

NEEDLE-FREE INHALABLE VACCINE AND ANTIBIOTIC POWDER AEROSOLS

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ROOM-TEMPERATURE-STABLE VACCINES, ENZYMES, ANTIBODIES, ANTIBIOTICS, AND PHARMACEUTICALS

Strategy: Stabilize and dry powders near room temperature by supercritical or near-critical fluid carbon dioxide processing

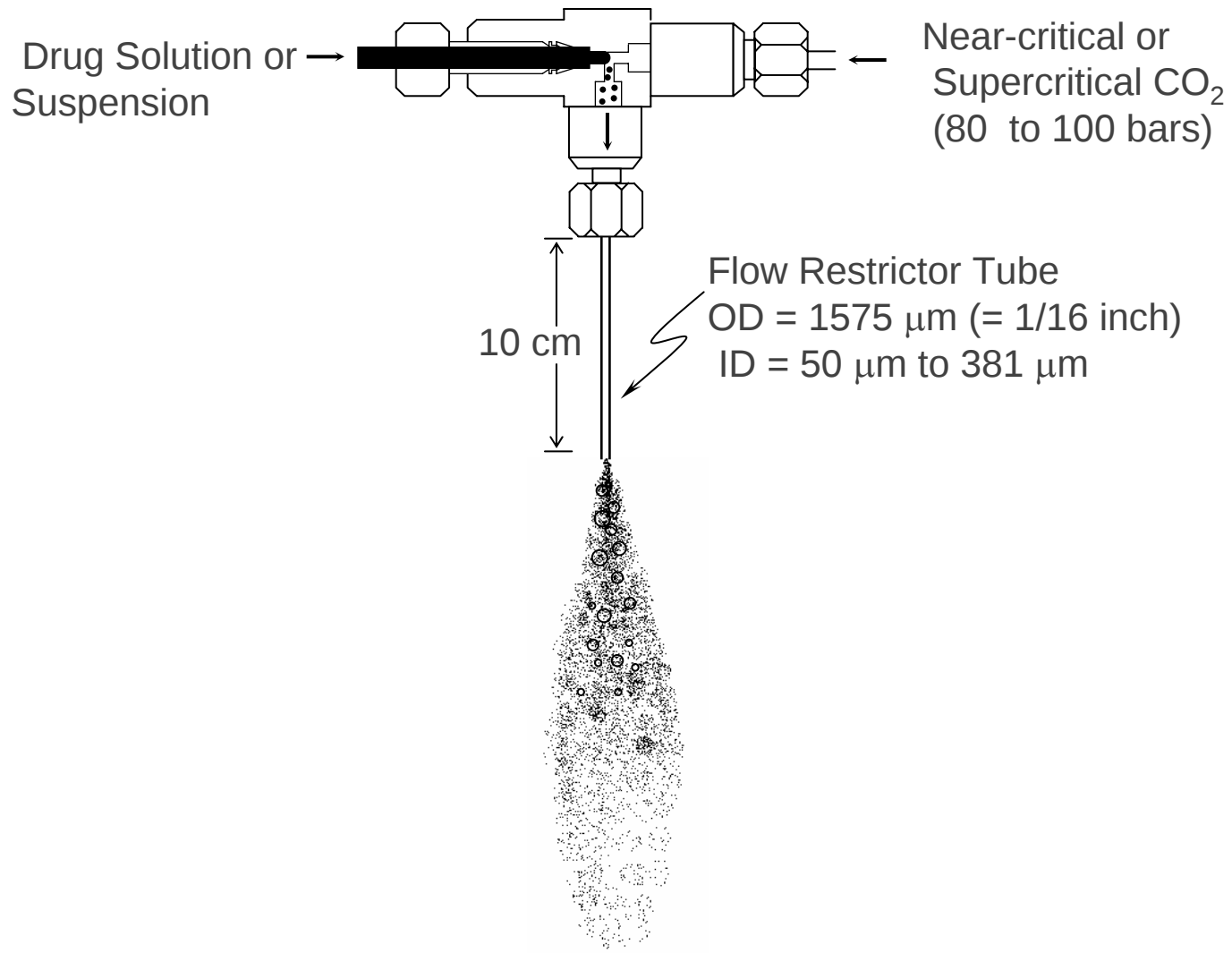
- Needle-free delivery of respirable vaccines (e.g., measles), antibiotics, and pharmaceuticals
- Nanoparticle and microparticle synthesis and coating
- Micronize dry powder antibiotics, enzymes, antibodies, e.g., Cipro Moxifloxacin, rifampicin, antitrypsin, IgG, for delivery into
- lungs (1-3 microns), or into nasal passages (10-30 microns)
- Stabilize in glassy sugar matrices, then micronize and desolvate
 - Avoid aggregation and create rapidly redissolved microparticles
 - Enhance bioavailability by increasing surface areas of particles, e.g., with smaller particles or leached composite particles

The Principle of a New CAN-BD Process: (Carbon Dioxide Assisted Nebulization with a Bubble Dryer®)

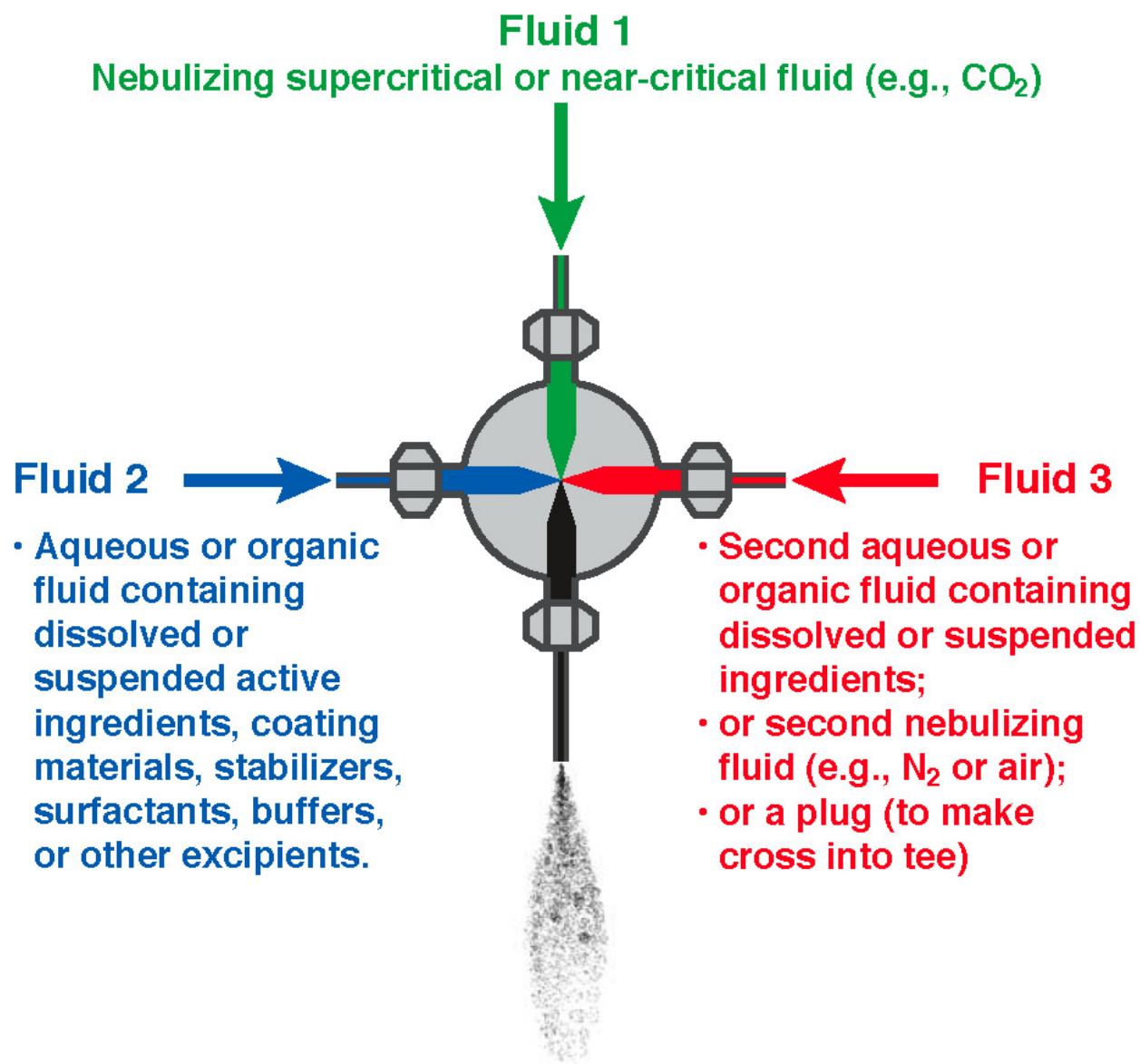
- In CAN-BD a solution or suspension in water (or, when necessary, an organic solvent) is mixed intimately in a low volume tee (or cross) with CO₂ at 100 bar to form an emulsion.
- The emulsion is rapidly expanded to atmospheric pressure through flow restrictor to generate aerosols of microbubbles and microdroplets.
- The aerosol plume is dried at temperatures below 60 °C as it mixes with nitrogen or air in the drying chamber.
- Dry fine powders are collected and packaged.

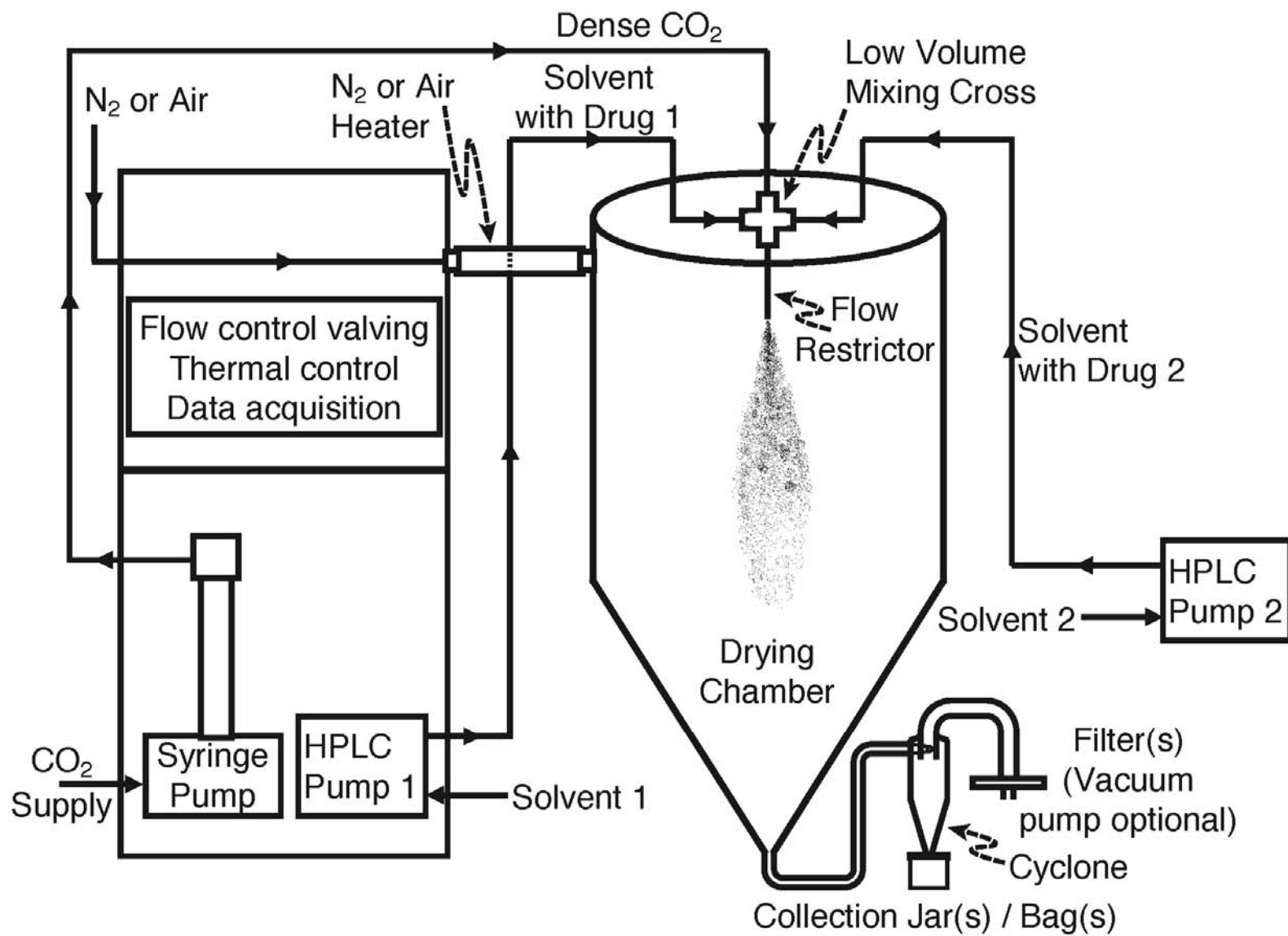
CAN-BD Mixing Tee

Drying Temperature: 1°C to 60°C



LOW DEAD VOLUME CROSS FOR CAN-BD



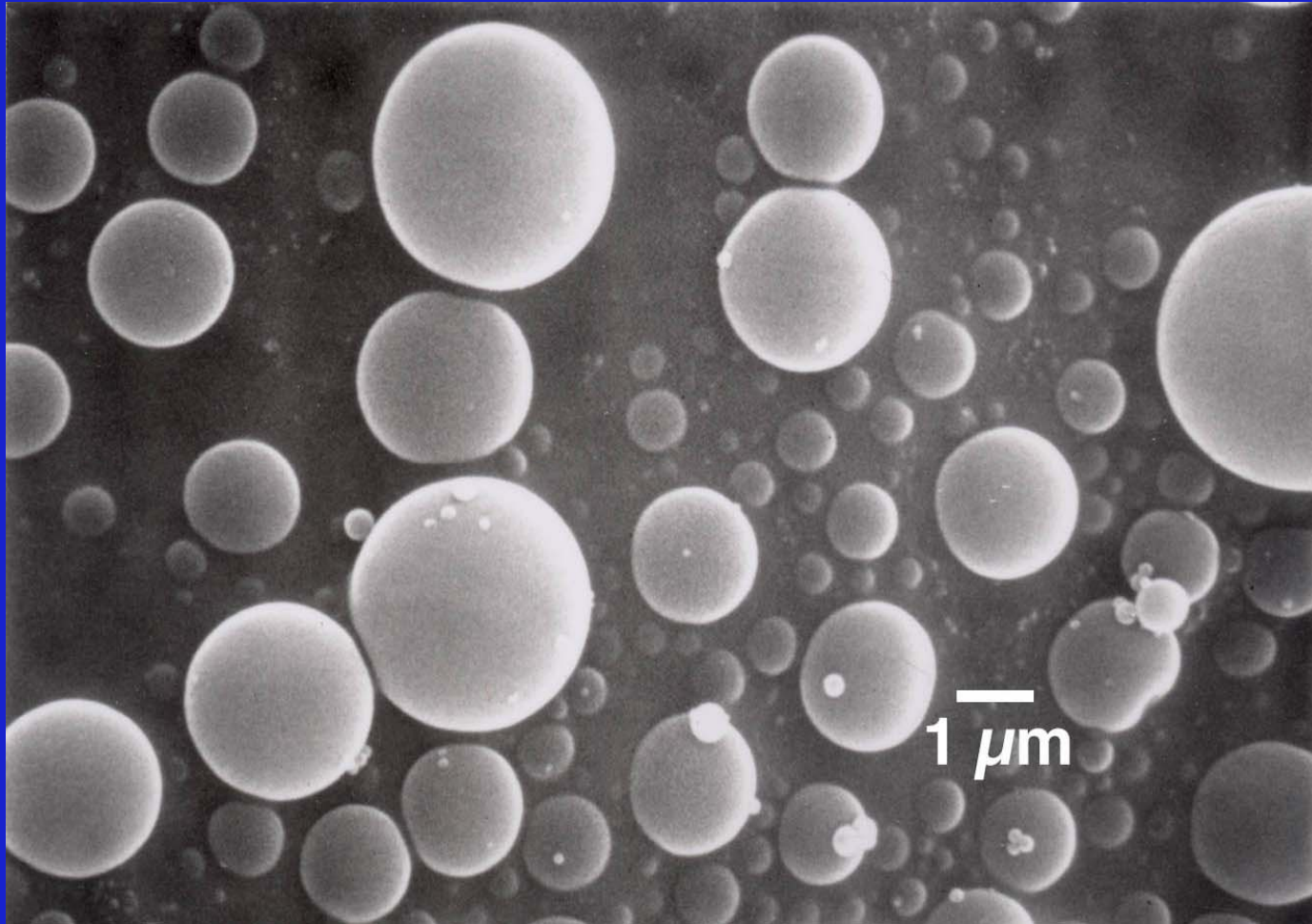






**Aerosol spray of microbubbles and droplets generated by the
CAN-BD process**

LYSOZYME

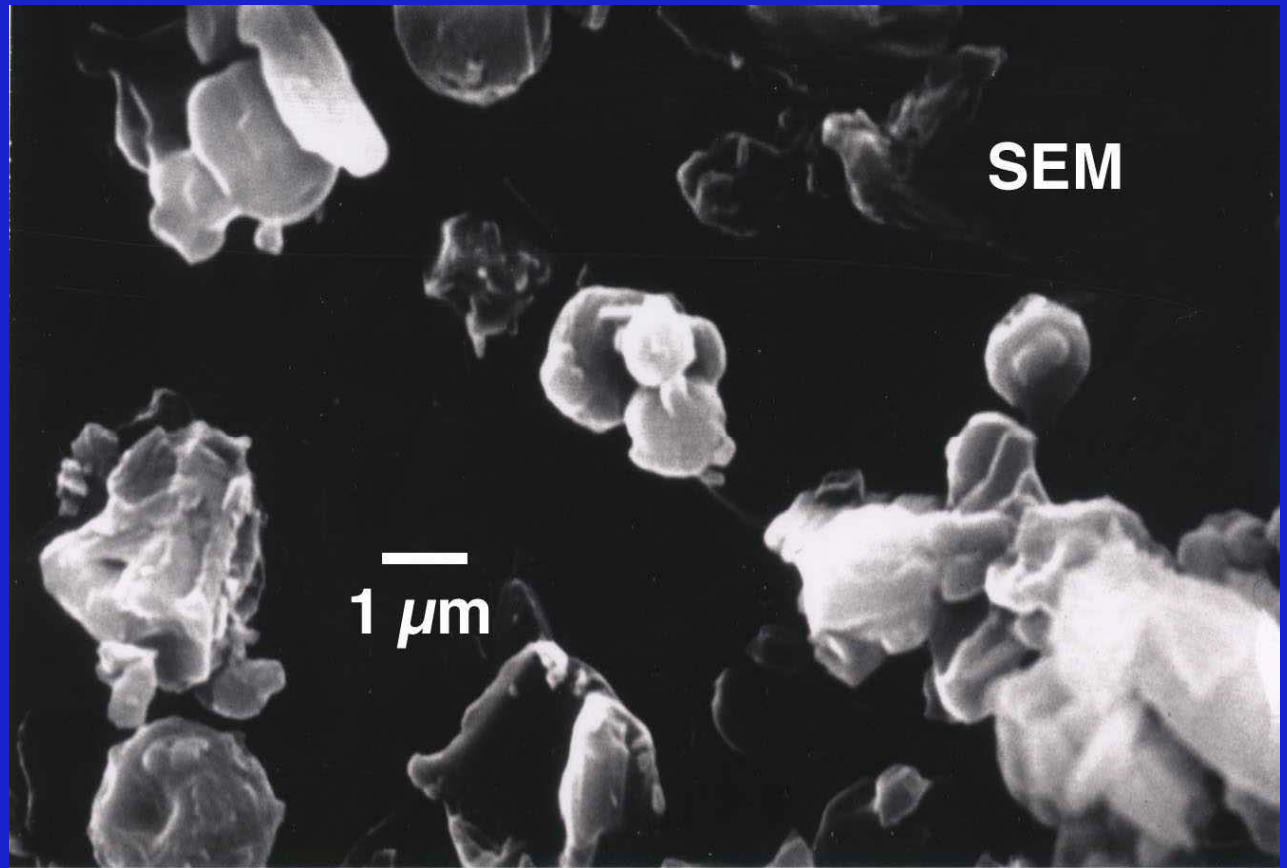
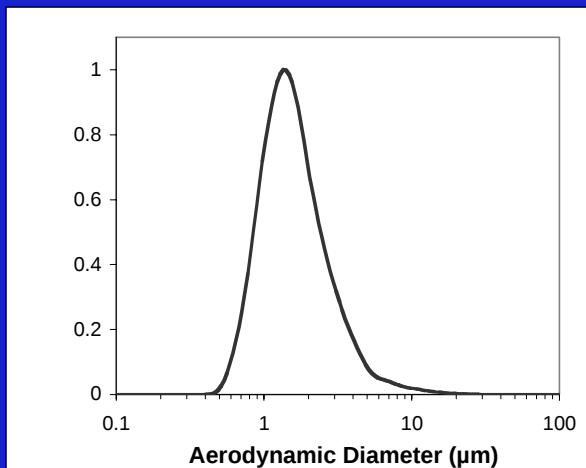


- Particles formed at 50 °C from an aqueous solution containing 10% lysozyme, 0.01% PEG 3350 and 0.01% Tween 80
- Mean aerodynamic diameter = 1.4 μm (95% < 3.0 μm)

Aerosol Delivery of Antibiotics to Lungs

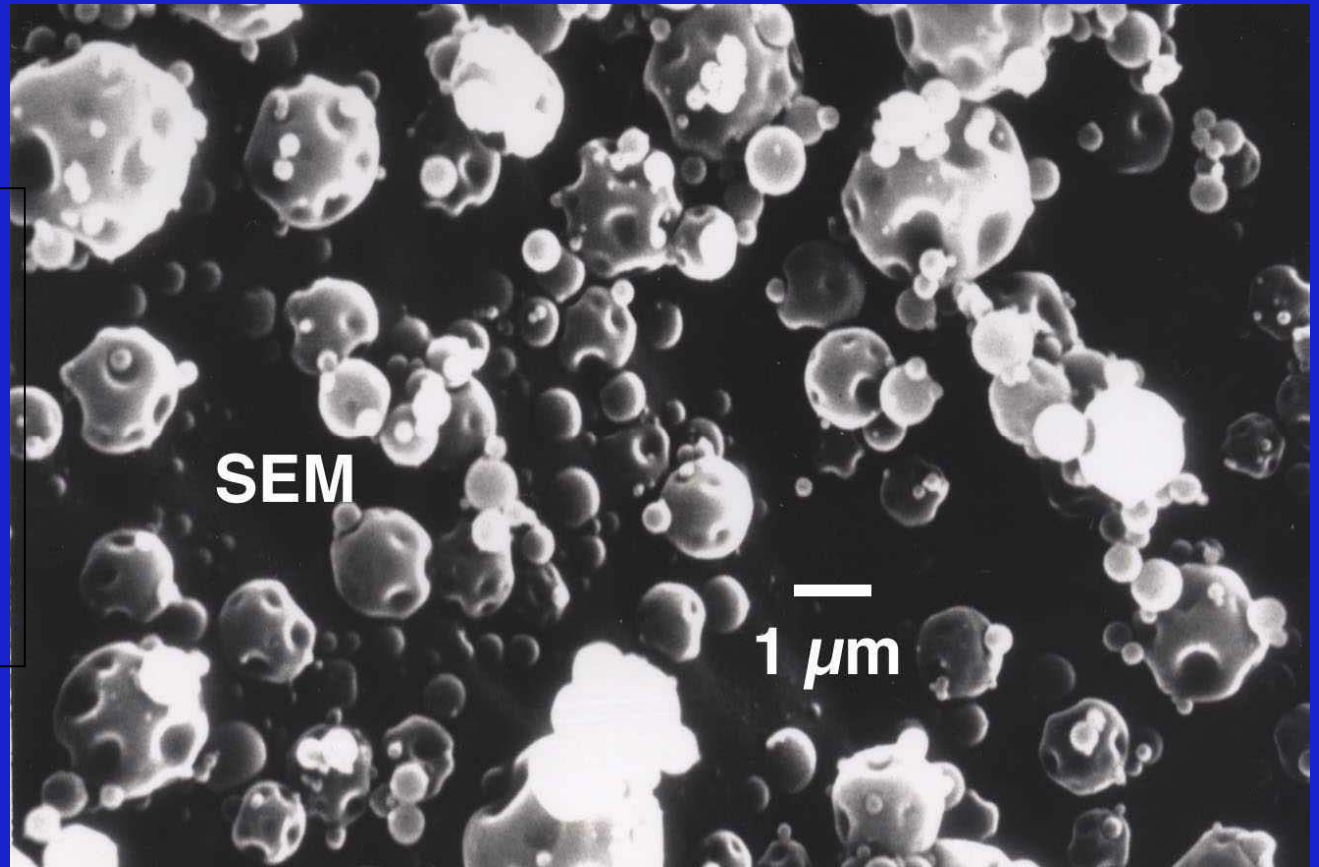
- Higher doses delivered locally to treat lung infections
- Reduced whole body burden of antibiotics
- Longer persistence in lung air space and tissue possible

Ciprofloxacin hydrochloride



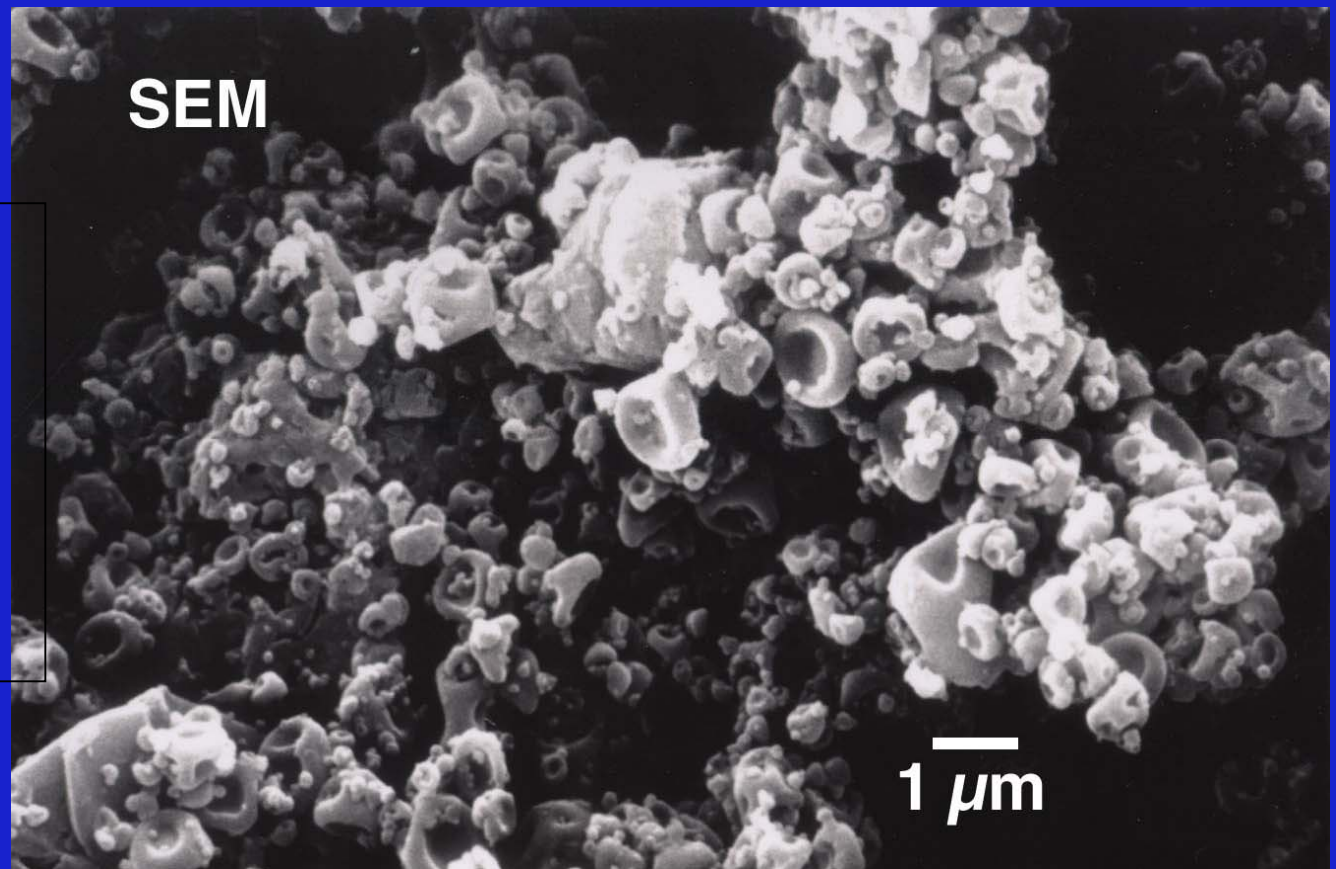
- Size distribution (by Aerosizer) and SEM of ciprofloxacin hydrochloride micronized by CAN-BD from a 3% aqueous solution. Dried at 60°C.
- Mean aerodynamic diameter = 1.6 μm; with 95% under 4.1 μm

Moxifloxacin hydrochloride



- Size distribution (by Aerosizer) and SEM of moxifloxacin hydrochloride micronized by CAN-BD from a 2% aqueous solution. Dried at 60°C.
- Mean aerodynamic diameter = 1.3 μm; with 95% under 2.5 μm

Rifampicin

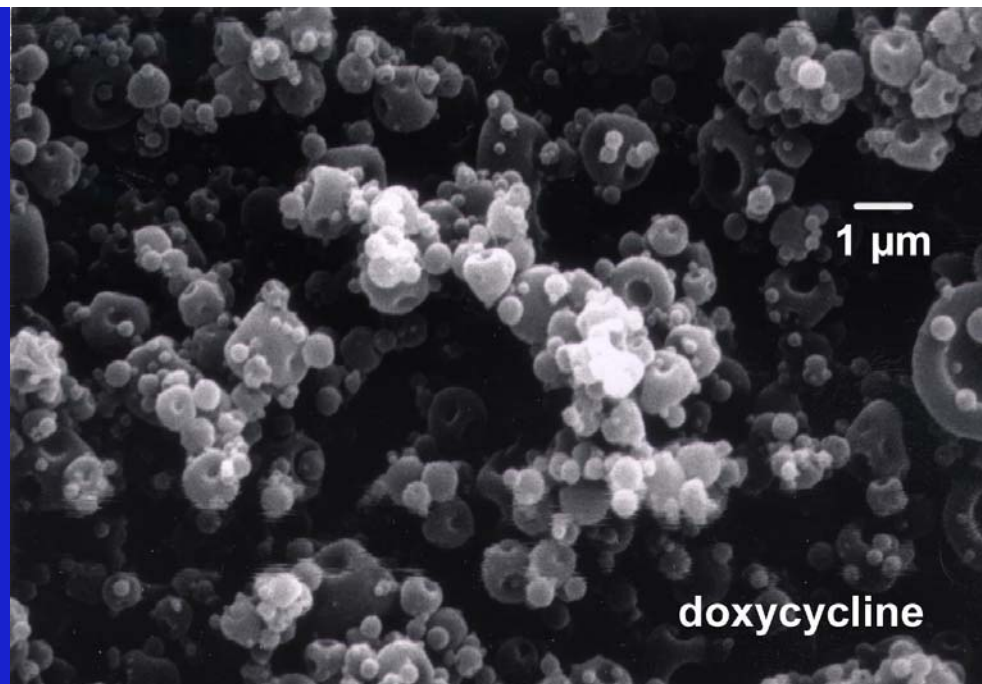


- Size distribution (by Aerosizer) and SEM of rifampicin micronized by CAN-BD from a 2% solution in ethyl acetate. Dried at 30°C.
- Mean aerodynamic diameter = 0.78 μm ; with 95% under 1.3 μm

0.4% solution in H₂O
Avg. particle size = 0.88 μ m
95% $\leq$ 1.42 μ m

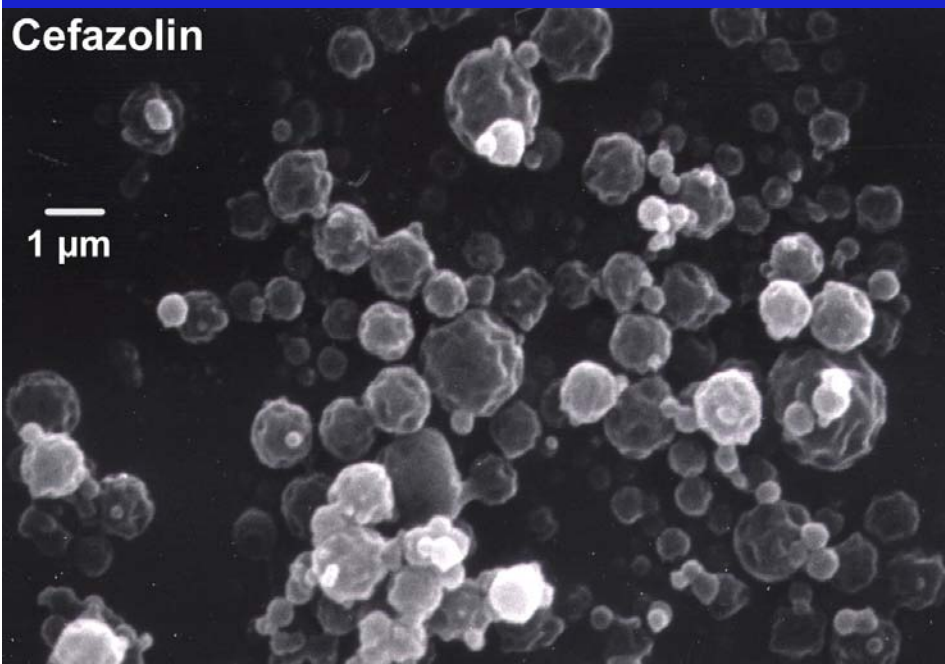
Amoxicillin

1 μ m



Cefazolin

1 μ m



0.5% solution in H₂O
Avg. particle size = 0.97 μ m
95% $\leq$ 1.60 μ m

**Particles of antibiotics
generated from
aqueous solutions**

0.5% solution in H₂O
Avg. particle size = 1.05 μ m
95% $\leq$ 1.82 μ m



Mexico-INSP

Measles aerosol immunization

SAFE ✓ no serious AEFIs, fewer than SC route

IMMUNOGENIC ✓ induced >80% response among infants < 9 months of age

✓ 86-100% response in studies (1961-2002) among ≥ 9 months & school-aged children

✓ **good response with rubella vaccine**

EFFECTIVE ✓ lower attack rate (outbreak Mexico 1988-90):

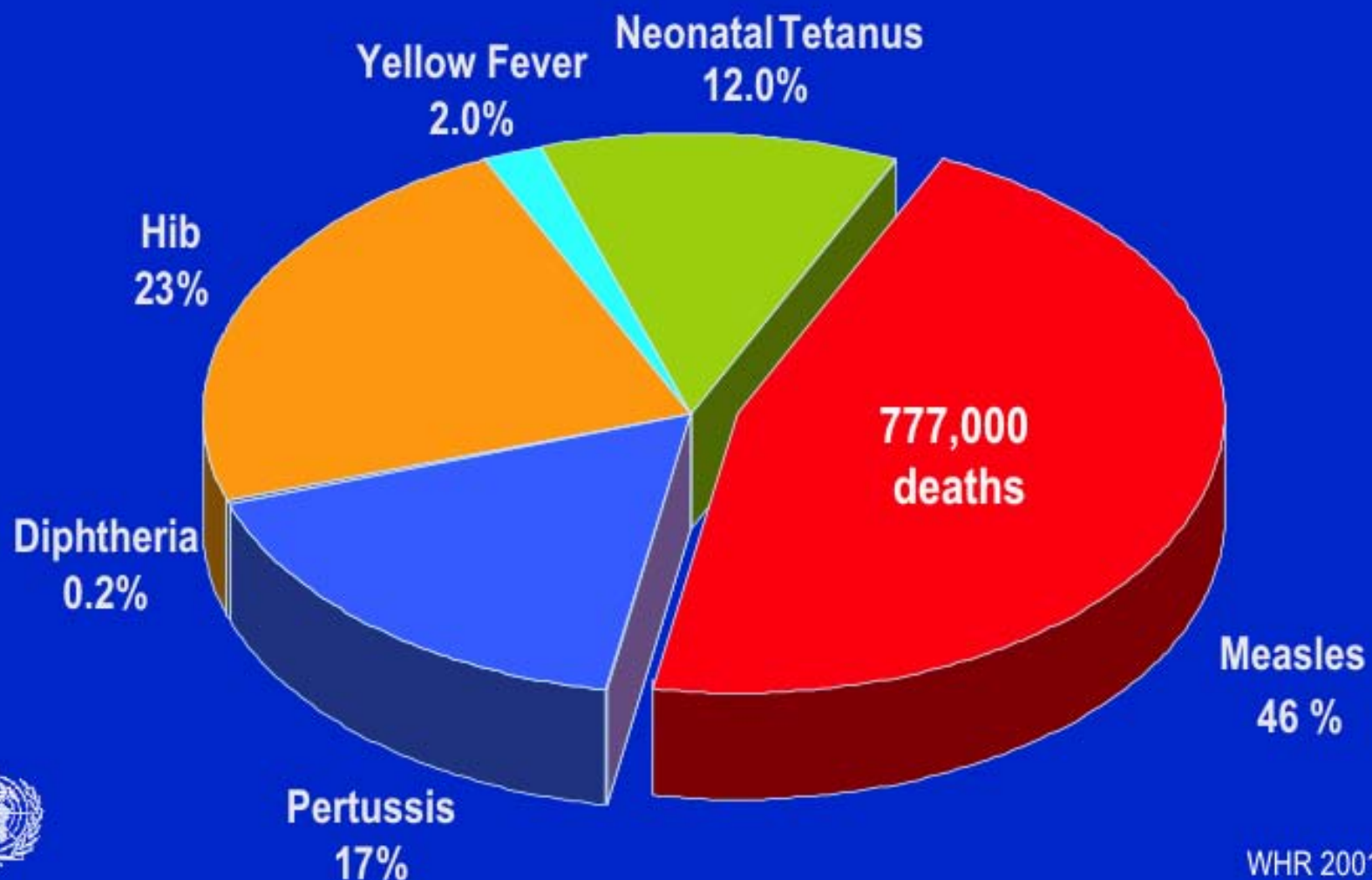
- immunized with aerosol (0.8%)
- immunized with s-c (14%)
- unvaccinated group (26%)



EZ strain retains potency during nebulization process

Henao-Restrepo and Papania, NIH Vaccines Conference, Rockville, Dec 2003

Causes of 1.7 million vaccine-preventable deaths among children < 15 years, 2000



Henao, An Overview of Aerosol Immunization, Meeting of the WHO Steering Committee on New Delivery Systems, 2004

CAN Processing of Live Virus Measles Vaccines



P. Rota, MD and M. Papania, MD, CDC, Atlanta, provided viral assays;
J. Burger, S. Cape, PhD, and E. Huang, PhD, A-D and CU, performed CAN-BD;
* B. Shekunov et al., Ferro, have achieved 34% yield by CAN-SFD.
Partial funding provided by Creare, Inc.

Measles Vaccine Team

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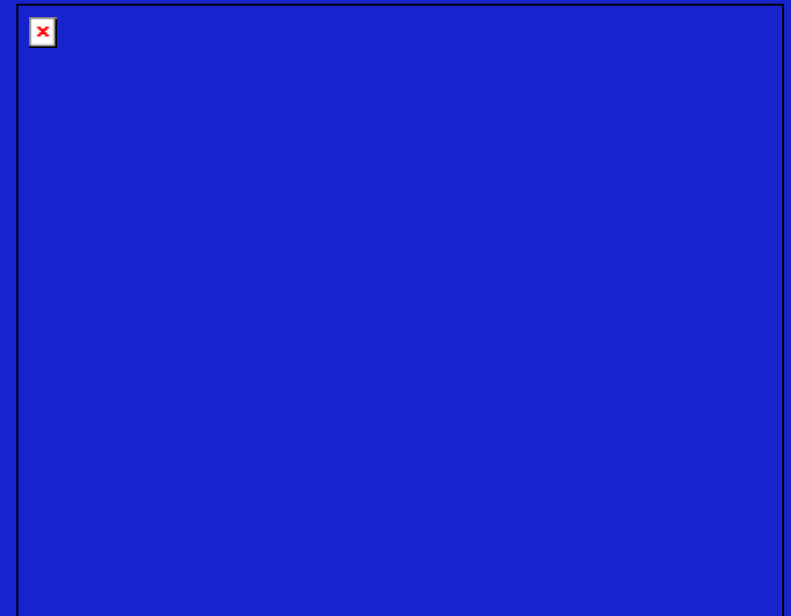
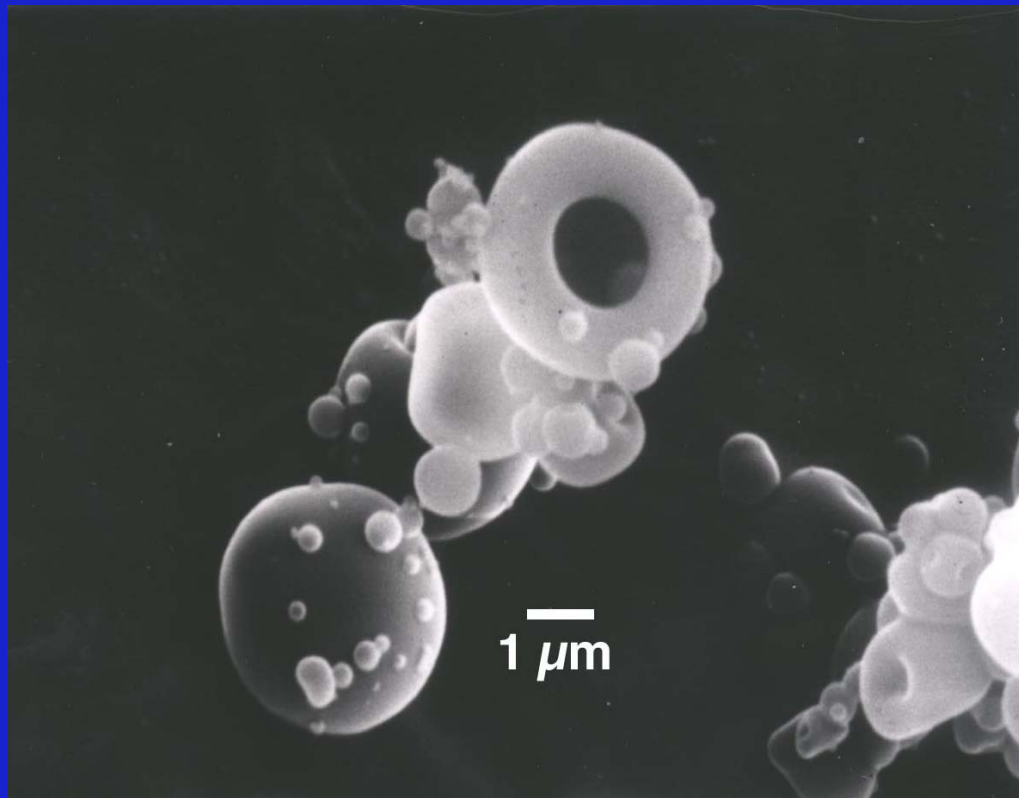
ZymoGenetics, Inc.

Eric Sievers

Human Intravenous Immune Globulin (IVIG) Particles

SEM Image

Size Distribution



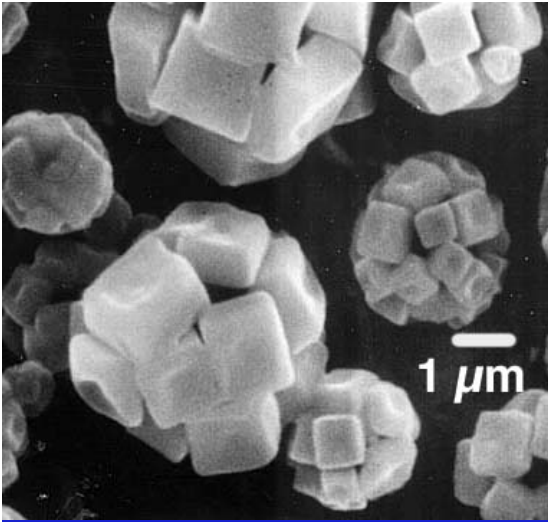
- Mean aerodynamic diameter = 1.45 μm
- 95% of particles < 2.75 μm

SOLVENTS FOR CAN-BD

- Water
- Carbon Dioxide
- Methanol
- Ethanol
- Acetone
- Ethyl acetate
- Methylene chloride (only when required)
- Mixtures of solvents

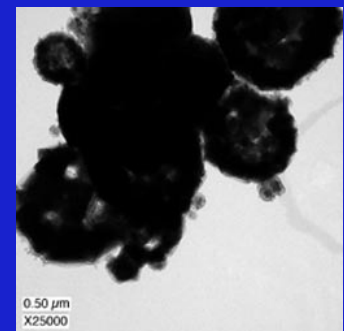
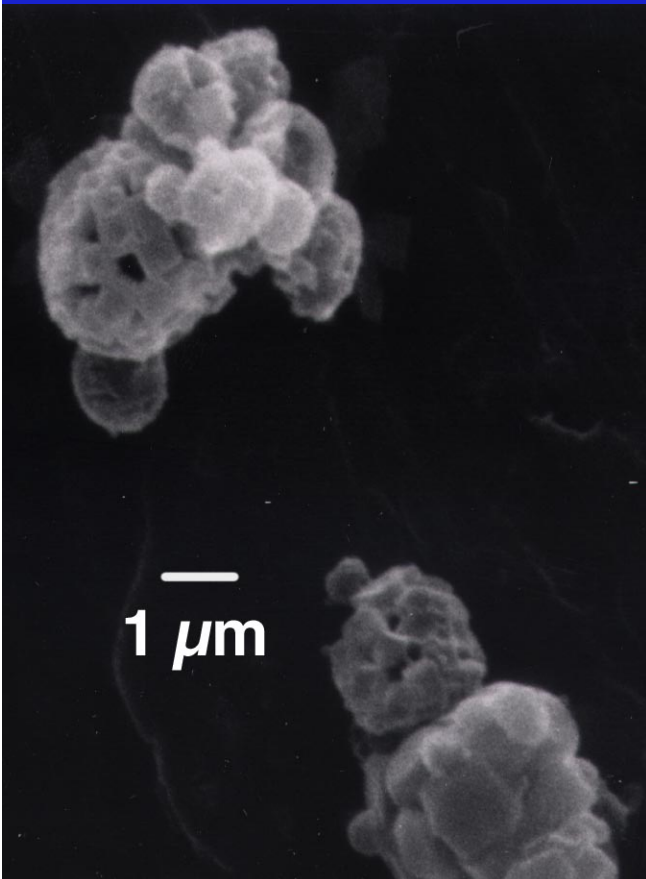
Two Fluid Particle Formation (Tee)

SEM of particles generated from a 10% aqueous solution of NaCl with CO₂



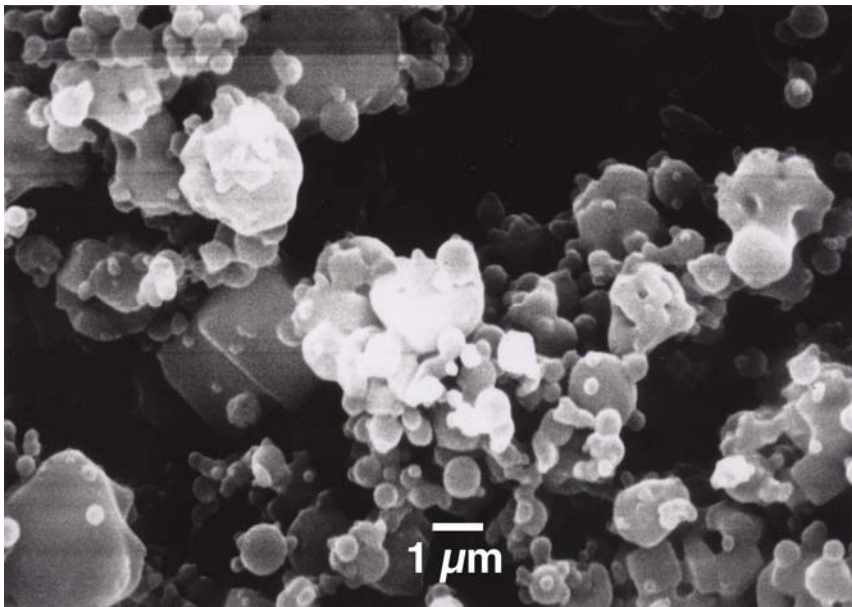
Three Fluid Particle Formation (Cross)

SEM/TEM of particles generated from a 10% aqueous solution of NaCl + 0.5% solution of PLGA in acetone with CO₂



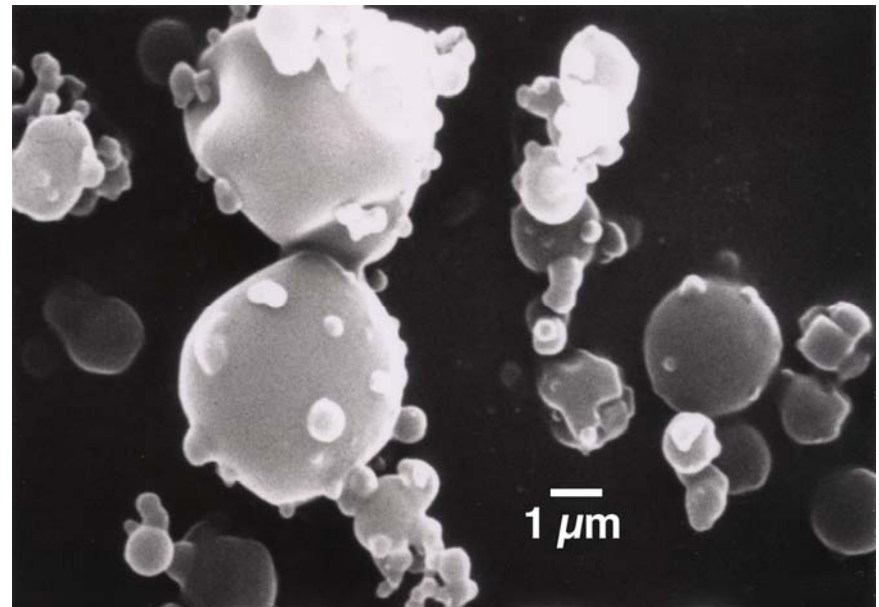
Coating of Particles by CAN-BD of Suspensions From a Tee

Light Coating
PLGA : NaCl (1:1)

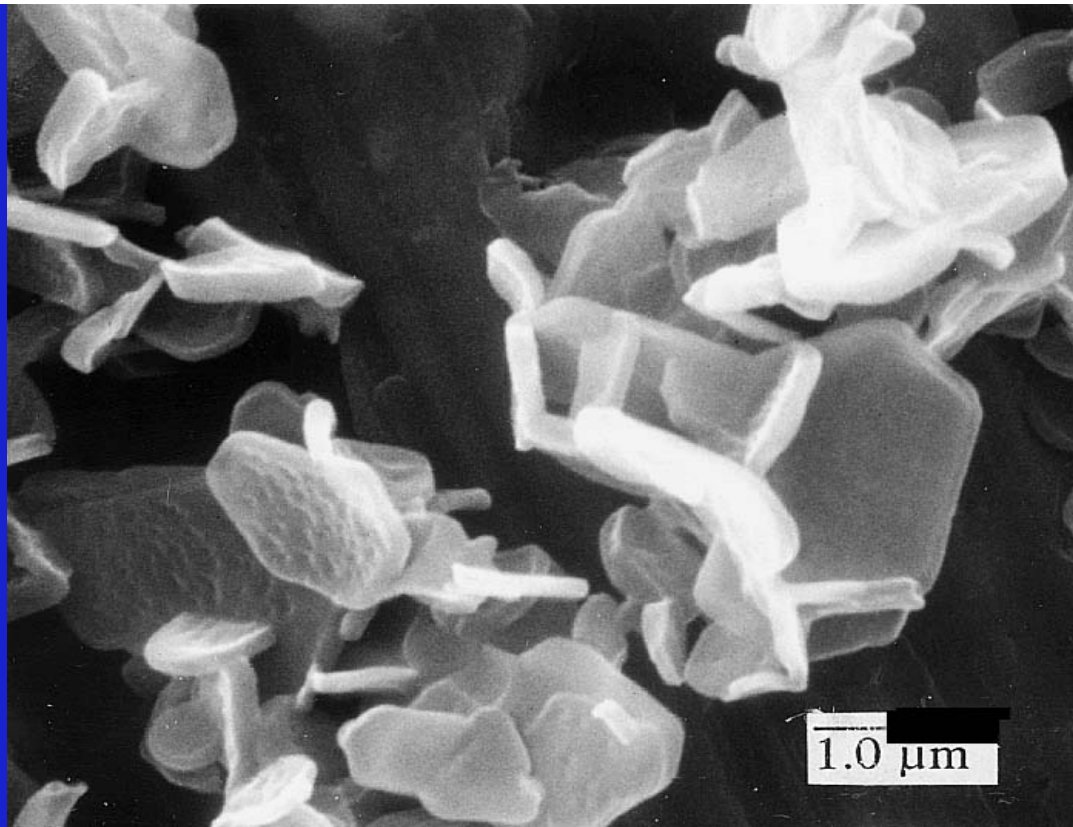


- Coated particles from a suspension containing 0.5% NaCl particles and 0.5% dissolved PLGA
- Mean aerodynamic diameter = 1.8 μm (95% 4.2 μm)

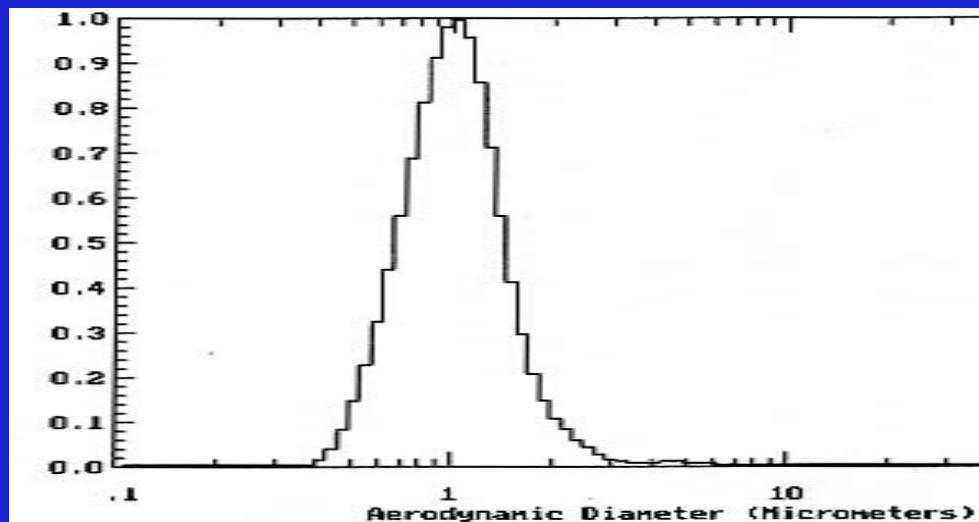
Heavy Coating
PLGA : NaCl (4:1)



- Coated particles from a suspension containing 0.5% NaCl particles and 2% dissolved PLGA
- Mean aerodynamic diameter = 2.0 μm (95% 5.2 μm)

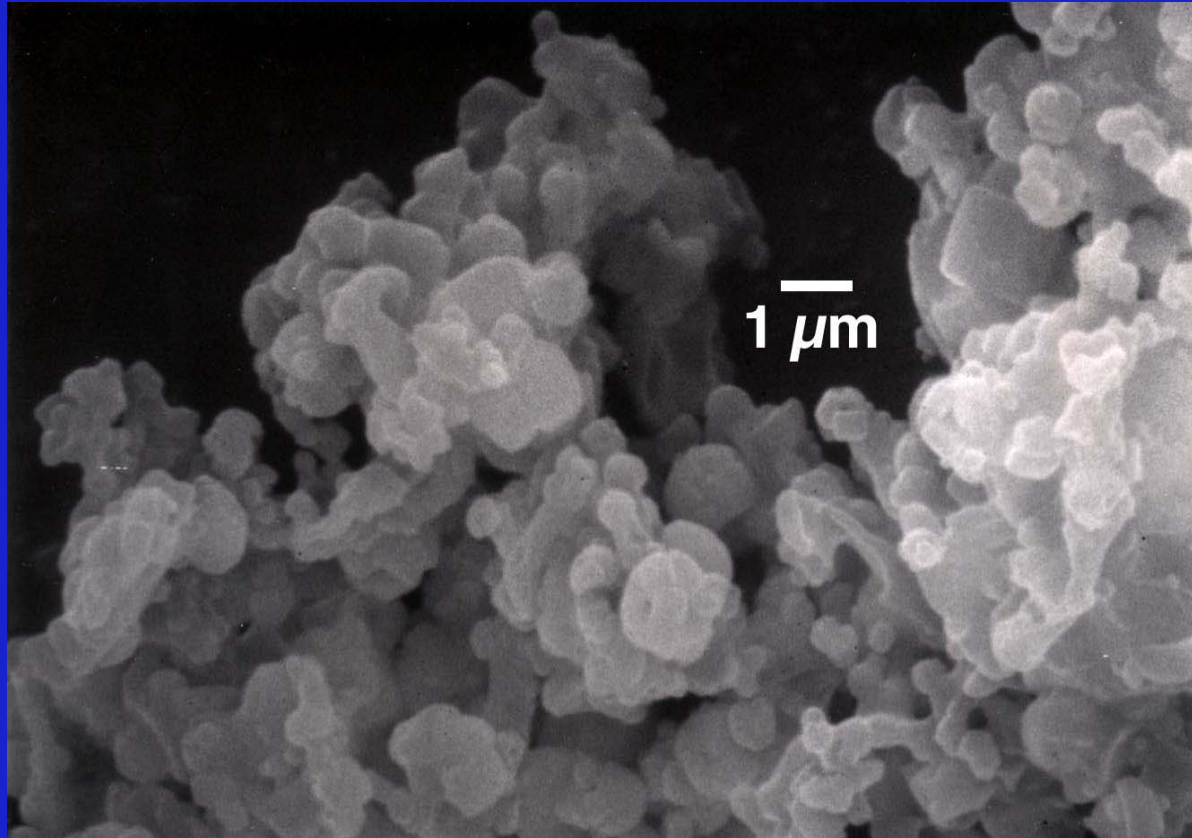


**SEM of crystalline
palmitic acid particles
generated by CAN-BD
from an ethanolic
solution containing
4% palmitic acid**



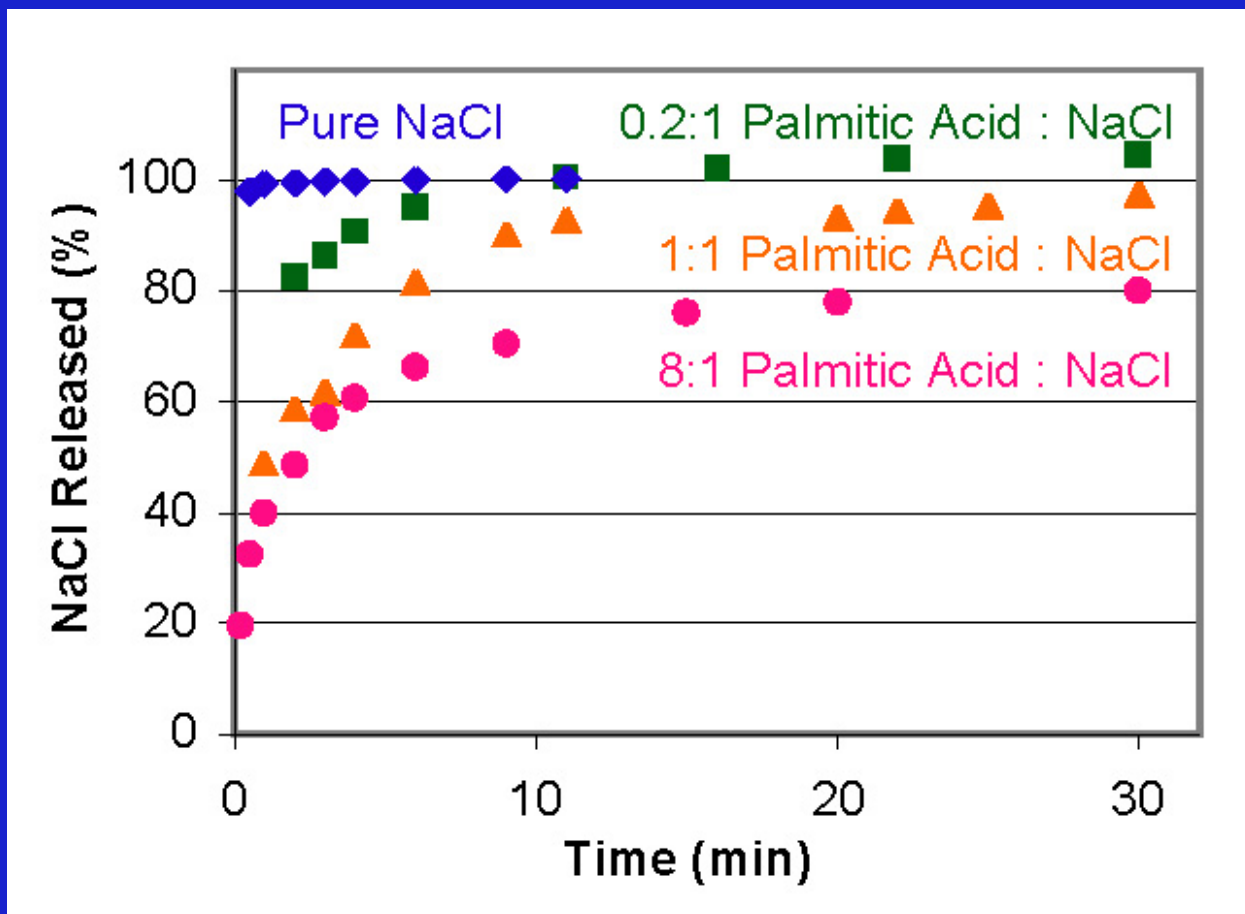
**average
aerodynamic
diameter
= 1.03 μm
99% < 3.40 μm**

Heterogeneous Particles of Palmitic Acid Mixed with NaCl

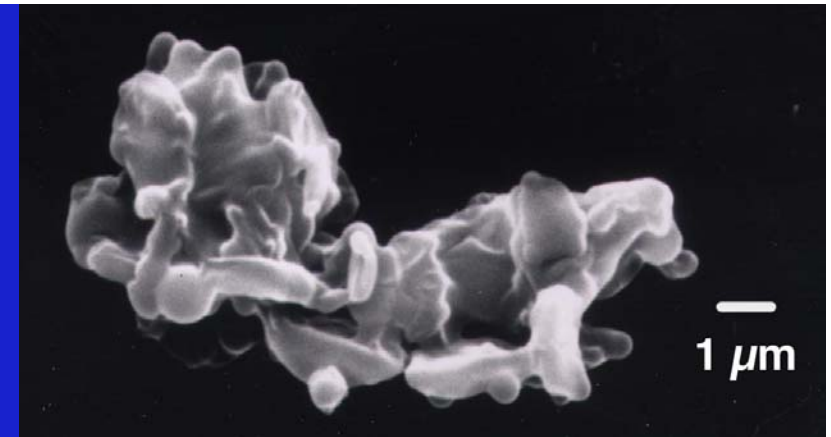
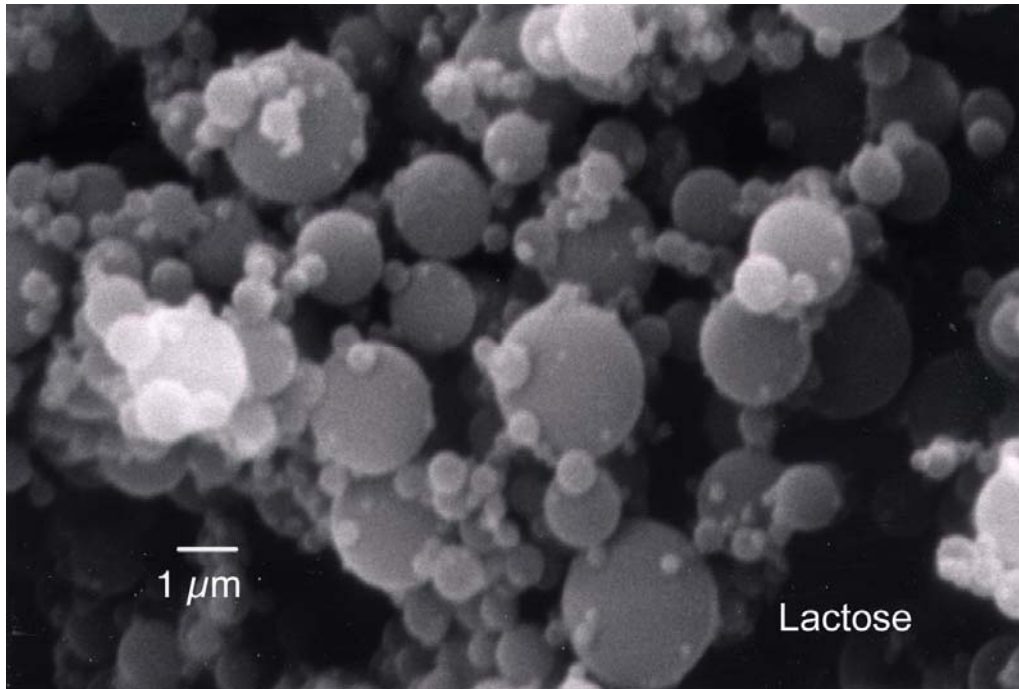


- Particles generated by CAN-BD from multiple fluids in a cross at 60 °C
 - 2% NaCl (water) and 2% Palmitic Acid (ethanol)
- 1 to 1 mass ratio of microcrystalline palmitic acid and NaCl formed
- Mean aerodynamic diameter = 1.5 μm (95% < 3.3 μm)

In Vitro Controlled Release of NaCl from Heterogeneous Particles of Palmitic Acid Mixed with NaCl



Particles generated by CAN-BD from ethanolic and aqueous solutions in a cross at 60 °C

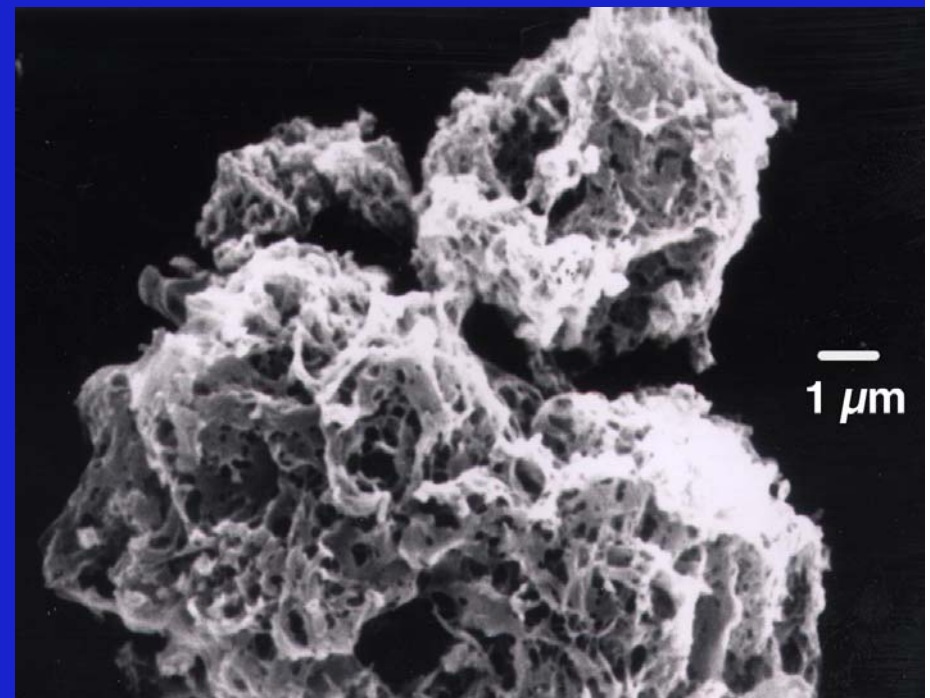


SEM of particles generated from a 2% aqueous solution of lactose + 2% solution of palmitic acid in ethanol with CO₂ in a **CROSS**

SEM of particles generated from a 10% aqueous solution of lactose with CO₂ in a **TEE**

SELECTIVE LEACHING OF HETEROGENOUS PARTICLES

Particles suspended in EtOH,
stirred for 0.5 hr, and filtered
Avg. aerodynamic diam. = 1.03 μm



REPRESENTATIVE SOLUTES AND SUSPENSIONS THAT HAVE BEEN MICRONIZED BY SUPERCRITICAL PROCESSING

Vaccines:	attenuated live measles virus vaccine, live B/Harbin influenza virus vaccine
Antibodies:	anti-CD4, IVIG, anti-human lambda light chain
Enzymes:	α_1 -antitrypsin, trypsinogen, lysozyme, lactate dehydrogenase
Antibiotics:	ciprofloxacin, moxifloxacin, rifampicin, isoniazid, tobramycin sulfate, amoxycillin, doxycycline
Water-soluble drugs:	albuterol sulfate, cromolyn sodium
Alcohol-soluble drugs:	naproxen, budesonide, betamethsone, amphotericin B, cyclosporin, DPPC
Sugars:	lactose, sucrose, trehalose, mannitol
Polymers:	PLA, PLGA, PEG
Amino acids:	methionine, arginine

Conclusions

- Multiple constituents can be incorporated in three fluids in a low volume cross to make heterogenous and homogenous nanoparticles and microparticles.
- Pharmaceuticals, proteins, antibodies, and vaccines can be stabilized, dried, and micronized by a newly patented process, CAN-BD.
- A SF nebulization, desolvation and coating method (CAN-BD) has been developed that can manufacture dry powders and coatings of many materials.
- Coating and drying requires only seconds.
- Water and many organic solvents yield nanoparticles and microparticles with unique compositions and properties.
- Fluid ratios, pressures, and solute concentrations determine particle size.
- For more data & applications, visit www.AKTIV-DRY.com

