation

Nanoemulsion – Ocular Nanoemulsion

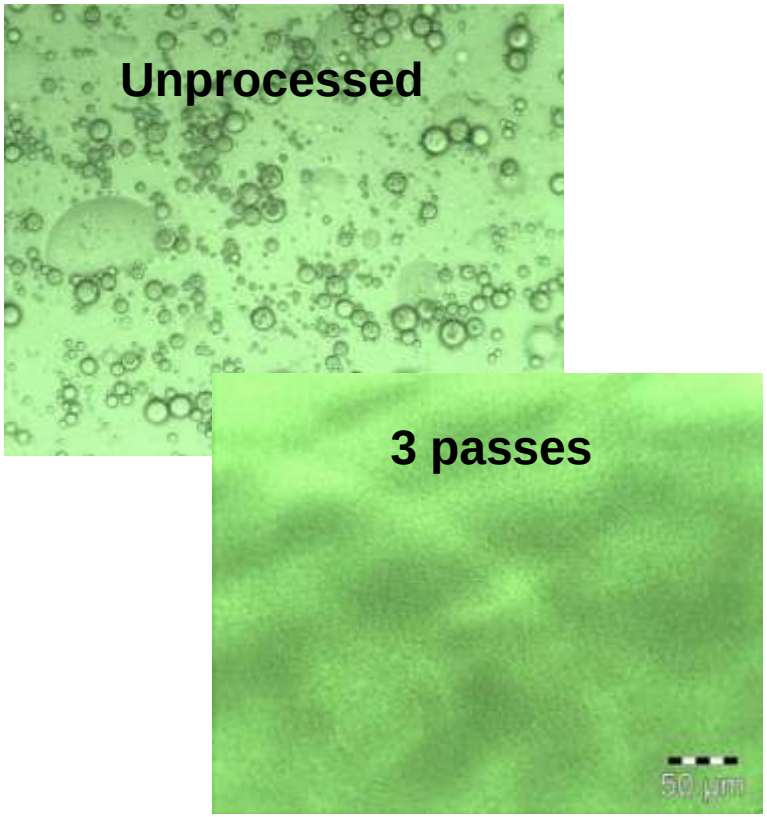
- An ophthalmic nanoemulsion used to treat dry eyes
- Castor oil in water nanoemulsion



Nanoemulsion – Anesthesia Nanoemulsion

- Used for induction and maintenance of anesthesia
- Soybean oil in water nanoemulsion

Pressure (psi)	# Passes	Particle Size (µm)		
		D10	d50	d90
Unprocessed	0	0.954	13.678	24.201
20,000	1	0.200	0.290	0.401
	2	0.187	0.247	0.323
	3	**0.134**	**0.181**	**0.244**



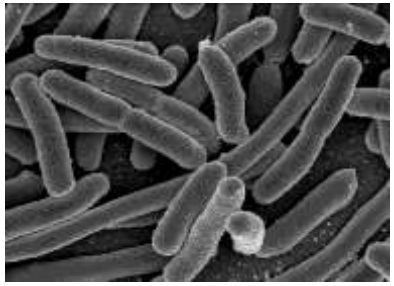
Cell Disruption

Cell Disruption

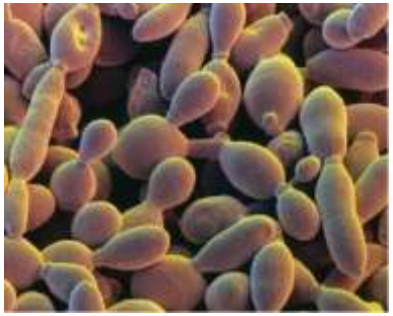
- The generation of important enzymes, proteins and other products form microbes has been developed and used for the last 40 years
- Cell rupture is required any time that products from cell sources must be removed from inside the cell
- Recover the most product
  - Rupture the highest percentage of cells
  - Minimize the potential for denaturing the protein (Shear, Temperature, etc.)
- Microfluidizer is a well known “Technology” within the biotech industry

Cell Disruption

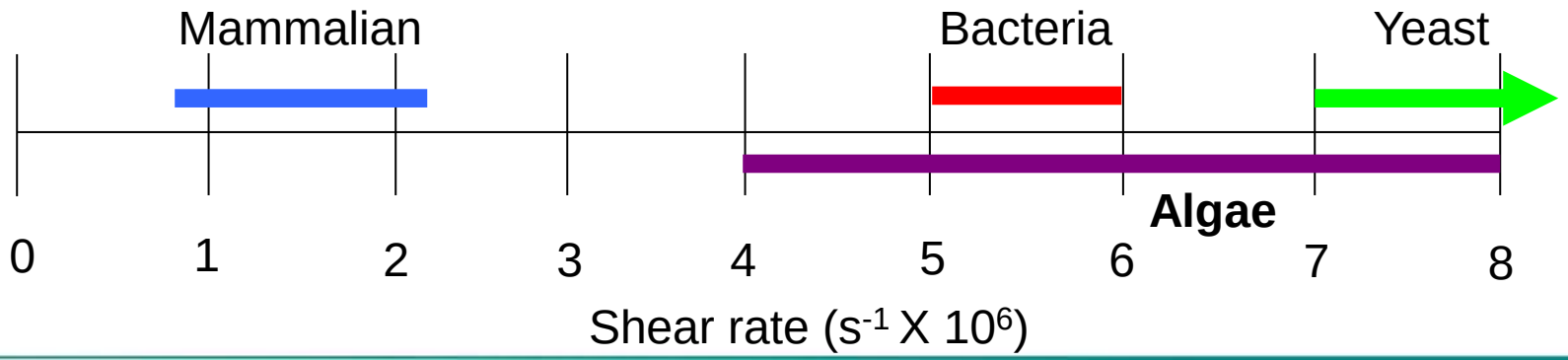
- Bacterial cells are the most commonly used cells for production of simple proteins
  - Usually only require 1P on a Microfluidizer® to achieve >90% rupture efficiency
- Yeast cells have the benefit of creating complex proteins
  - Among the hardest to rupture: high shear and multiple passes



Source: NIAID, NIH



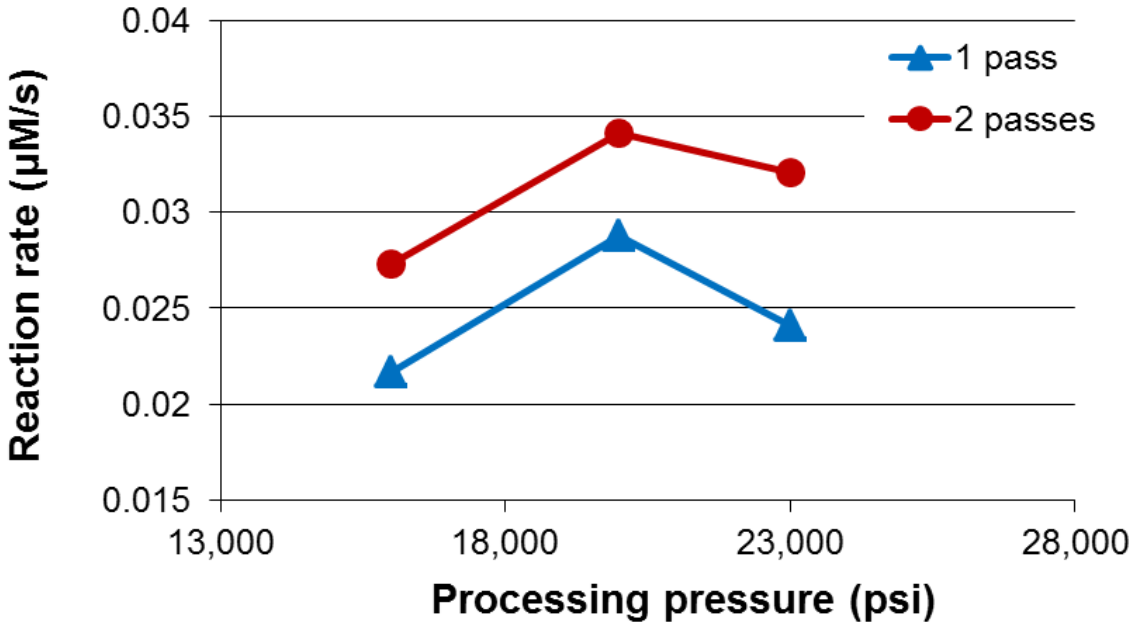
Source: Johns Hopkins Univ.

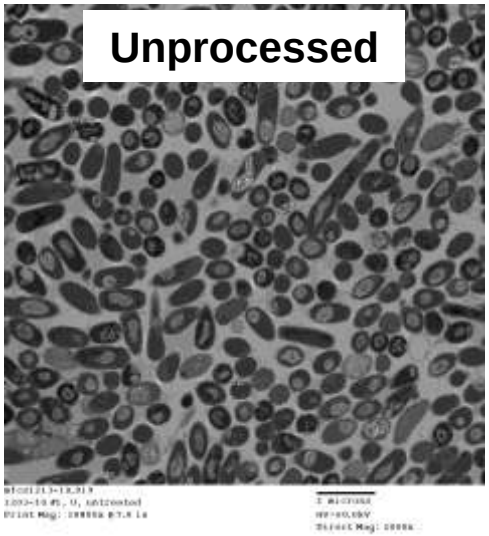


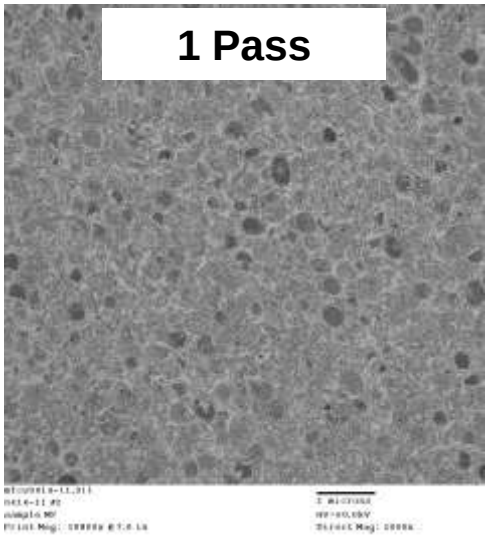
Cell Disruption – *E.coli*

- Optimization of *Eschericia coli* W3110 pTrcHisB:opd cell disruption and Organophosphate Hydrolase recovery
- Grow cells in a 2L bioreactor
- Rupture cells at various conditions:
  - 1 and 2 passes
  - Three different pressures
- Enzyme activity assay after IMCA purification
  - Determines activity of target enzyme vs. Bradford assay which determines total protein recovery
- TEM analysis of cells before and after rupturing

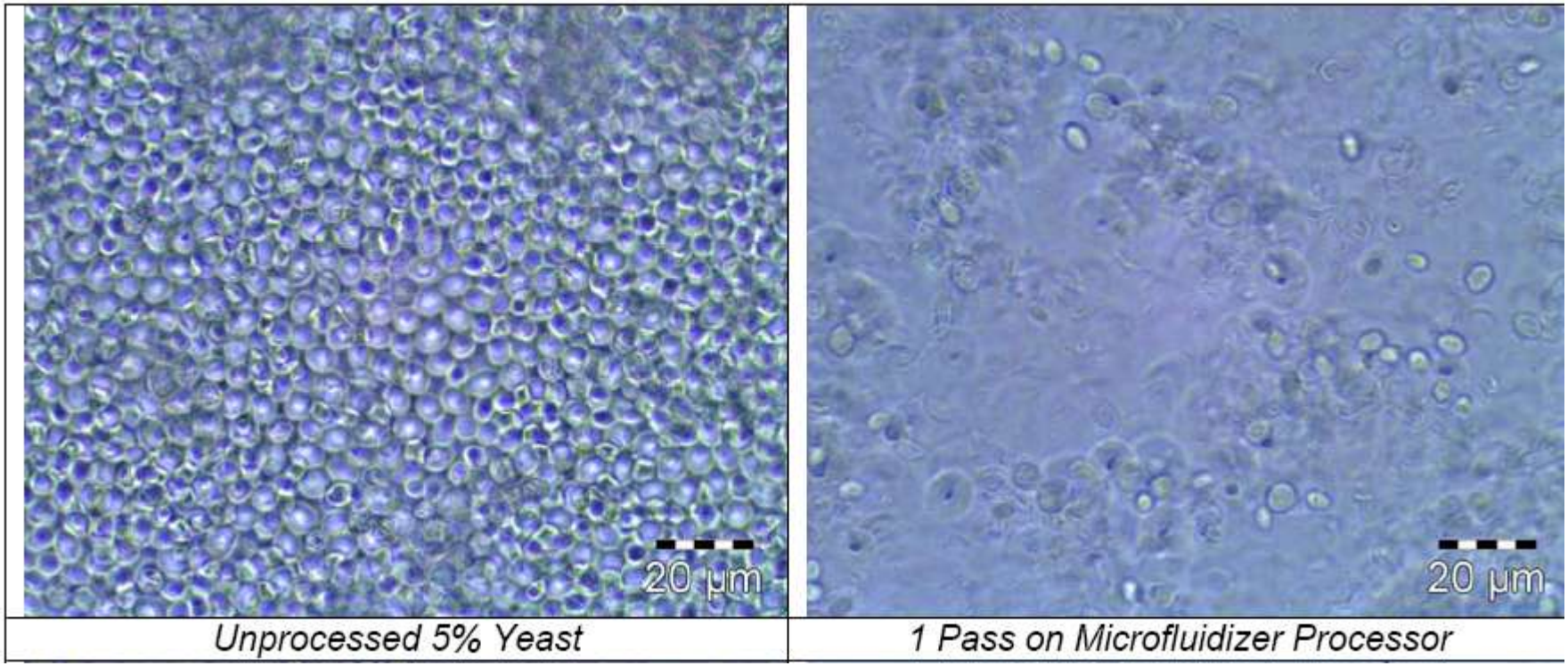
Cell Disruption – *E.coli*







Cell Disruption – Yeast (*Pichia Pastoris*)

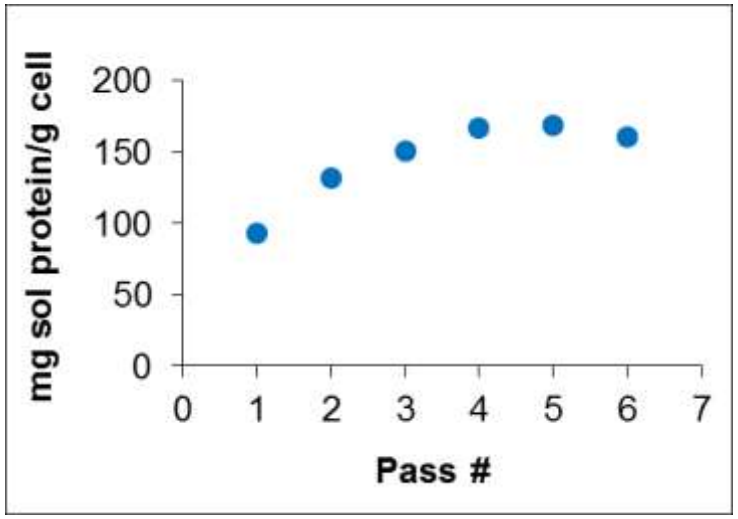


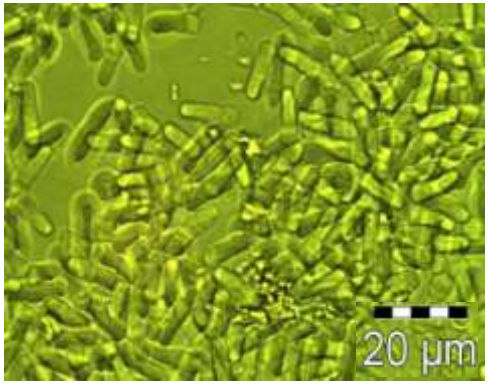
Unprocessed 5% Yeast | *1 Pass on Microfluidizer Processor*

***Process conditions*: 1 pass 30,000 psi (2070 bar)**
***Chamber:* H10Z (100 microns)**
***Shear rate:* 6.94 X 10^6 s^{-1}**

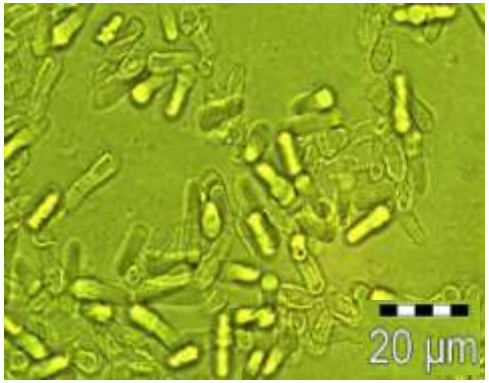
Cell Disruption – Yeast (*S. pombe*)

***Process conditions*:**
30,000 psi (2070 bar)
Chamber:
G10Z (87 microns)
Shear rate per pass:
7.37 X 10^6 s^{-1}

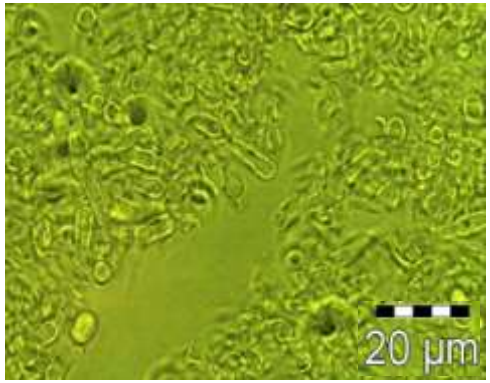




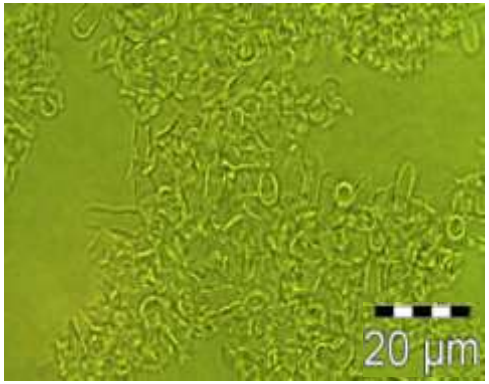
Unprocessed



1 pass ~60% lysis



5 passes ~95% lysis



10 passes ~99% lysis

Size Reduction of Solid API Suspension

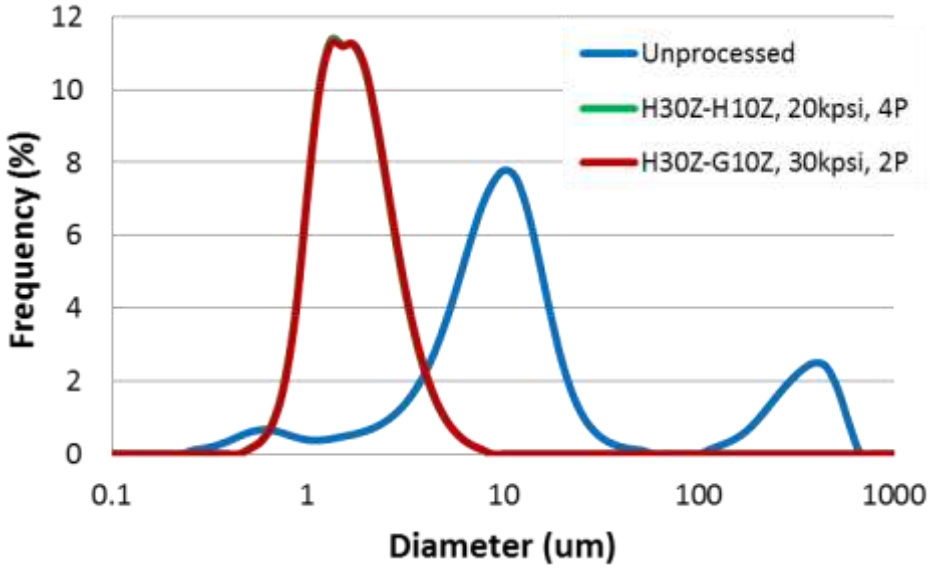
Size Reduction of Solid API Suspension

- Many new APIs are “poorly water soluble”
- Typical time that actives will stay in the body are 12-18 hours.
- In order to achieve therapeutic doses, large quantities of active must be administered.
- Active ingredients that are not dissolved are typically removed by the liver
- Particle size and size distribution reduction
  - Does not change the solubility
  - Changes (increases) surface area, which greatly affect the dissolution rate and in most cases the bioavailability

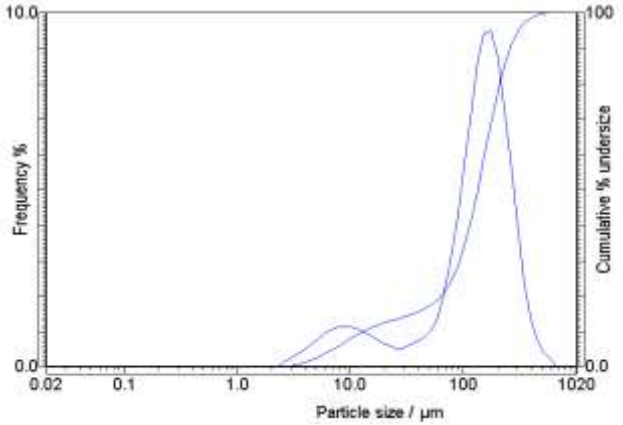
Size Reduction of Solid API Suspension – Atovaquone

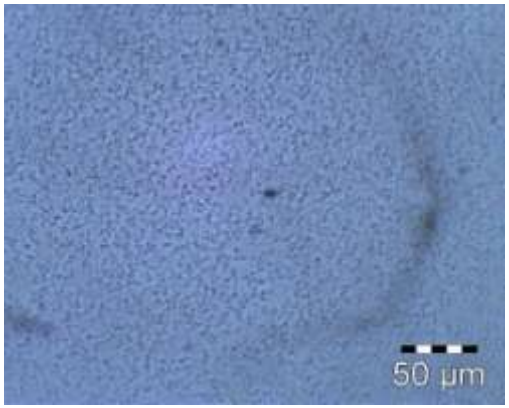
- Used to treat many conditions including
  - > Malaria
  - > Toxoplasmosis
  - > Babesia
  - > Pneumocystis pneumonia (PCP)
- Generally used in combination with other drugs
  - > Proguanil
  - > Azithromycin
- It prevents the electron transport of enzymes

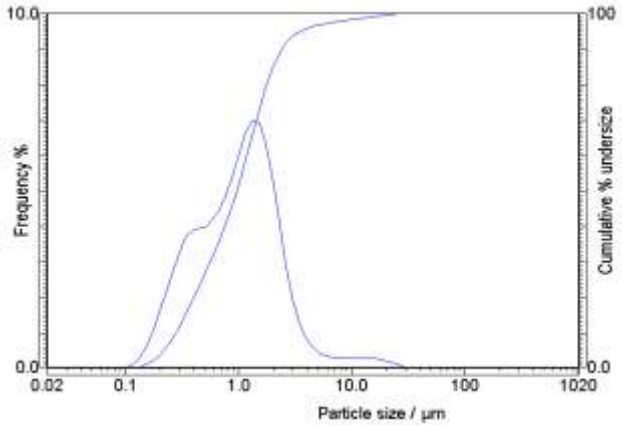

Source: lymewithpurpose.wordpress.com



Size Reduction of Solid API Suspension – High Blood Pressure Drug



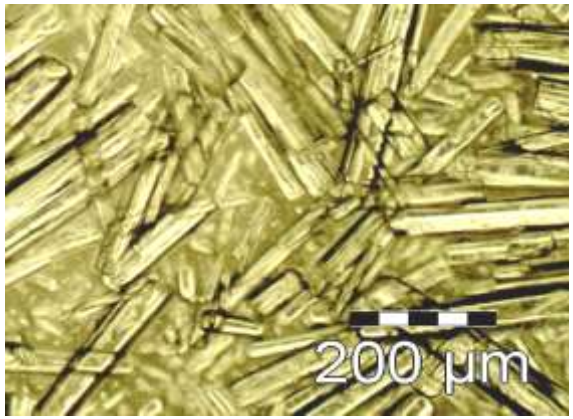


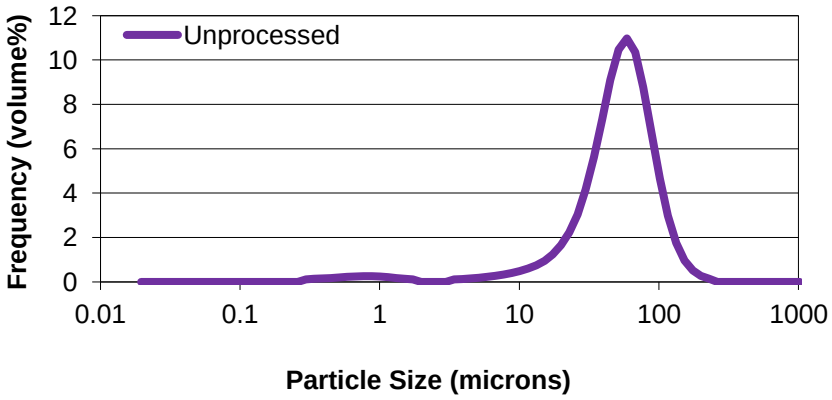


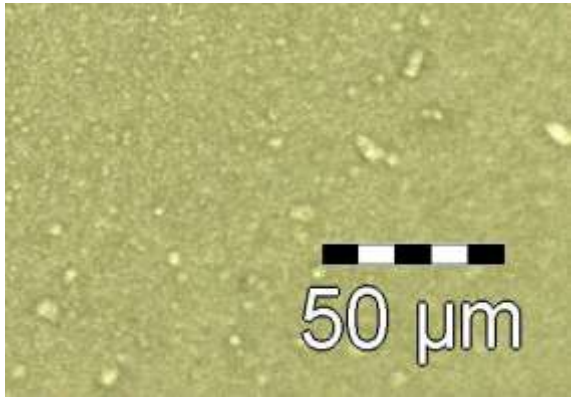
1 pass

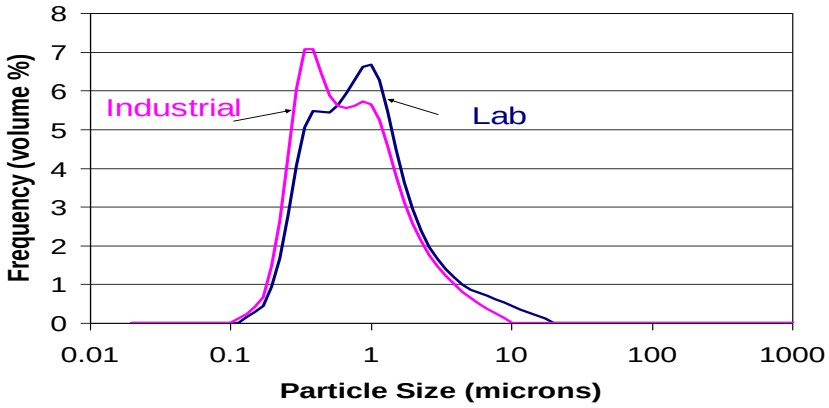
Median particle size (D50) after 1 pass: ***955 nm***

Size Reduction of Solid API Suspension – Epilepsy Drug







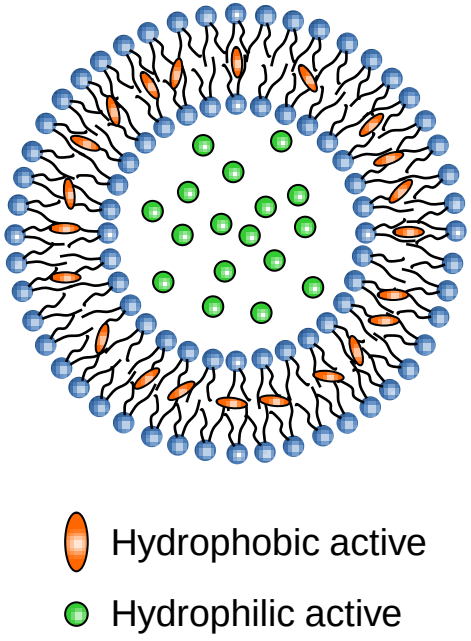


- **Median particle size** (D50) with ***lab*** machine: ***773 nm***
- **Median particle size** (D50) with ***production*** machine: ***614 nm***

Liposome

Liposome

- Liposomes are spherical lipid vesicles with a bi-layered membrane structure
- Can encapsulate either hydrophobic or hydrophilic active, or both
- One of the most successful delivery systems currently in clinical use



Liposome – Liposomal Active for the Treatment of Cancer

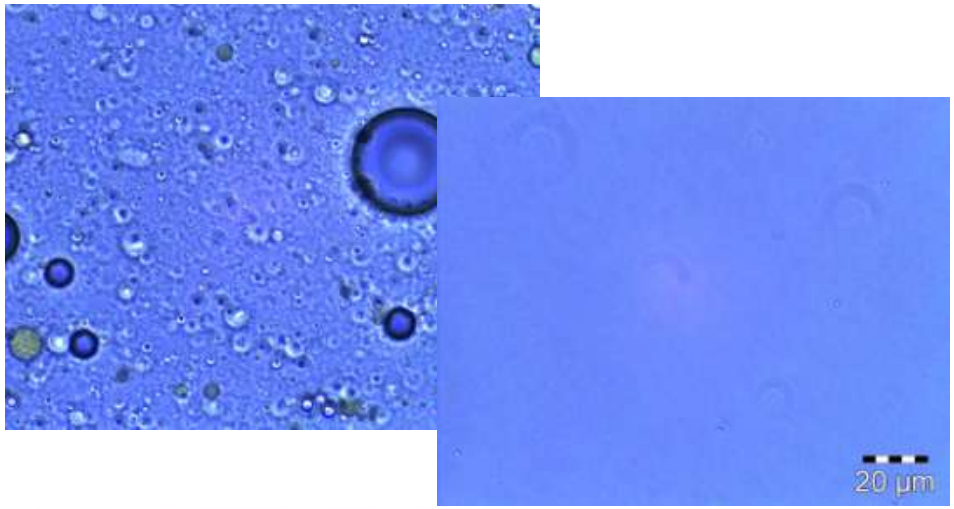
Company: A large US based pharmaceutical company

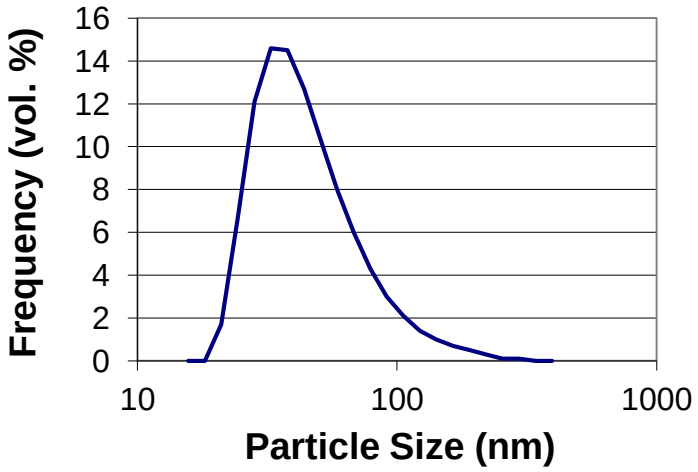
Application: Treatment of carcinomas

Material: A poorly water soluble active pharmaceutical ingredient with a melting point of < 60ºC

Goal: To analyze various processing conditions and formulations to produce liposomal formulations of the active in the range of 50 nm to 150 nm.

Results:

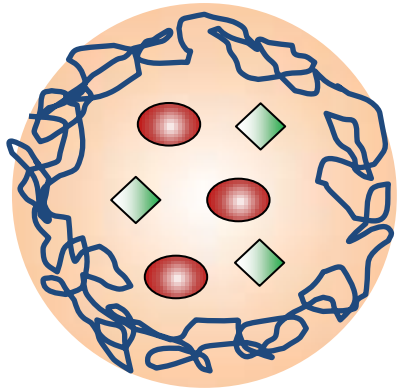




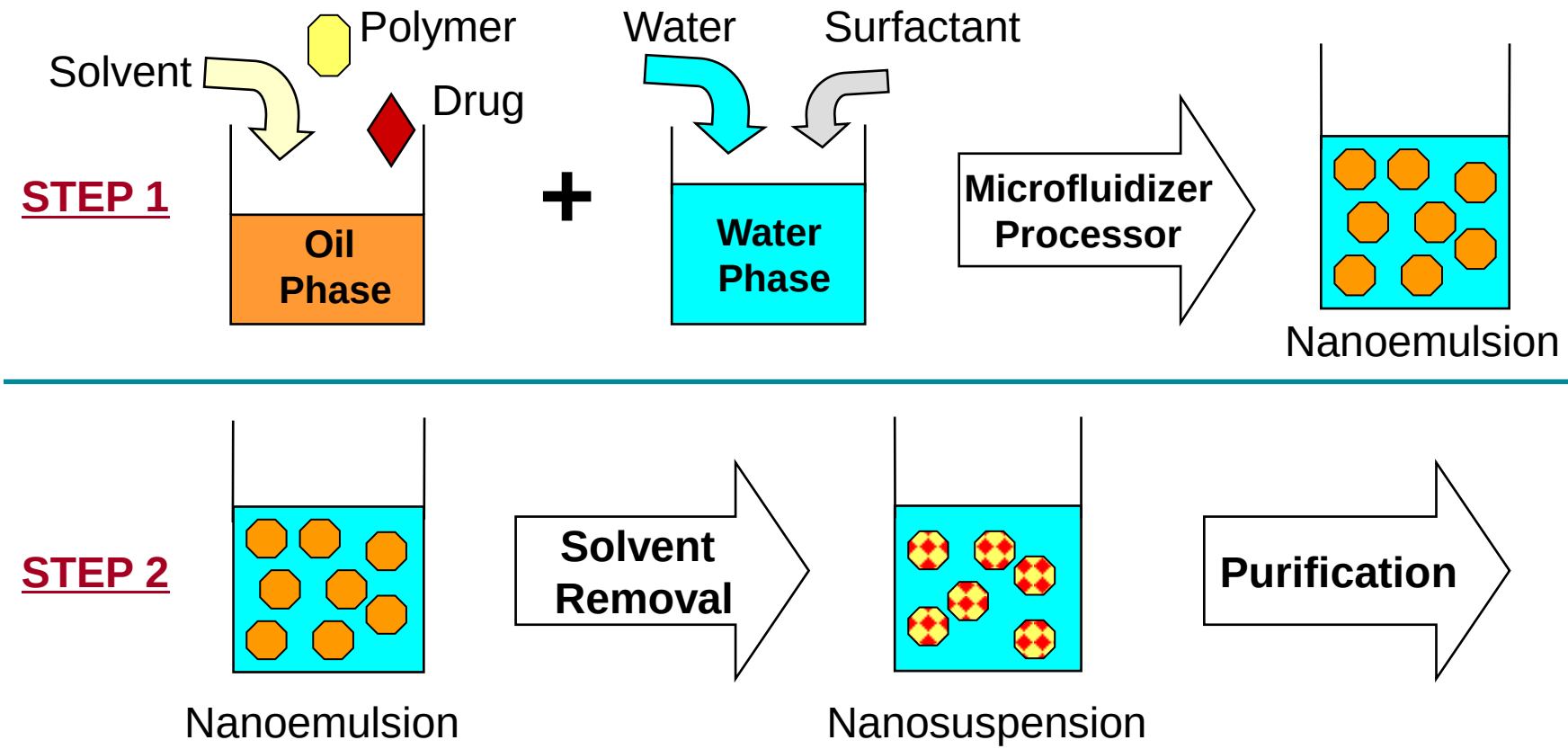
Polymer Nano-Suspensions

Polymeric Nanoparticles

- Combination drug products
- Drug and resistance modulator
- Drug and energy delivery (heat, light, and sound)
- Drug and imaging agent



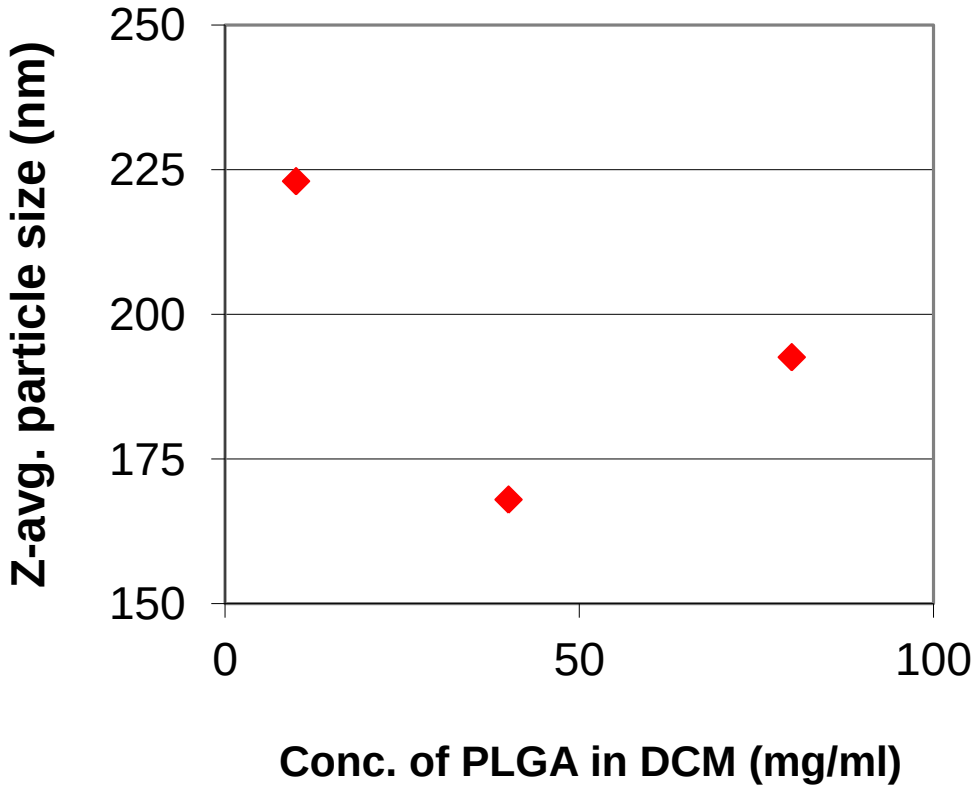
Polymer Nanoparticles – Emulsion Evaporation Method

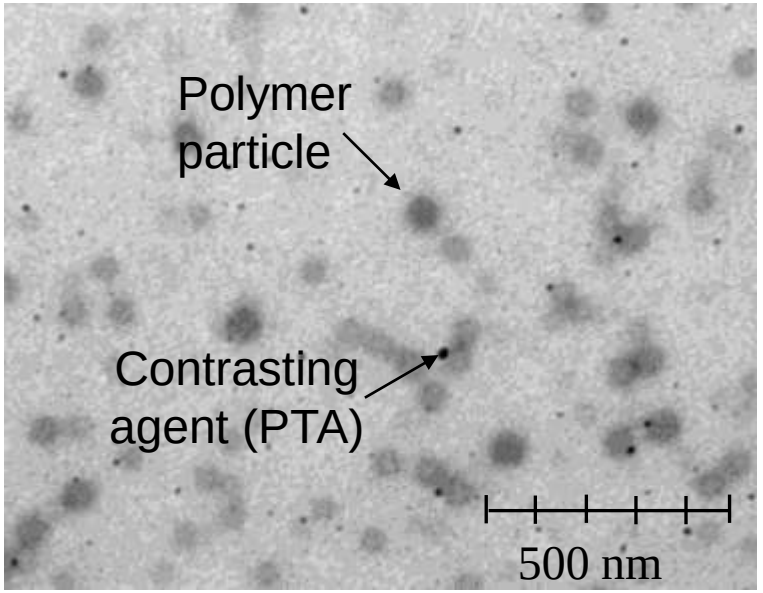


- Solvent is ***not*** miscible with water
- Post processing is ***critical*** and includes removal of solvent and the formation of nanosuspensions

Polymer Nanoparticles – Emulsion Evaporation Method

- Polymer/solvent/non-solvent system: poly(lactic-co-glycolic acid (PLGA) / dichloromethane (DCM) / D.I. water
- Processed with M-110EH for 1 pass at 10,000psi (70 Mpa)





TEM images were taken to verify particle size

T. Panagiotou, S.M. Mesite, J.M. Bernard, K.J. Chomistek and R.J. Fisher, NSTI-Nanotech 2008, ISBN 978-1-4200-8503-7 Vol.1

Polysaccharide Molecular Weight Reduction

Polysaccharide Uses in Pharmaceuticals

- Polysaccharides are utilized in many pharmaceutical applications
  - Biocompatible and biodegradable in the body
  - Naturally broken down to building blocks, allowing for drug release without inflammatory immune response
- Polysaccharides have diverse molecular weights (MW) and structures
  - Hydrophilic groups enable bond formation with tissues and mucosal membranes, and extended circulation in blood

    → increased probability of targeting of tumors
  - Hydrophobic groups also prevalent on polymer backbone

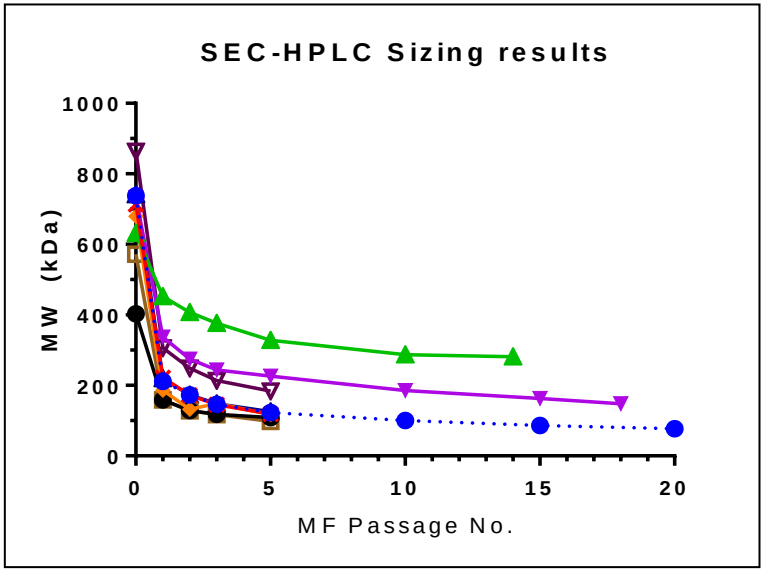
    → good carriers for water-insoluble drugs
  - **Low MW polysaccharides show positive Zeta potential and higher solubility at a neutral pH**

Exercise physiology: energy, nutrition, and human performance. Lippincott Williams & Wilkins. 2006

Polysaccharide MW Reduction

- Material: Polysaccharide vaccine recently developed to enhance production of type-specific antibodies against pneumococcus in high-risk patients
- Goal: Reduce molecular weight of the polysaccharide to below 250 kDa
- The LV1 was utilized to process polysaccharide formulations

➢ Results indicate that the MW of 7 out of 8 of the polysaccharides were reduced below the targeted MW using the Microfluidizer®



Summary

- Microfluidizer high shear fluid processors can produce nanomaterials with a wide variety of multiphase applications

- Through the demonstrated case studies, Microfluidizer™ technology has been proven to:
  - Be superior to conventional technology.
  - Be very efficient and reliable.
  - Offer precise, repeatable and scalable results.

- For biopharma industry, production Microfluidizers® are designed for compliance with cGMP and are capable of CIP/SIP operations.

Any Questions?

I will be happy to address any questions

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Learn More

Visit www.microfluidicscorp.com to view our complete technology capabilities, or submit a sample for Proof of Concept testing

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