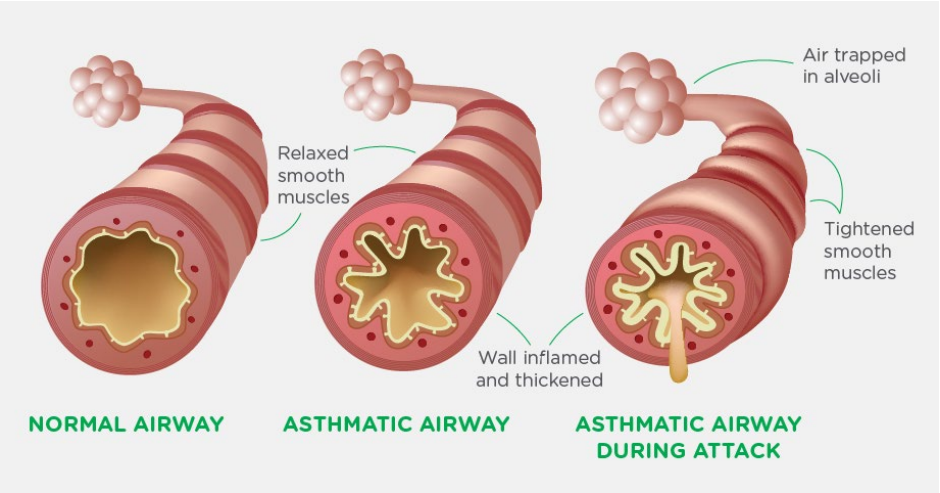
UAB SCHOOL OF ENGINEERING

The University of Alabama at Birmingham



Materials for Aerosol Treatment of Disease: New Microscopy for Bioequivalence and Improving Therapeutic Index

Mark Banaszak Holl

Department of Mechanical and Materials – School of Engineering
Division of Pulmonology, Allergy, and Critical Care Medicine – Heersink School of Medicine

January 24, 2023

Engineering for the Human Process - Aerosols for Drug Delivery

Banaszak Holl Lab

UAB Monash University

Mark Banaszak Holl | Jing Zhang | Jinhee Kim | Jared Smith | Sheikh Tanzina Haque | Blessy Joseph

University of Sydney

Hak-Kim Chan | Dipesh Khanal | Wei-Ren Ke

$$$ ARC DP 210102526
Understanding bacteriophage deactivation and stabilisation in formulations

$$$ US FDA Contract 75F40123C00001
Identification of Drug Distribution in Aerosols A Nanospectroscopy and NanoThermal Analysis

$$$ ARC LP160101498
Novel nano-medicine technology using nanocrystals in liposomes

Engineering for the Human Process - Aerosols for Drug Delivery

Mark Banaszak Holl

Jing Zhang

Jinhee Kim

Jared Smith

Hak-Kim Chan

Dipesh Khanal

Wei-Ren Ke

Nanoscale morphological, chemical, and thermal characterization of pharmaceutical powder aerosols. J. Zhang, D. Khanal, H. K. Chan, M. M. Banaszak Holl, submitted.

Applications of AFM-IR for drug delivery vector characterization: infrared, thermal, and mechanical characterization at the nanoscale. J. Zhang, D. Khanal, M. M. Banaszak Holl. *Advanced Drug Delivery Reviews* **2023**, *192*, 114646.

Optical photothermal infrared spectroscopy for nanochemical analysis of pharmaceutical dry powder aerosols. D. Khanal, J. Kim, J. Zhang, W.-R. Ke, M. M. Banaszak Holl, H. K. Chan. *International Journal of Pharmaceutics* **2023**, *632*, 122563

Bulk to Nanometer-Scale Infrared Spectroscopy of Pharmaceutical Dry Powder Aerosols. D. Khanal, J. Zhang, W.-R. Ke, M. M. Banaszak Holl, H.-K. Chan. *Analytical Chemistry* **2020**, *92*, 8328-8322.

Asthma

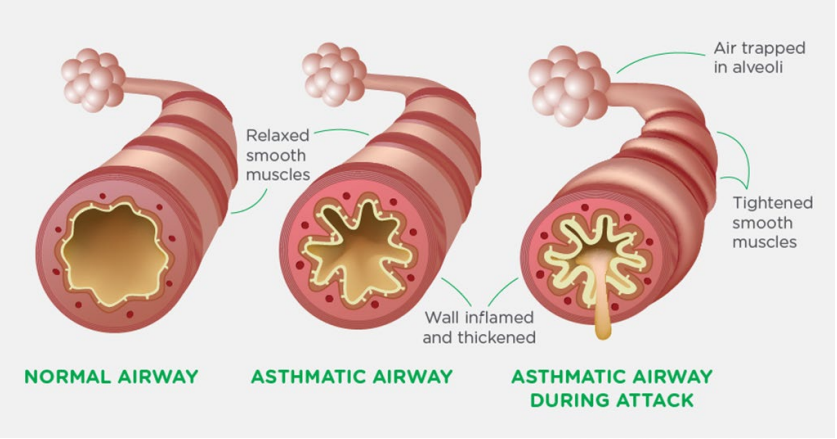
Chronic, inflammatory disease of conducting airways affecting 300 million people worldwide. Affluent societies have occurrence in 1 in 10 children and 1 in 12 adults. Estimated economic impact is estimated to exceed $18B. A mixture of genetic susceptibility and environmental risk factors lead to a variety of endotypes and consideration of asthma as a syndrome.

Both innate and adaptive immune systems act with epithelial cells to cause:

- Bronchial hyper-reactivity (non-specific response to exercise and cold air)
- Mucus overproduction
- Airway wall remodeling
- Airway narrowing

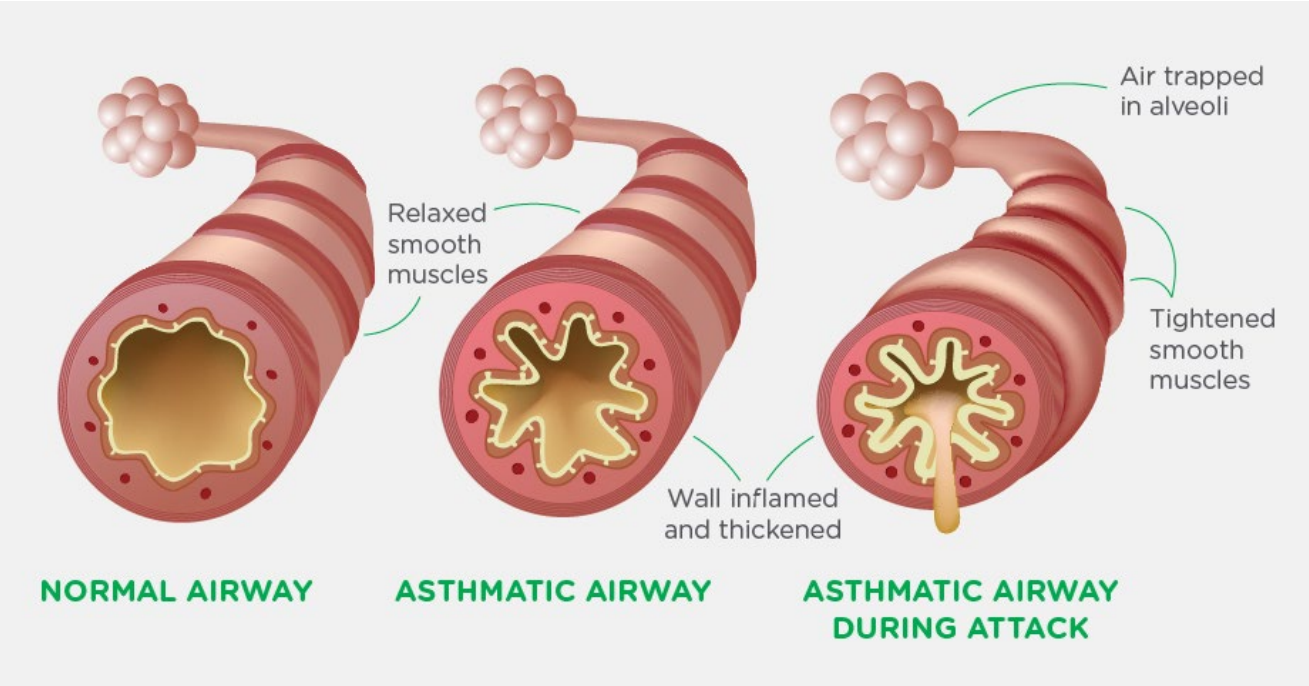
Leading to:

- Shortness of breath
- Wheezing
- Chest tightness



Lambrecht, Hammad *Nature* **2015**, *16*, 45-56

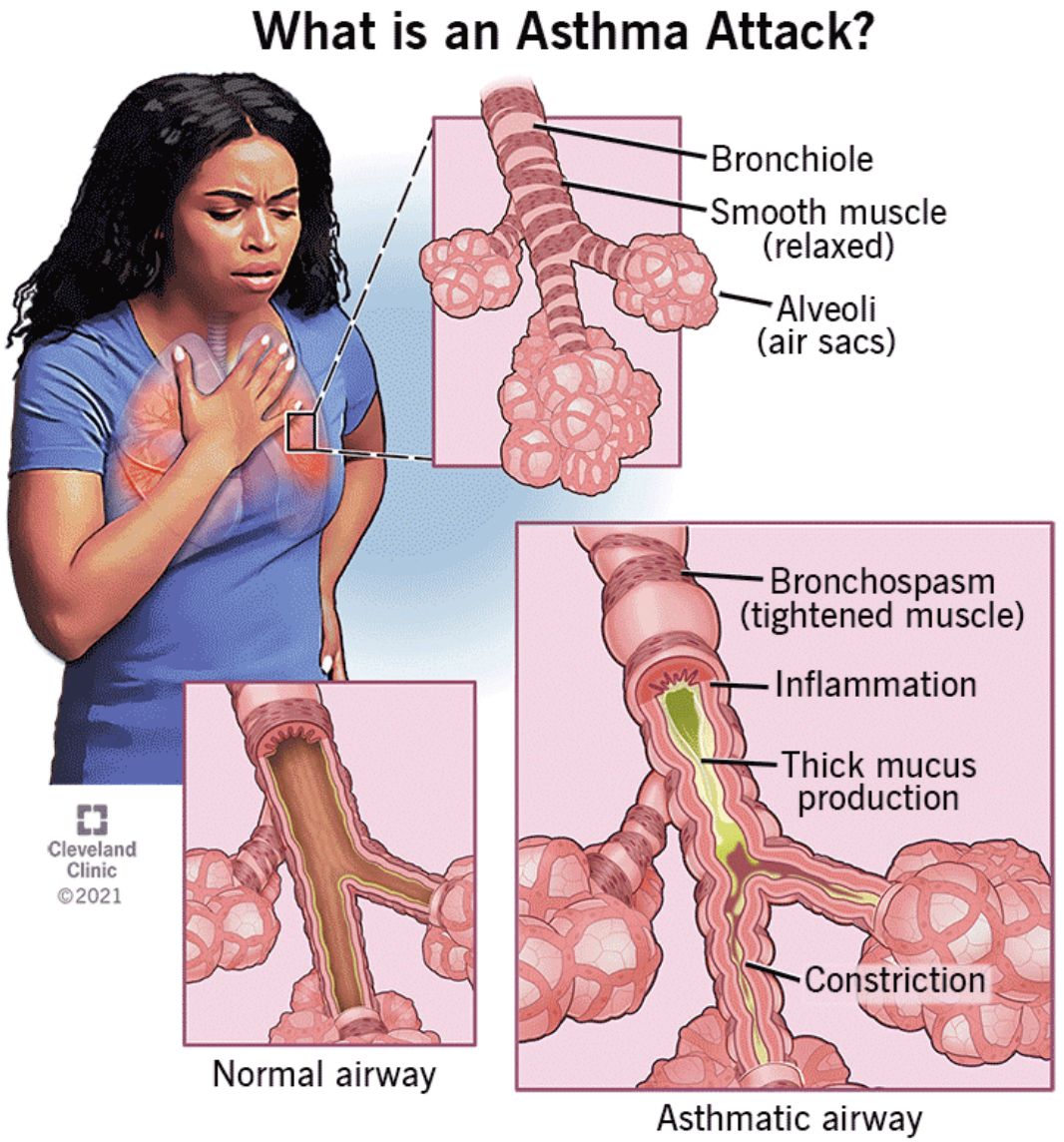
Asthma flare-ups are caused by triggers, which are irritants like high smog levels, colds and flu, cigarette smoke, scented cleaning products or even seasonal changes in the weather.



What happens during an asthma attack?

The airways get swollen and inflamed.
Thick, sticky mucus fills up the airways.
The muscles that wrap around the airways squeeze tight.
Air becomes trapped in alveoli

https://www.choa.org/parent-resources/asthma/asthma-attacks



https://my.clevelandclinic.org/health/diseases/6424-asthma

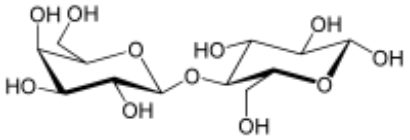
Inhaled Corticosteroid and β2-agonist for Asthma Treatment

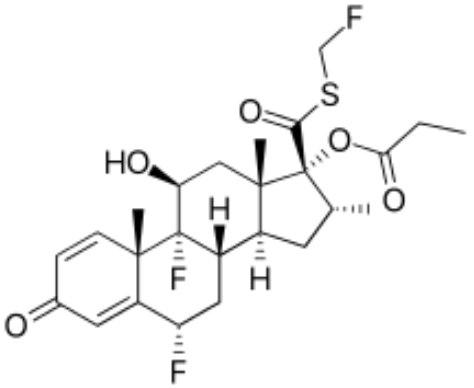
Corticosteroids (i.e. steroids): anti-inflammatory medicine. Synthetic version of hormones normally produced by the adrenal glands (two small glands that sit on top of the kidneys).

Effective for ~90% of Asthma cases

Fluticasone Propionate: Aka Flovent®, Flonase®, and Flixotide®

Mixed with lactose excipient

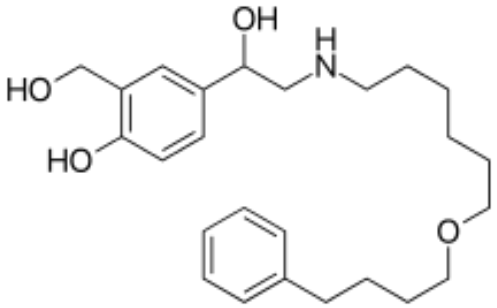




agonist at the glucocorticoid receptor producing anti-inflammatory and vasoconstriction effects.

β2-agonist:

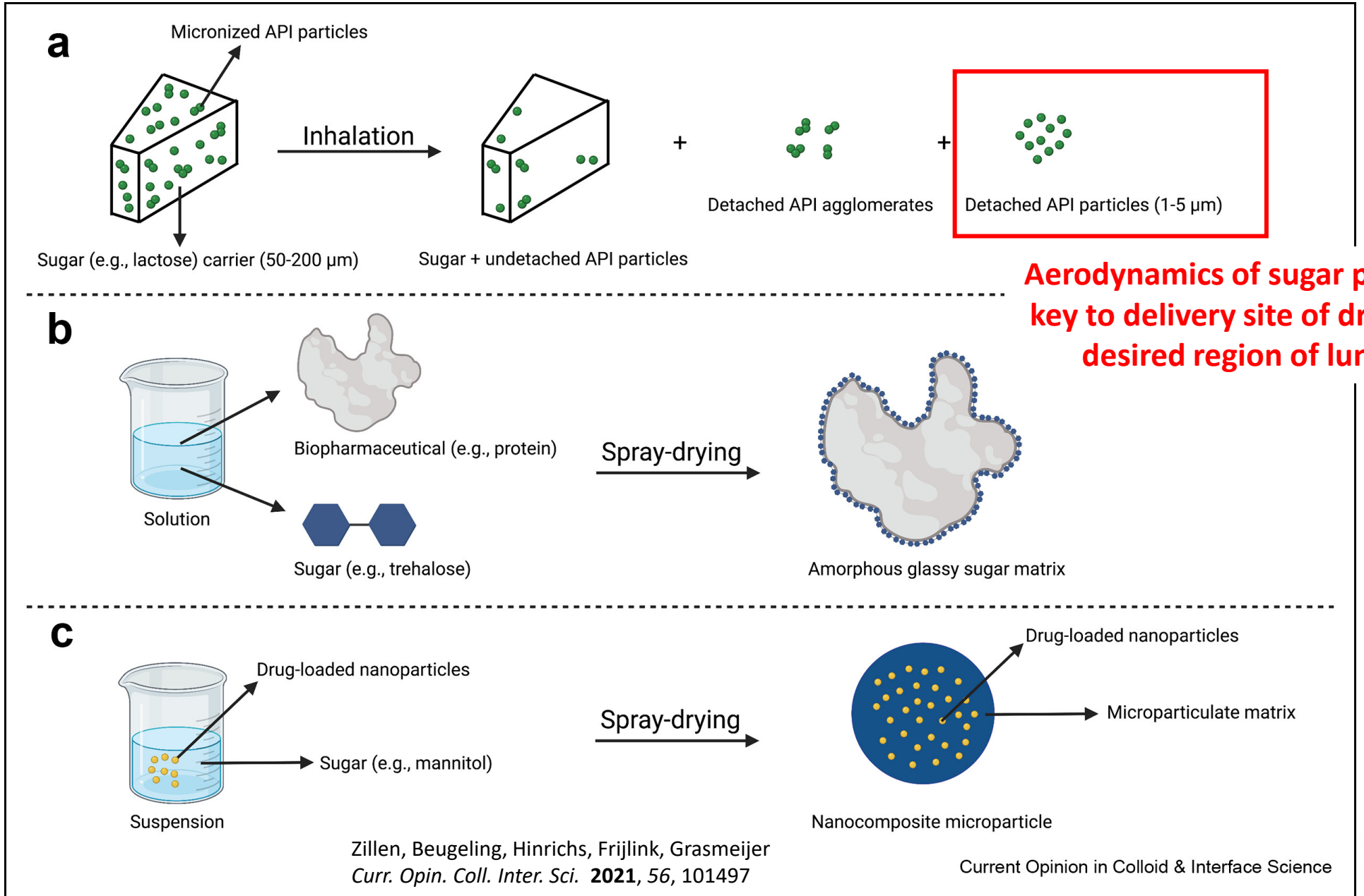
Salmeterol Xinofate: Aka Serevent® and Aeromax®



β2-agonist that relaxes bronchial muscles in the lungs – 12 hour action

Jetzer, M. W.; Morrical, B. D.; Fergenson, D. P.; Imanidis, G. *Int. J. Pharm.* **2017**, *532*, 218−228.

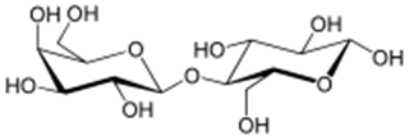
Formulation of Drugs with Sugar Excipients



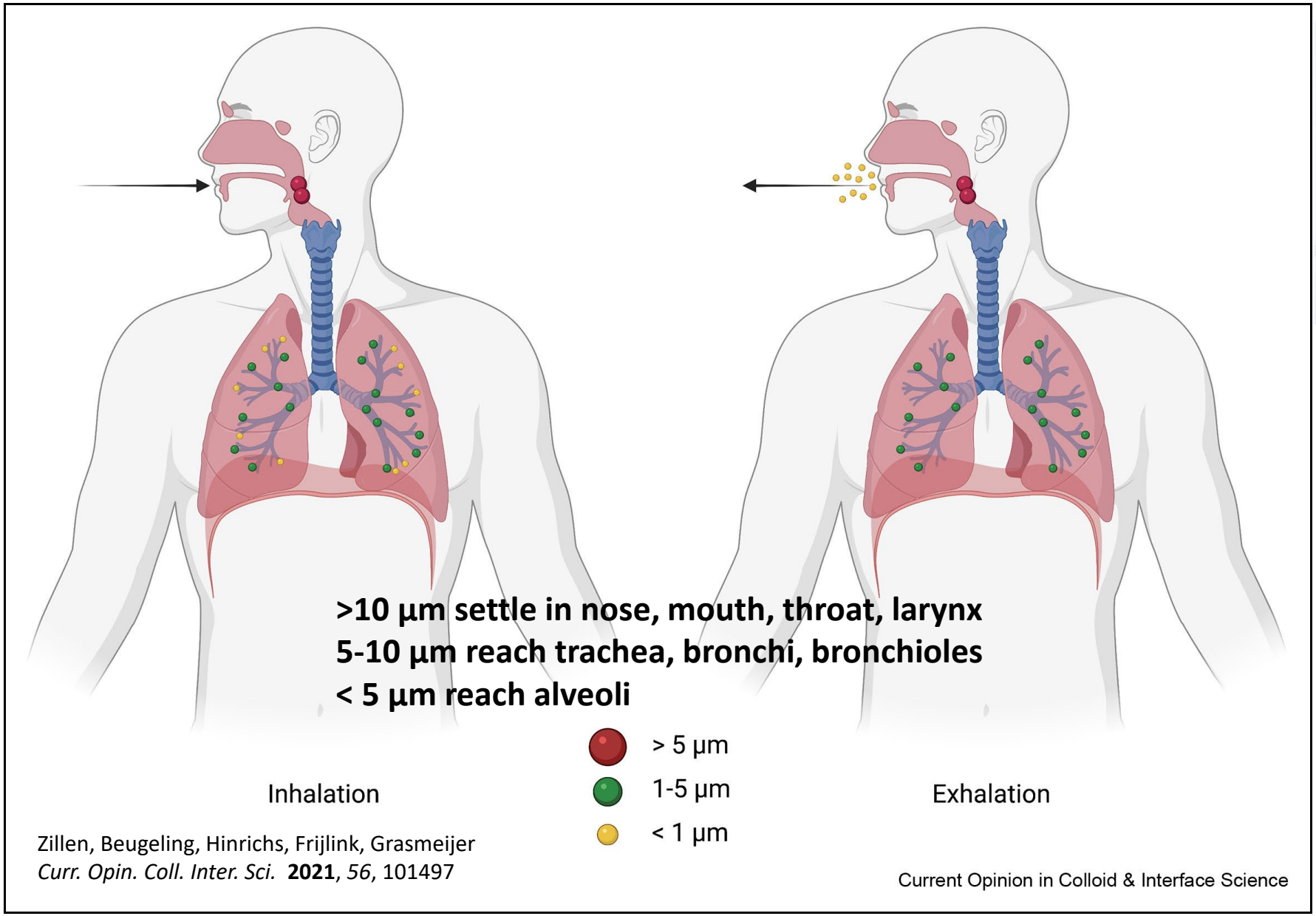
Aerodynamics of sugar particle key to delivery site of drugs to desired region of lungs

Zillen, Beugeling, Hinrichs, Frijlink, Grasmeijer
Curr. Opin. Coll. Inter. Sci. **2021**, *56*, 101497

Current Opinion in Colloid & Interface Science



Dispersion into lungs with inhaler achieved by absorbing drugs on micron-size lactose particles.



Zillen, Beugeling, Hinrichs, Frijlink, Grasmeijer
Curr. Opin. Coll. Inter. Sci. **2021**, *56*, 101497

Current Opinion in Colloid & Interface Science

Seretide® Combination Inhaler

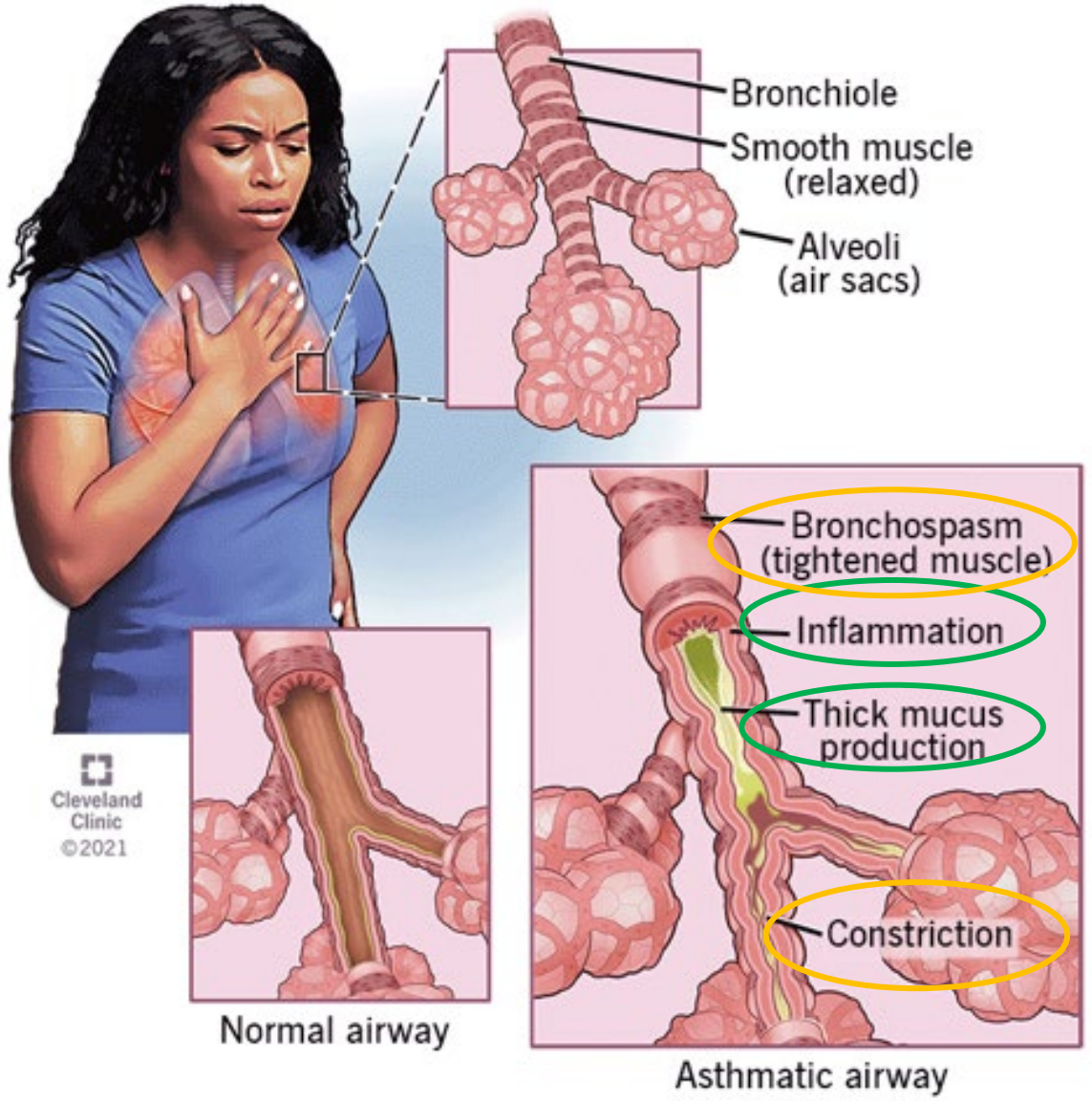
Deliver directly to lung cells to obtain therapeutic dose

while

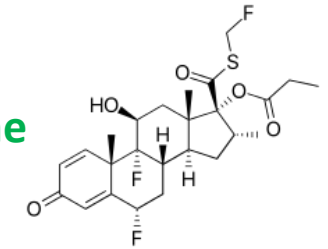
Reducing systemic delivery and associated side effects

Common: Headaches, sore throat, cramps, increased heart rate

Rare: allergic reaction, bone density loss, glaucoma, slowed growth (children)



Action of corticosterioid fluticasone

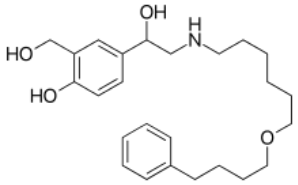


"significant clinical and in-vitro evidence to suggest that the co-association of **steroid** AND **β2-agonist** at the level of the single administered aerosol particle leads to a synergetic effect with improved clinical outcomes"

Synergistic effects operating at both the molecular receptor and cellular levels

Important to deliver **BOTH** drugs to the **SAME** cell in the lung tissue

Action of β-2 agonist salmeterol

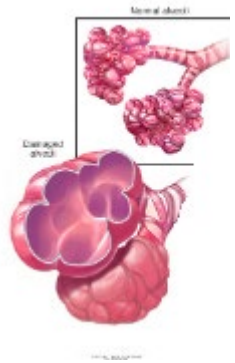


Jetzer, M. W.; Morrical, B. D.; Fergenson, D. P.; Imanidis, G. *Int. J. Pharm.* **2017**, *532*, 218–228.

Treatments also relevant to

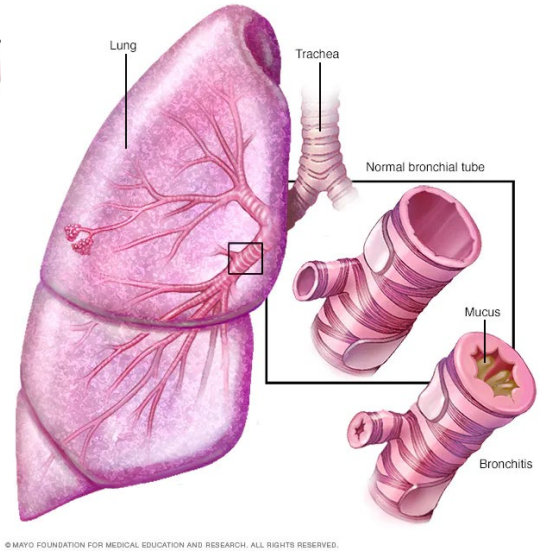
Chronic Obstructive Pulmonary Disease (COPD)

Affects about 10% of people over 45 years of age with a similar prevalence in males and females in developed counties reflecting smoking pattern



Emphysema

destruction of the fragile walls and elastic fibers of the alveoli. Small airways collapse when you exhale



Bronchitis

bronchial tubes become inflamed and narrowed and your lungs produce more mucus

In developing countries over half of the COPD patients are non-smokers, particularly women exposed to biomass smoke in poorly ventilated homes

COPD is now ranked as the 4th commonest cause of death in the world although in developed countries it has risen to 3rd and is ranked as the 5th commonest cause of morbidity and a leading cause for emergency hospital admission with acute exacerbations

Substantial co-morbidity in Adults with Asthma

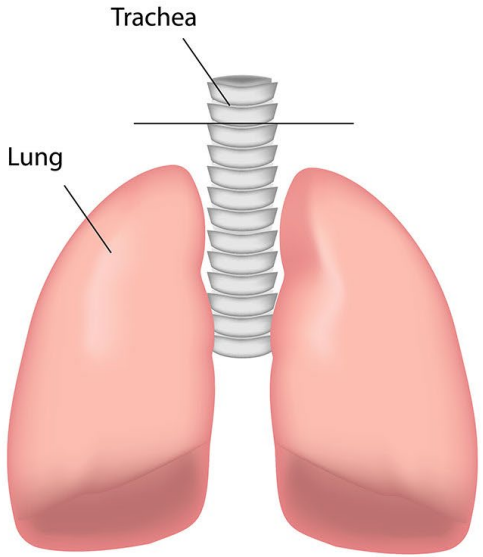
- Respiratory infection
- Heart Problems
- Lung Cancer
  - Depression

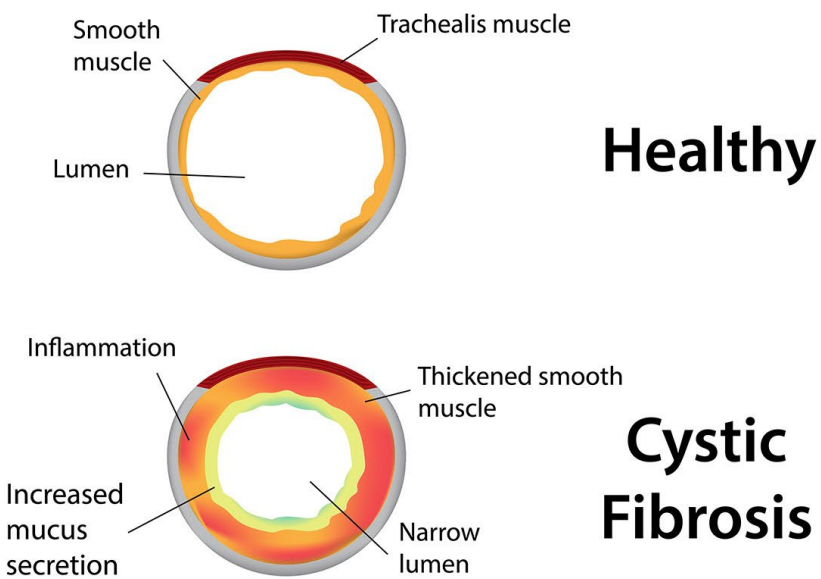
https://www.mayoclinic.org/diseases-conditions/copd/symptoms-causes/syc-20353679

Barnes *Allergy* **2019**, *74*, 1249-1256

Treatments also relevant to

Cystic Fibrosis





Inflammation and tissue changes reminiscent of Asthma and COPD

- Inhaled corticosteroids (fluticasone) used
  - to reduce endobronchial inflammation
  - Minimize systemic adverse effects of oral prednisolone

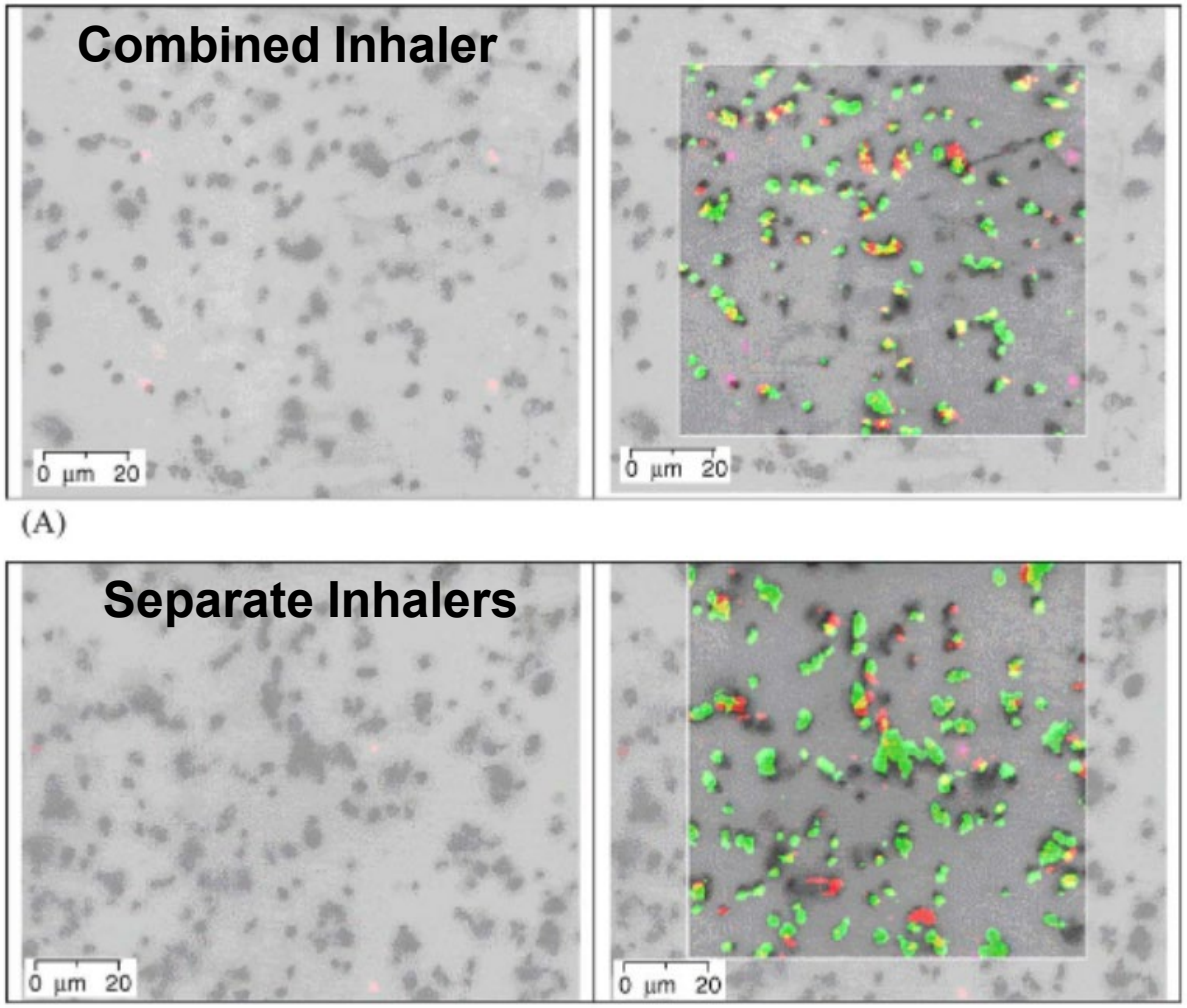
- Inhaled long-acting β2-agonist (salmeterol) used
  - to improve bronchial obstruction and hyperresponsiveness

No long-term clinical studies proving efficacy

J. Cyst. Fibrosis **2009**, *8*, 295-315

Antibiotics **2021**, *10*, 486

What is known about Aerosol Particle Content and Structure ?



Fluticasone and Salmeterol Particle Co-Association by Raman Microscopy

Fluticasone Propionate
Salmeterol Xinofate

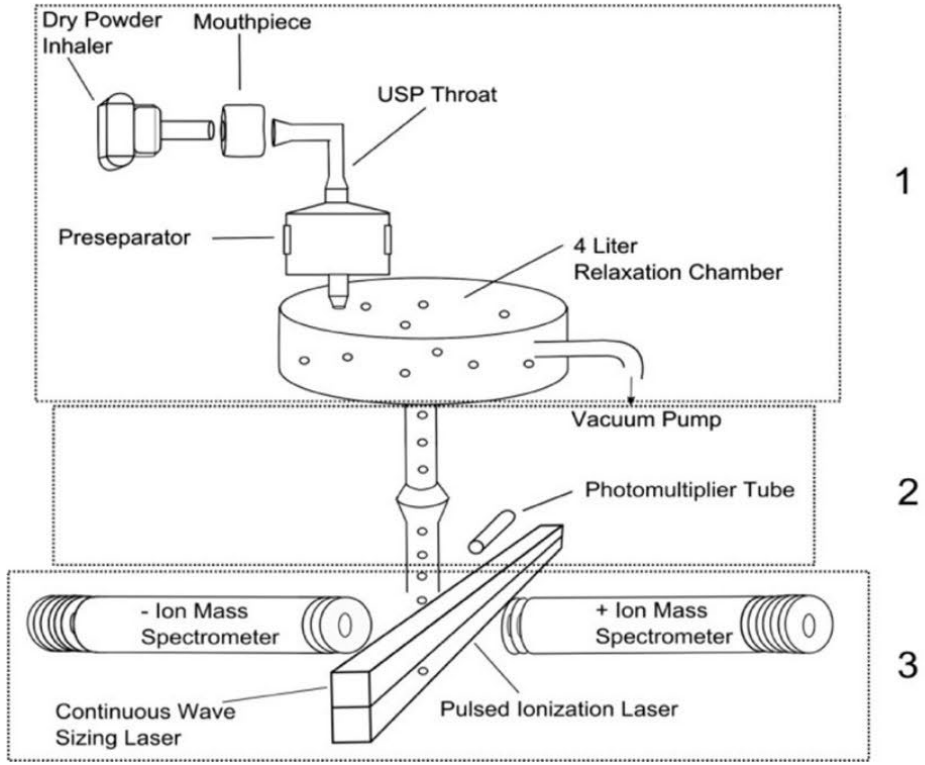
Drugs combined in particles Yellow

Spatial resolution ~ 3 µm or about size of many single particles

Theophilus, A.; Moore, A.; Prime, D.; Rossomanno, S.; Whitcher, B.; Chrystyn, H. *Int. J. Pharm.* **2006**, *313*, 14−22.

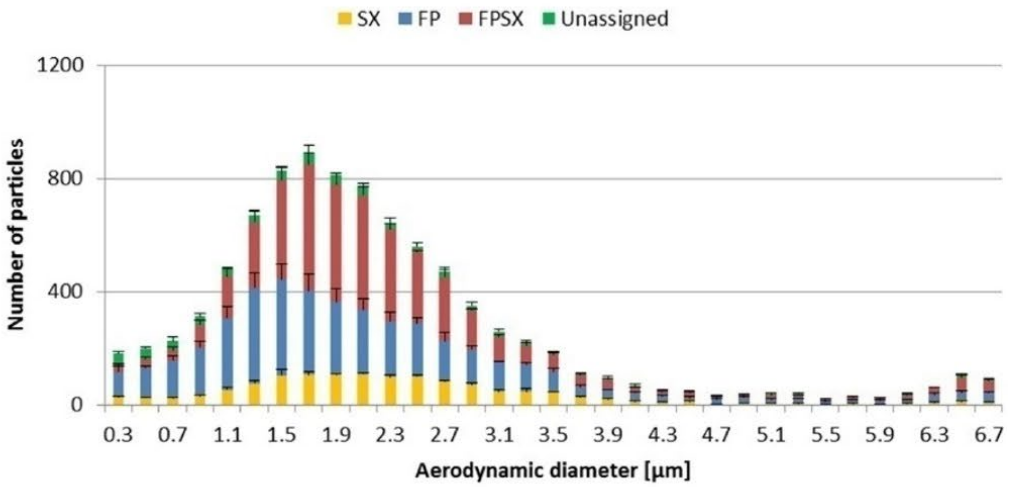
Fluticasone and Salmeterol Particle Co-Association by Mass Spectrometry

Novartis/University of Basel



Fluticasone Propionate : Salmeterol Xinofate

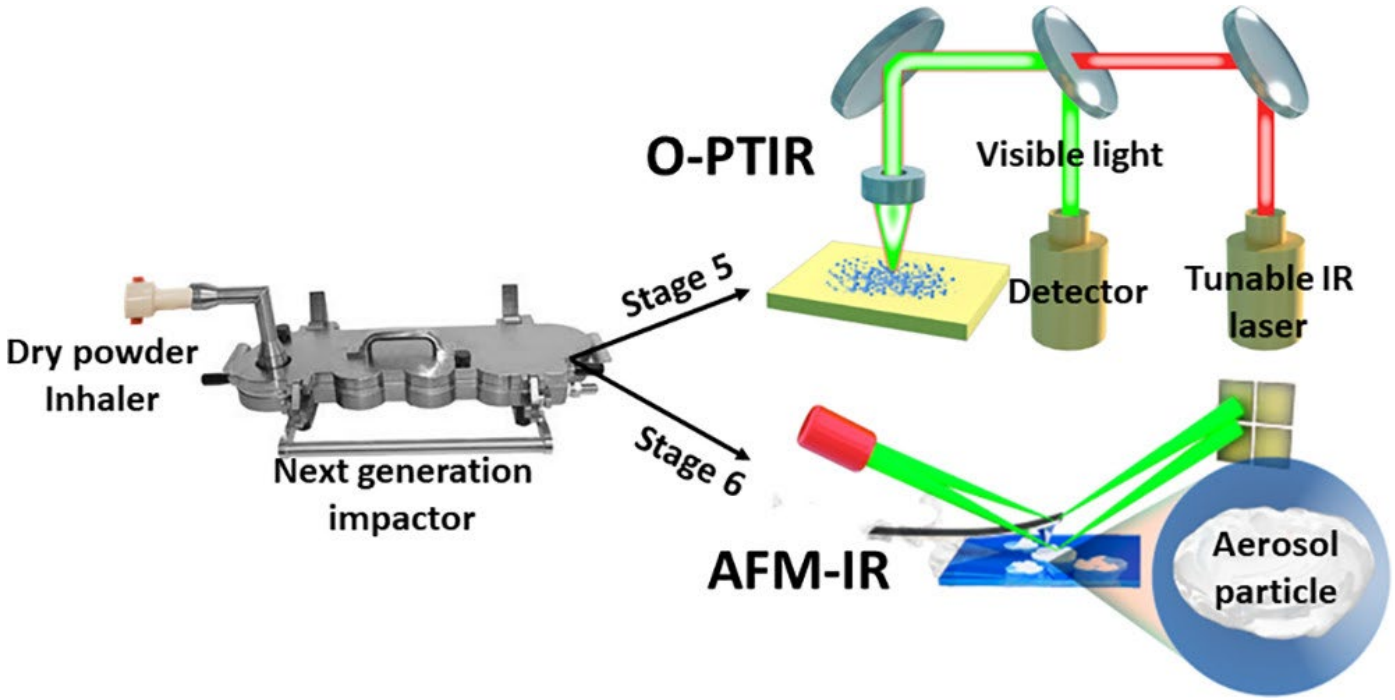
10-Fluticasone/ 1-Salmeterol co-associated particles



- **45% of particles contain fluticasone and salmeterol**
- **70% of particles containing salmeterol also contain fluticasone**

Jetzer, M. W.; Morrical, B. D.; Fergenson, D. P.; Imanidis, G. *Int. J. Pharm.* **2017**, *532*, 218−228.

Analytical Challenge: Solid-State Characterization of Pharmaceutical Inhalation Aerosols



- Nanometer to Micrometer Particles
- Complex Drug/Excipient Mixtures
- Assess variation at particle level?
- Use O-PTIR and AFM-IR

Optical Photothermal Infrared Microscopy - 0.3 µm (300 nm) spatial resolution on individual particles

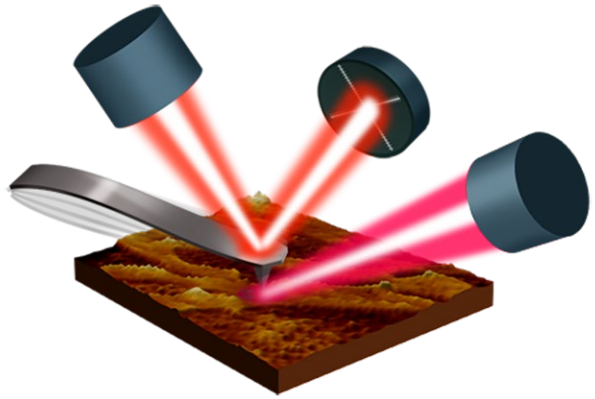
Atomic Force Microscopy – Infrared Spectroscopy - 0.01 µm (10 nm) resolution on individual particles

Rapidly assess drug/drug/excipient ratios at an interparticle and intraparticle level to 100s to 1000s of particles

D. Khanal, J. Zhang, M. M. Banaszak Holl, H.K. Kim Analytical Chemistry **2020**, *92*, 12, 8323-8332

Photothermal Infrared Spectroscopy

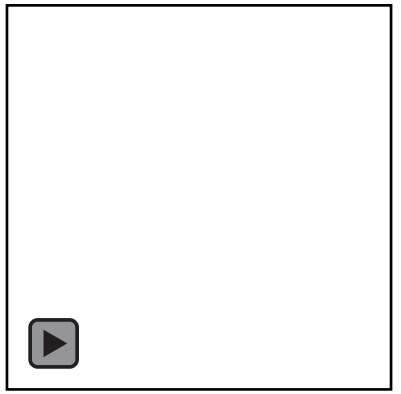
AFM-IR: Bruker IconIR



- **Nano-scale** (10 nm) thermal expansion detected via AFM tip for **IR spectrum**
- Simultaneous acquisition of **mechanical data** including modulus, adhesion, dissipation, and deformation)
- **nanoThermal** analysis (T_g, T_m)

OPTIR: Photothermal mIRage-LS

Coming to UAB in 2023 !



- **Micro-scale** (0.5 μm) thermal expansion detected via green laser for **IR spectrum**
- Simultaneous **Raman spectrum**
- Rapid all-optical system
- Co-located **fluorescence microscopy**

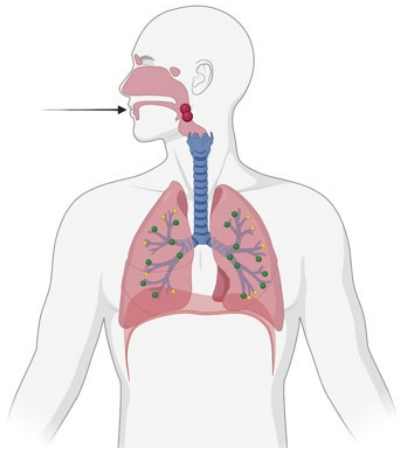
Conventional Raman and FTIR microscopy probe chemical information from the aggregates of aerosol particles in the size range greater than a few micrometers.

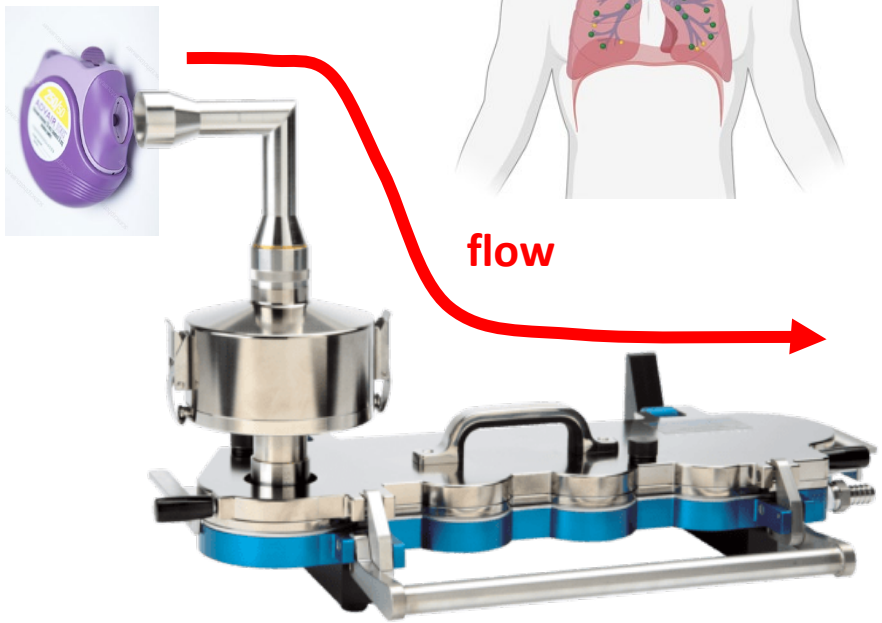
https://www.bruker.com/en/products-and-solutions/infrared-and-raman/nanoscale-infrared-spectrometers/dimension-iconir.html

https://www.photothermal.com/home/co-located-fluorescence-optir/

Evaluating Aerodynamics - Next Generation Impactor

Particles In Here





Particles collected on Si wafers or CaF_2 cover slips at different stages as a function of size

Analysis

OPTIR: mIRage-LS

AFM-IR: IconIR

Stage	1	2	3	4	5	6	7	8
Cutoff Diameter in microns	6.1	3.4	2.2	1.3	0.72	0.4	0.24	< 0.24

What do we hope to learn about drug delivery formulations?

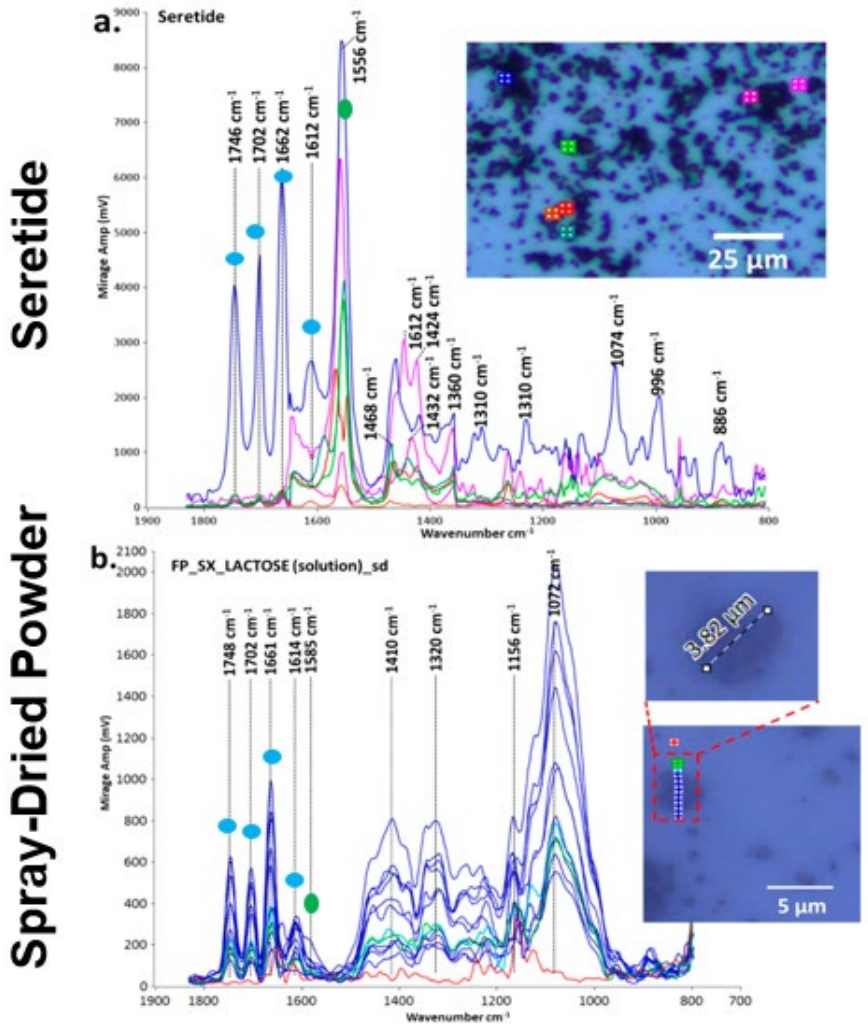
- Distribution of drugs on particles as a function of particle aerodynamic properties
  - Co-localization of drugs on within particles
  - Habit of drug crystals
- Distribution of particle melting points
  - As a function of drug distribution/co-localization/crystal habit

Optimize efficacy of each particle, particularly dual drug synergy

Melting points related to dissolution rate of drug crystals

- Develop as a Method for Quantitative comparison of “Name Brand” to “Generic” formulations in support of Bioequivalence Assessment
- Develop as Method for varying formulation to optimize therapeutic index

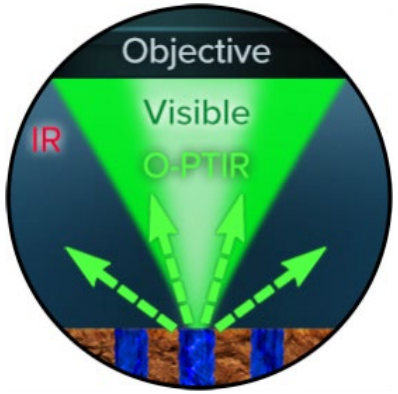
O-PTIR spectra of sample collected from stage 5 of NGI (a) Seretide; (b) spray-dried powder from solution.



● Fluticasone Propionate ● Salmeterol Xinofate

Analytical Chemistry **2020**, *92*, 12, 8323-8332

OPTIR



Both Drugs and Lactose Identified In IR spectra

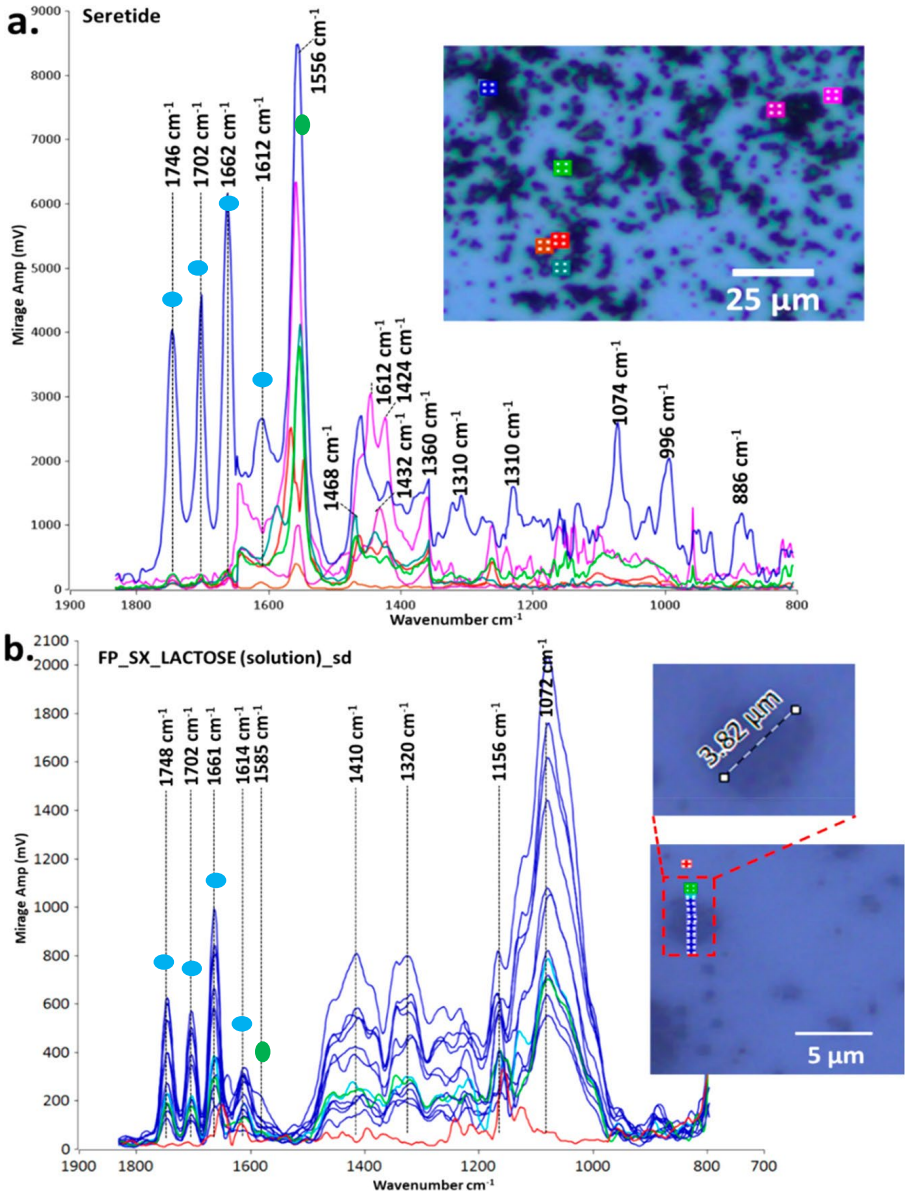
Rapid drug specific imaging of ~100,000 µm^3 ROIs and thousand of particles at ~ 0.3 µm resolution

Comparison of micronized aerosol powder inhaler (API) particles to spray-dried particles.

OPTIR spectra of sample collected from stage 5 of a NGI (a) Seretide; (b) spray-dried powder from solution.

Seretide

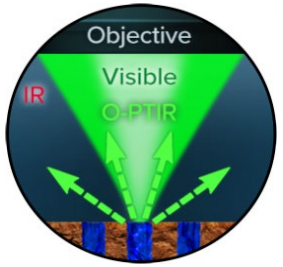
Spray-Dried Powder



● Fluticasone Propionate

● Salmeterol Xinofate

OPTIR



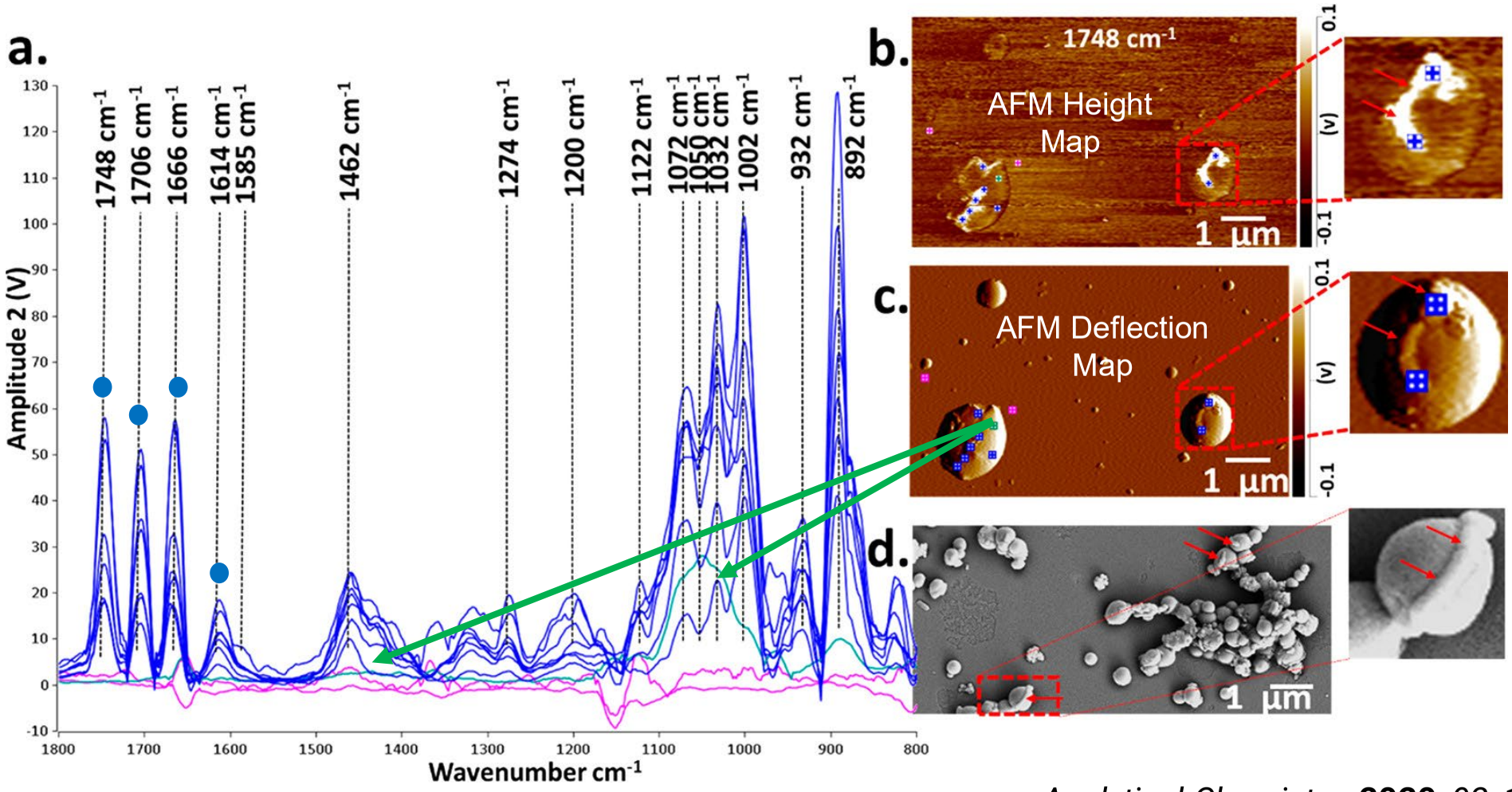
Both Drugs and Lactose Identified In IR spectra

Analytical Chemistry **2020**, *92*, 12, 8323-8332

Localization of Fluticasone Propionate in 1 µm Lactose Particle

● Fluticasone Propionate
Blue squares indicate location of spectra

aerosol particles of spray-dried powder



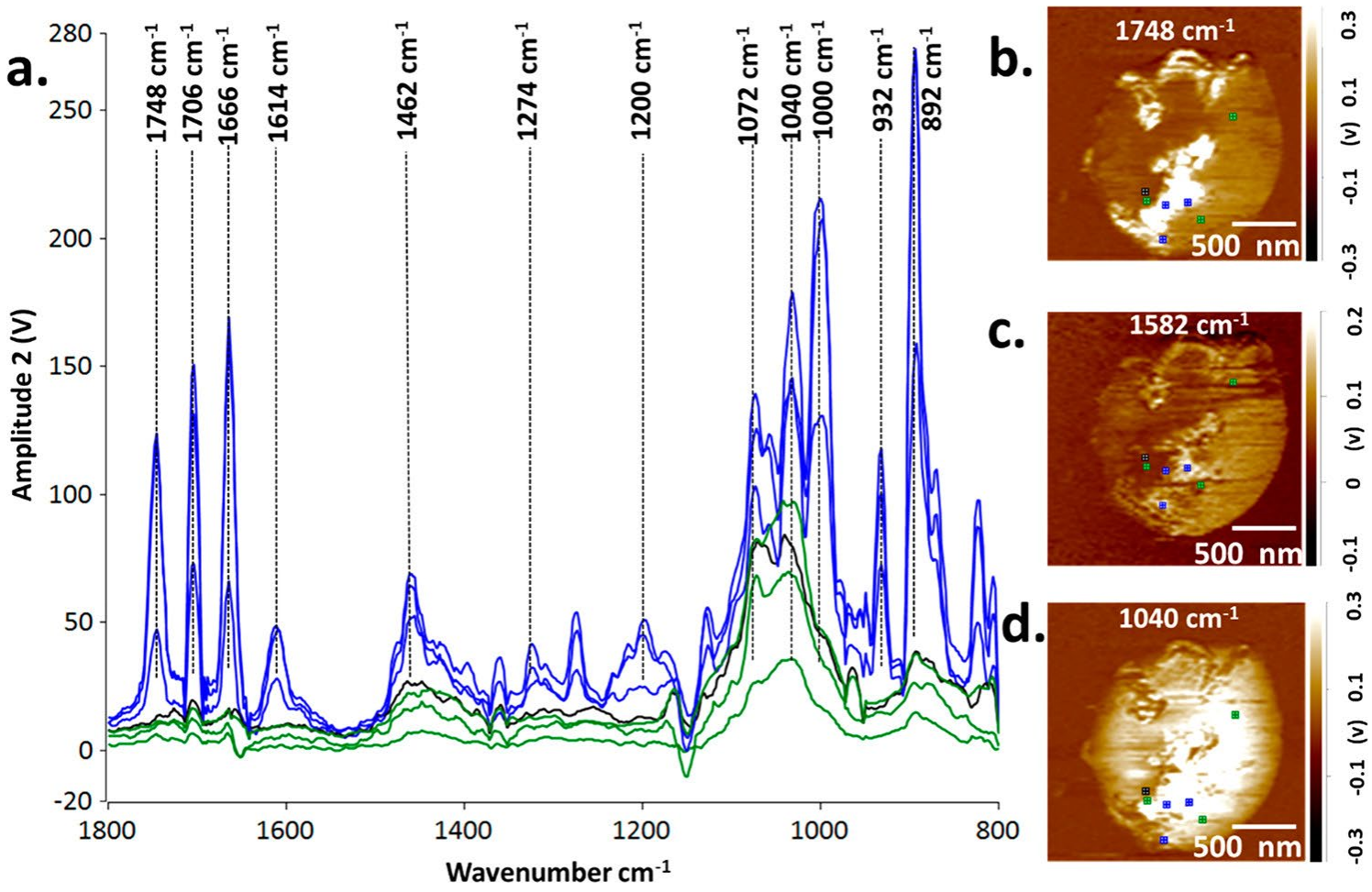
Fluticasone Propionate
IR Map (1748 cm^{-1})

IR of Lactose Green Square

SEM image
Drug Deposit Indicated

Analytical Chemistry **2020**, *92*, 12, 8323-8332

Singe Particle Characterization of Drug Aerosol



Fluticasone Propionate
IR Map (1748 cm^{-1})

Highly Localized

Salmeterol Xinofate
IR Map (1582 cm^{-1})

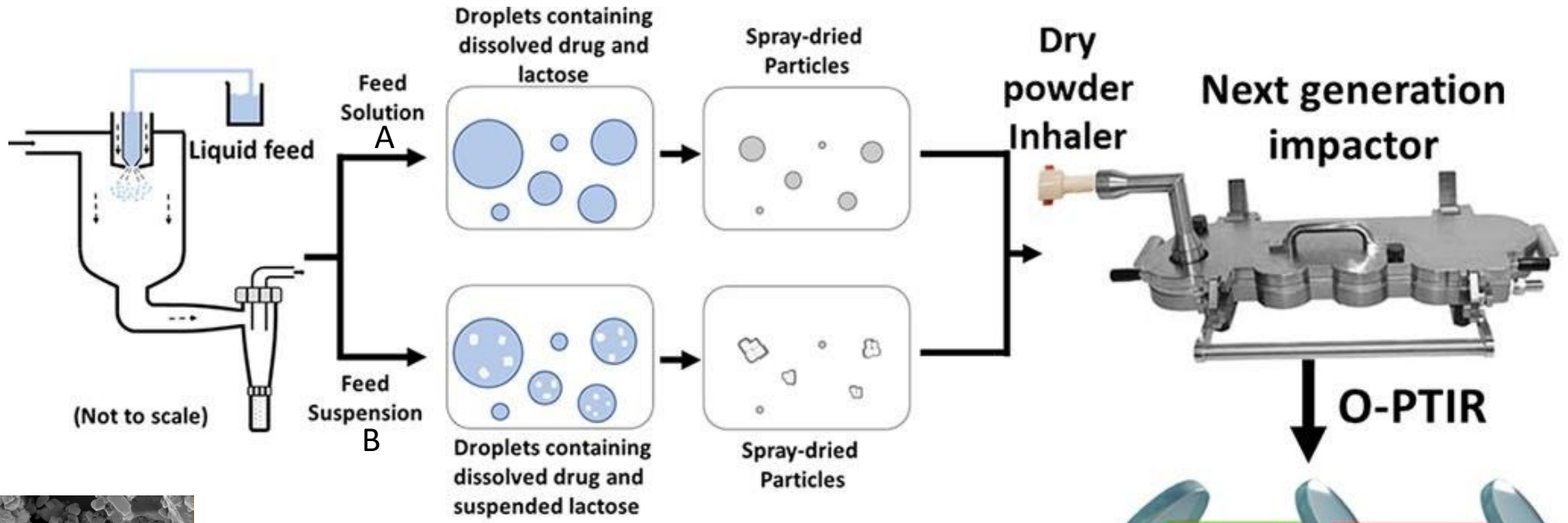
Smaller amount co-localized with Fluticasone

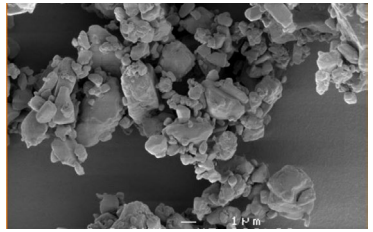
Lactose
IR Map (10-40cm^{-1})

Bulk of particle

Analytical Chemistry **2020**, *92*, 12, 8323-8332

Formulation effects on Drug Content with Aerosol Stage for Spray-Dried Alternatives





Lactohale® 300 is a very fine, micronised lactose with a D50 below 5 microns.

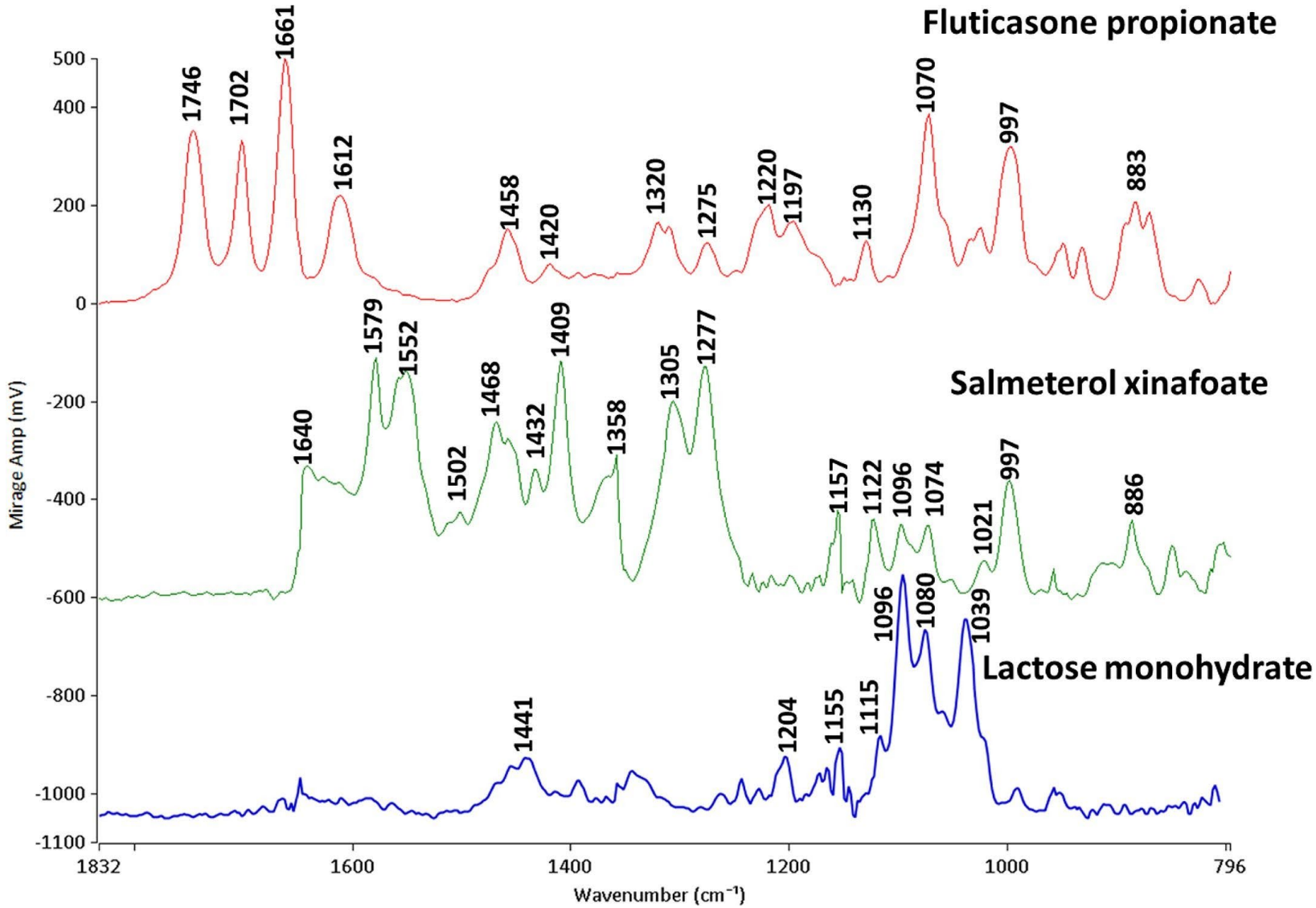
A: 8 FP to 1 SX w/ LH300 in 80/20 v/v isopropanol/water

B: 8 FP to 1 SX w/ LH300 in isopropanol

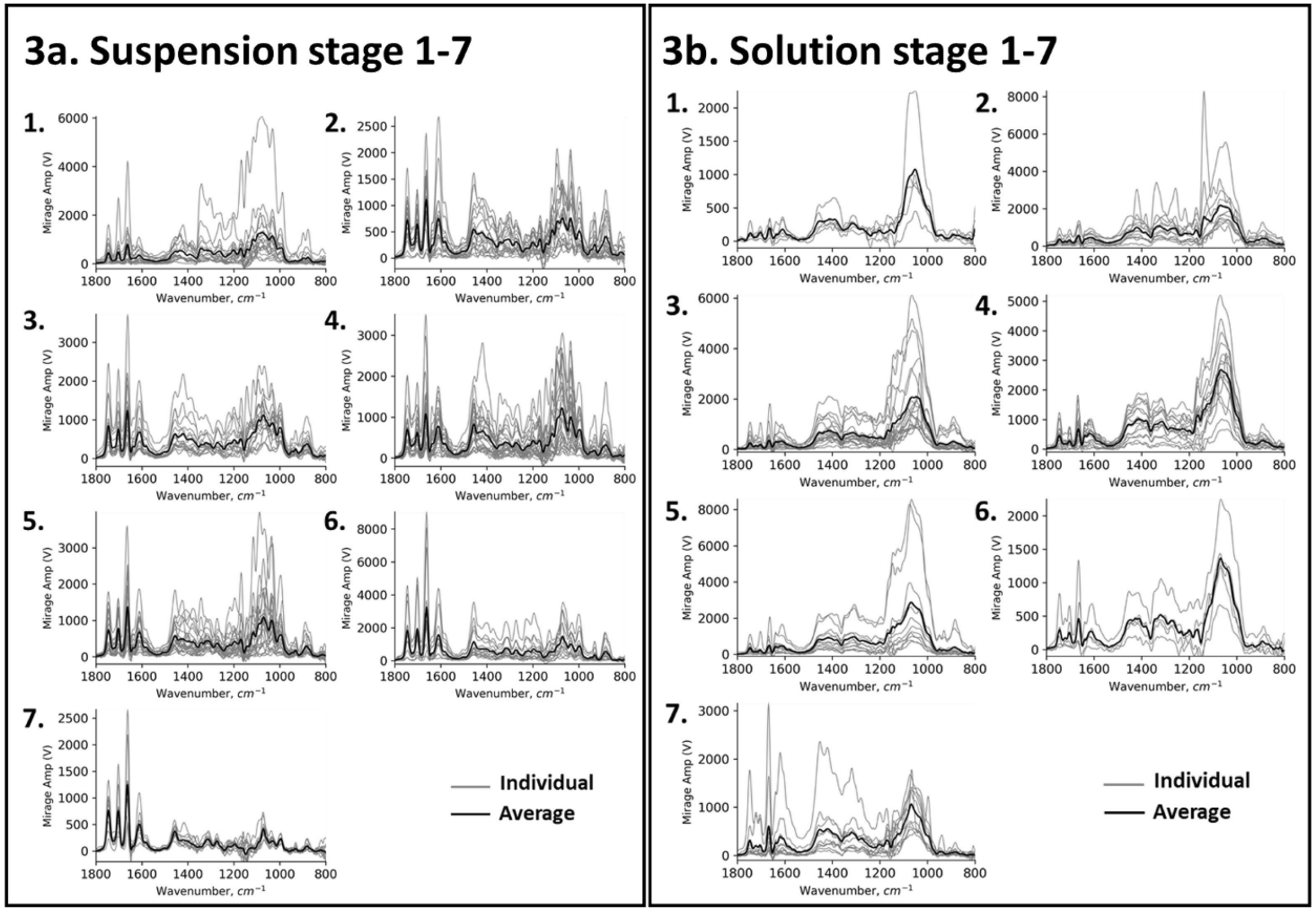
Khanal, Kim, Zhang, Ke, Banaszak Holl, Kim
Int. J. Pharm. **2023**, *632*, 122563

D50 indicates *Median* particle size is 5 microns

O-PTIR Spectra of Drugs and LH300

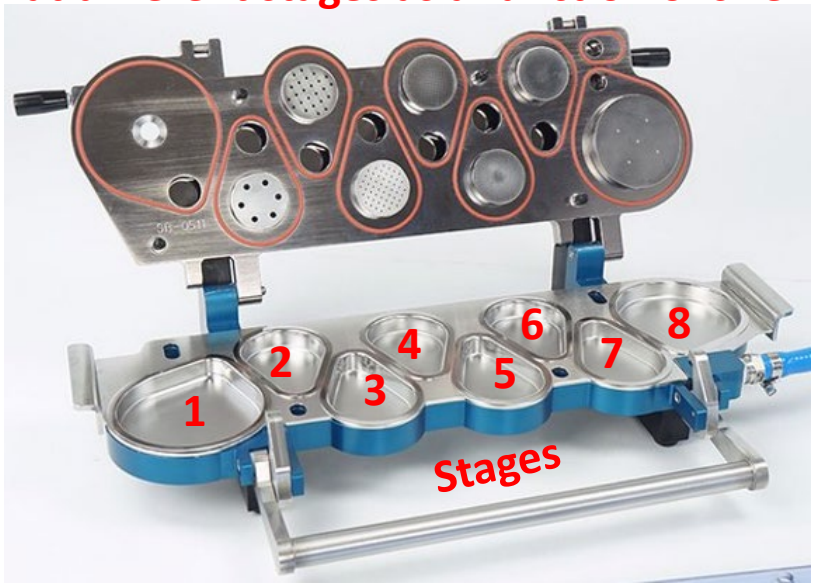


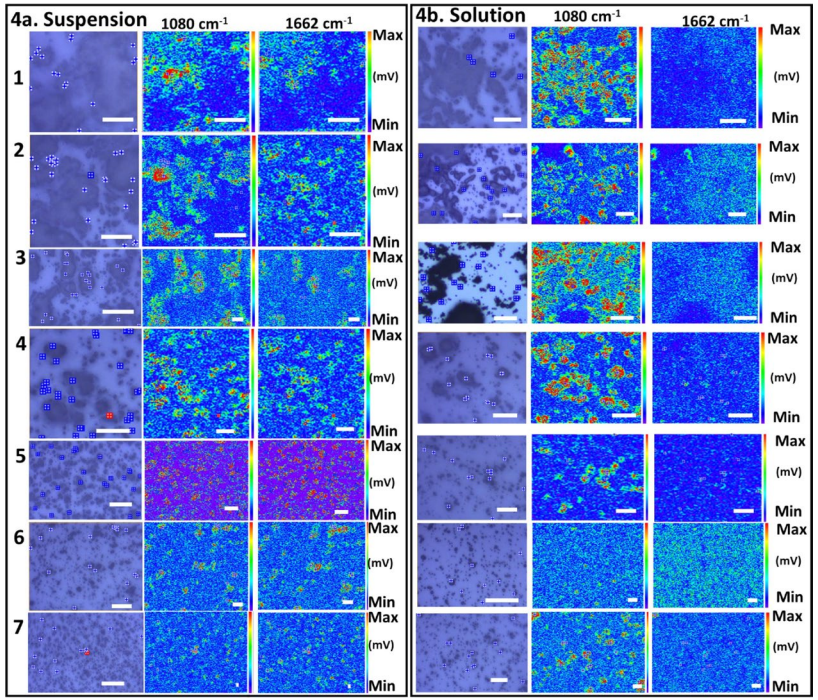
O-PTIR spectra collected of particles collected from stages 1–7 of the NGI



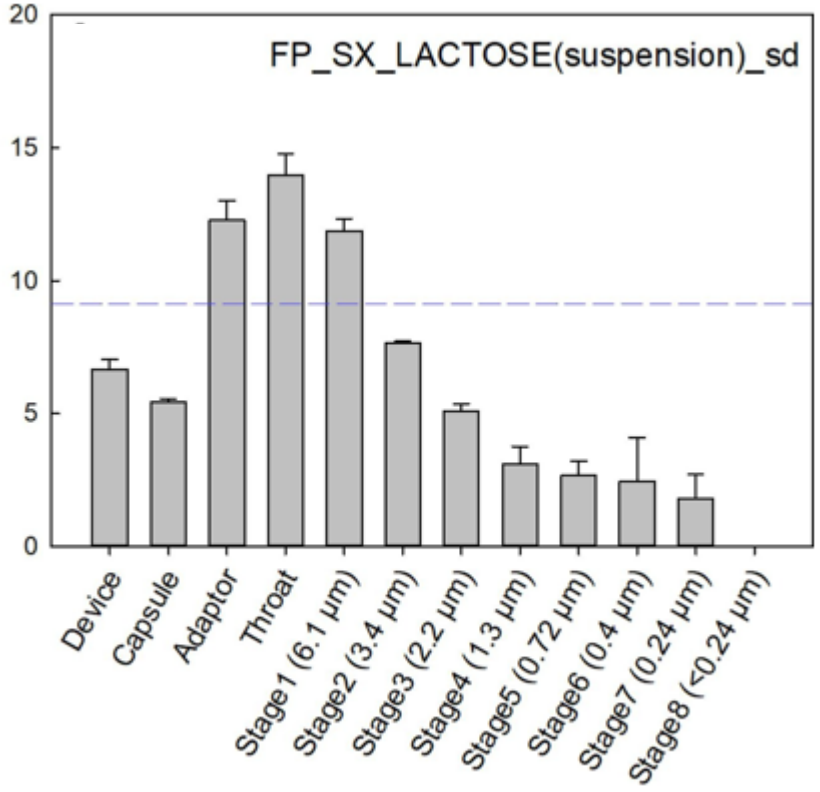
Stage	1	2	3	4	5	6	7	8
Cutoff Diameter in microns	6.1	3.4	2.2	1.3	0.72	0.4	0.24	< 0.24

Particles collected on Si wafers or CaF_2 cover slips at different stages as a function of size

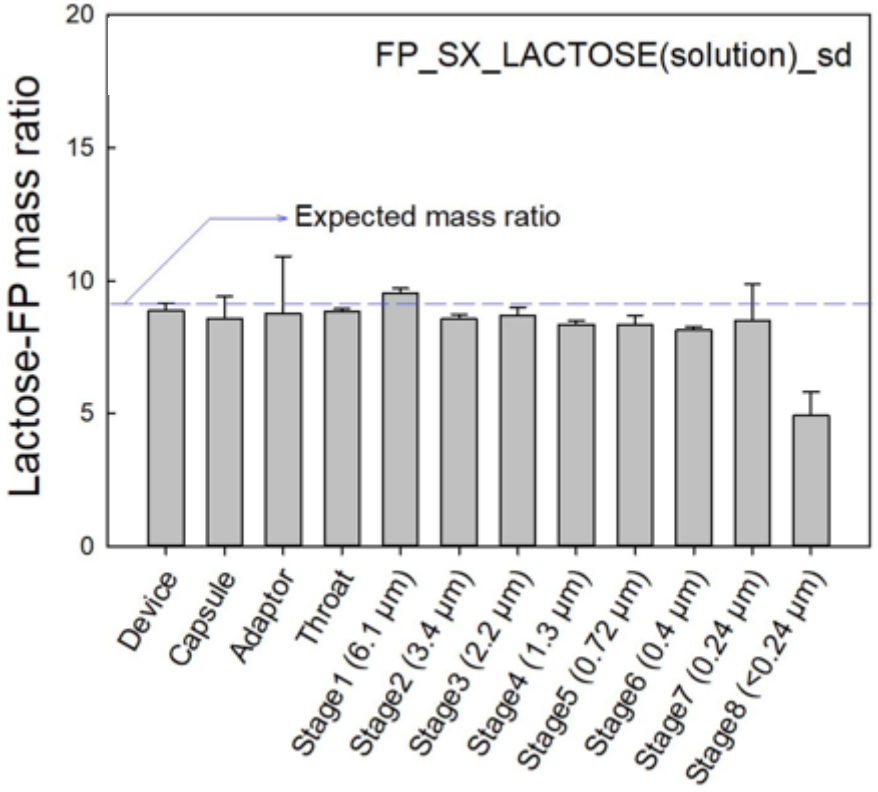




Mass ratio of lactose to fluticasone propionate based on bulk HPLC chemical analysis of particles collected from stages 1–7 of NGI

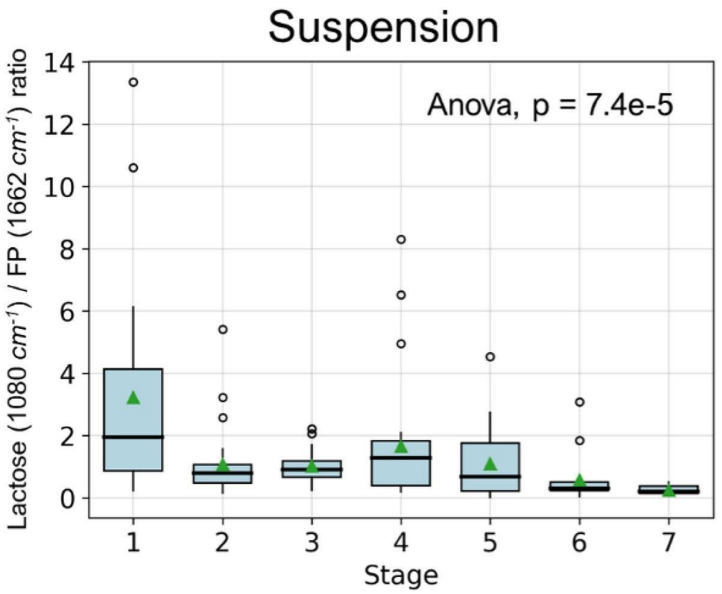


Solute composition in the suspension spray-dried particles varies with stage with less FP for smaller particles.

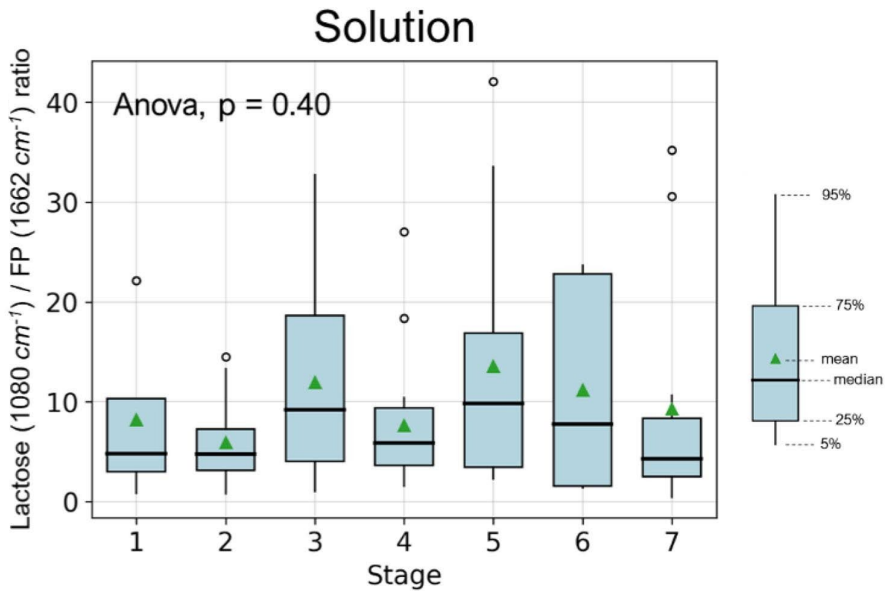


Solute composition in solution spray-dried particles is identical to that of the feed solution

Ratio of lactose to fluticasone propionate for particles collected from stages 1–7 of spray-dried powder for suspension



Stage	1	2	3	4	5	6	7
Mean	3.2	1.1	1.0	1.7	1.1	0.6	0.3
Median	1.9	0.8	0.9	1.3	0.7	0.3	0.2
n	18	24	17	26	24	16	12

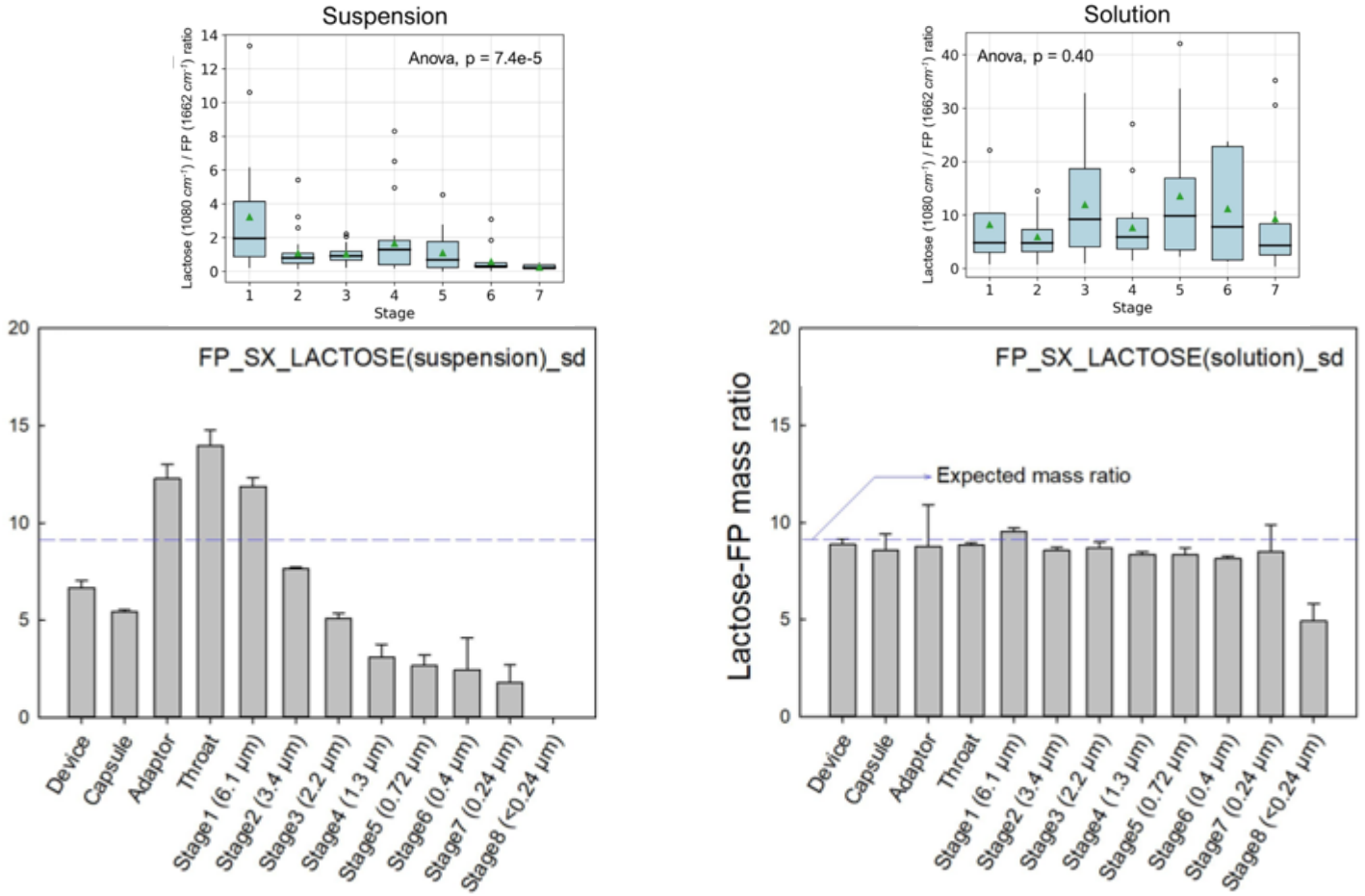


Stage	1	2	3	4	5	6	7
Mean	8.2	5.9	12.0	7.6	13.6	11.1	9.3
Median	4.8	4.8	9.2	5.9	9.8	7.8	4.3
n	5	12	20	16	12	8	11

Suspension formulation indicates a decrease in the average lactose-FP ratio by tenfold from 3.2 on stage 1 to 0.3 on stage 7

Solution formulation stages exhibit a relatively high lactose-FP ratio across all the stages with a minimum of 5.9 and maximum of 13.6 (9.9 ± 2.7)

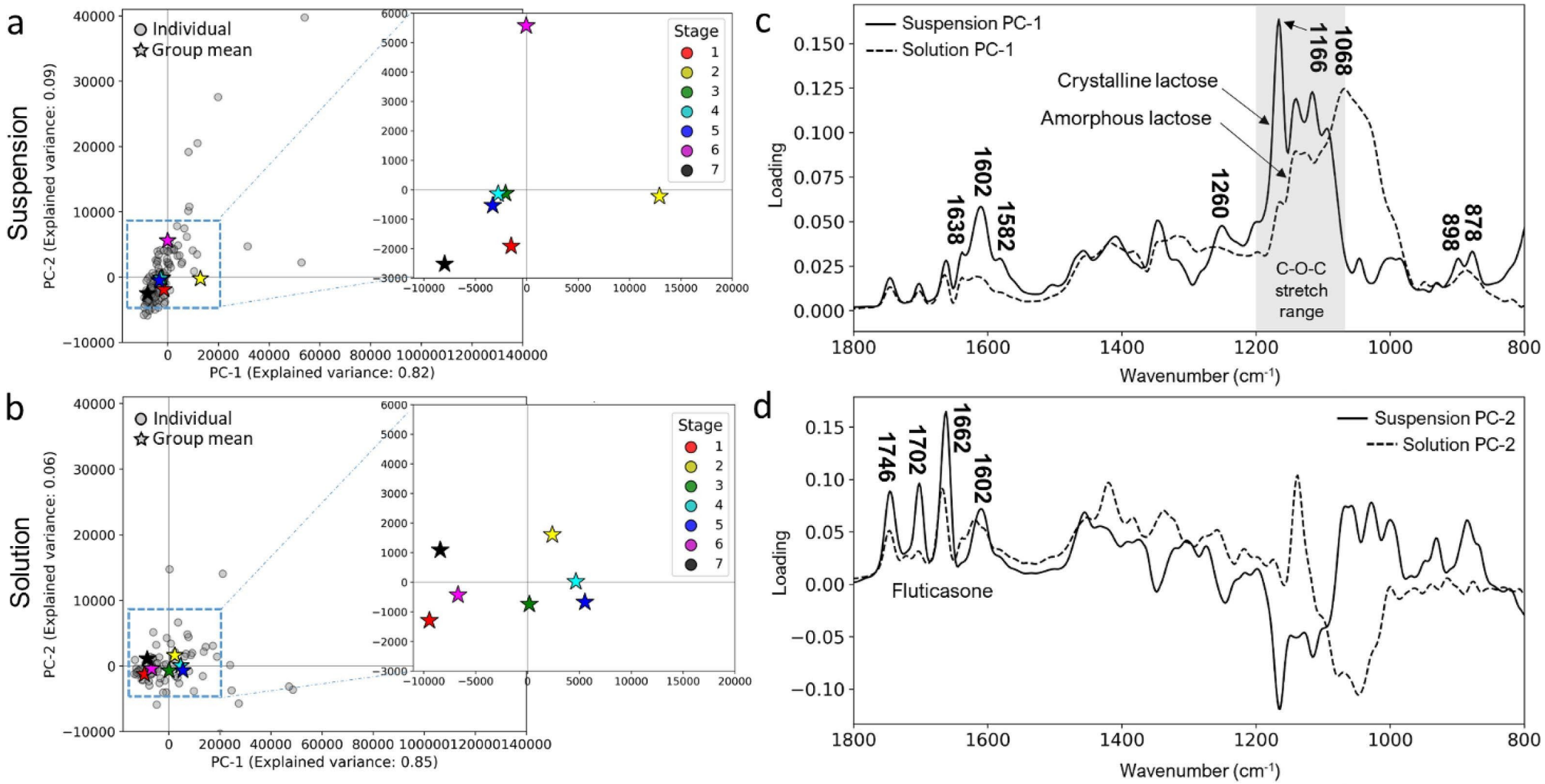
IR peak ratio of lactose:FP (1080 cm^{-1}:1662 cm^{-1}) from each stage of the suspension and solution formulations closely follows the bulk lactose-FP mass ratio.



IR data ALSO indicates particle to particle dispersion in ratio that is not observed in bulk analysis

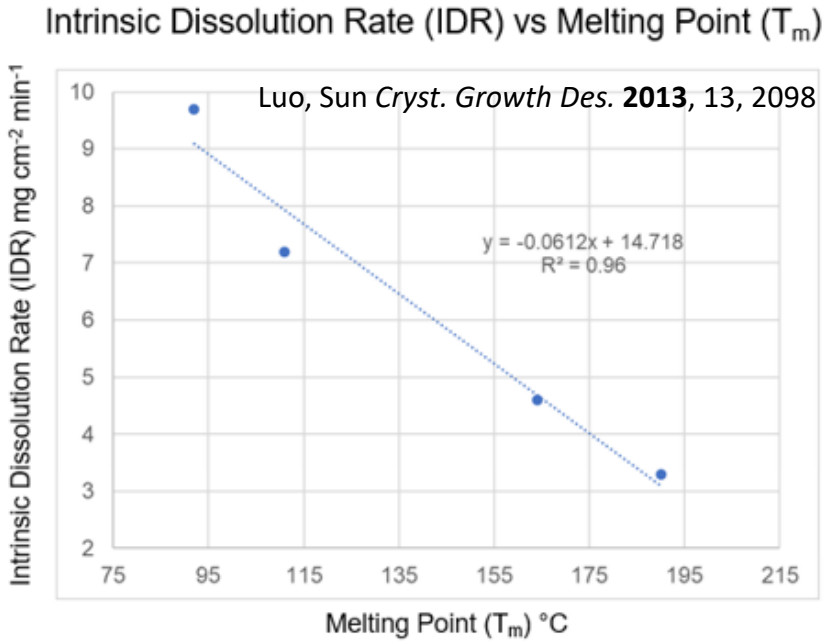
Principal component analysis indicates Lactose Present in Crystalline form in Suspension Formulation (LH300) and is amorphous in Solution Formulation

In both formulations variation largely results from changes in FP/lactose ratio



The Importance of Melting Points

- Criterion of purity
  - *Sensitive indicator of mixture for aerosol formulations*
- Strongly correlated with water solubility (thermodynamic)
  - *Sensitive indicator of dissolution rate? (kinetic)*
- Can we evaluate distribution of melting points for aerosol formulations?
  - *Role of distribution in formulation dissolution rate?*
  - *Particles and subparticle differences?*
- Importance of controlling melting points in aerosol formulation for tuning therapeutic index?



Intrinsic dissolution rate (IDR) versus melting point (T_m) for pyrazine carboxamide and co-crystalline mixtures with malonic, succinic, and glutaric acids

A powerful new tool in support of Bioequivalence Studies and Formulation Design?

Katritzky, Jain, Lomaka, Petrukhin, Maran, Karelson
Crystal Growth & Design **2001**, *1*, 4, 261–265

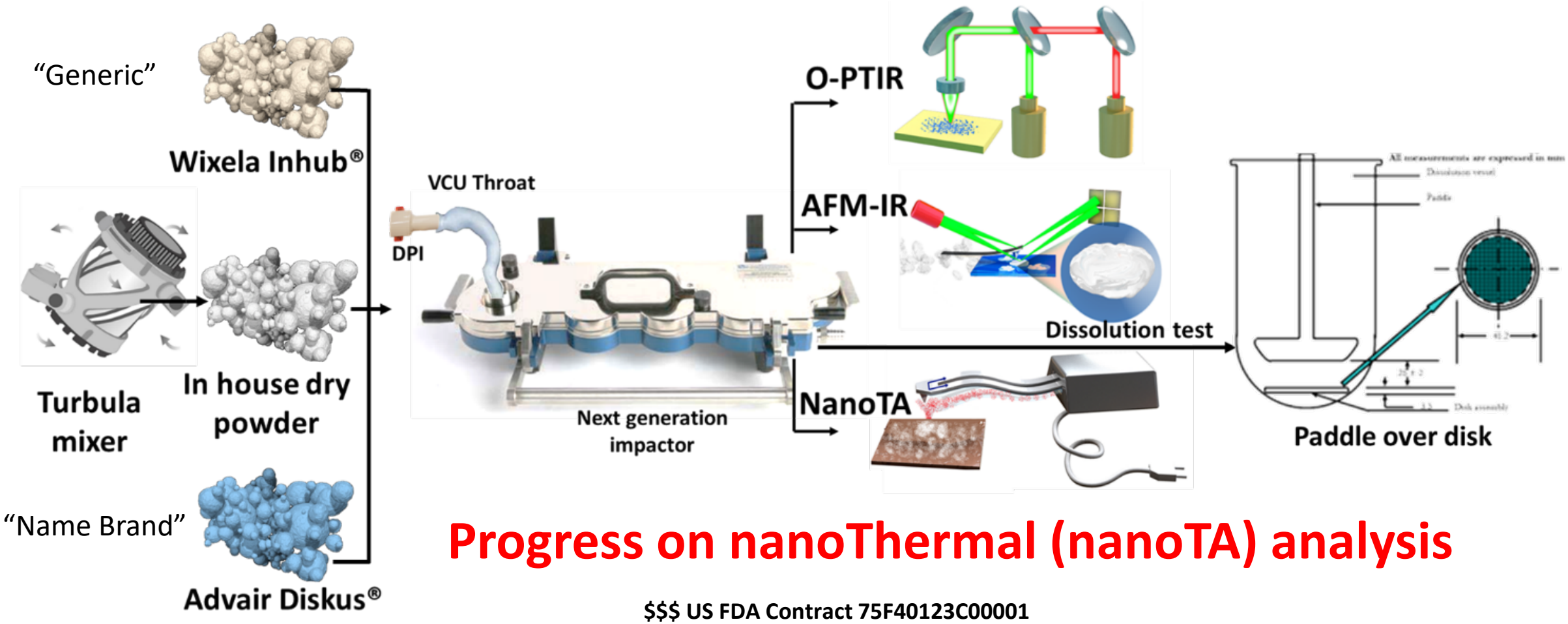
Kim, Grant *Int. J. Pharm*. **1989**, 57, 117
Teleki, Nylander, Bergstrom *Pharmaceutics* **2020**, 12, 493
Hanfeef, Chadha *J. Pharm. Sci.* **2019**, 108, 3792

Assessing the Relationships of

- Particle compositions
- Melting points
- Particle mechanics

as a function of

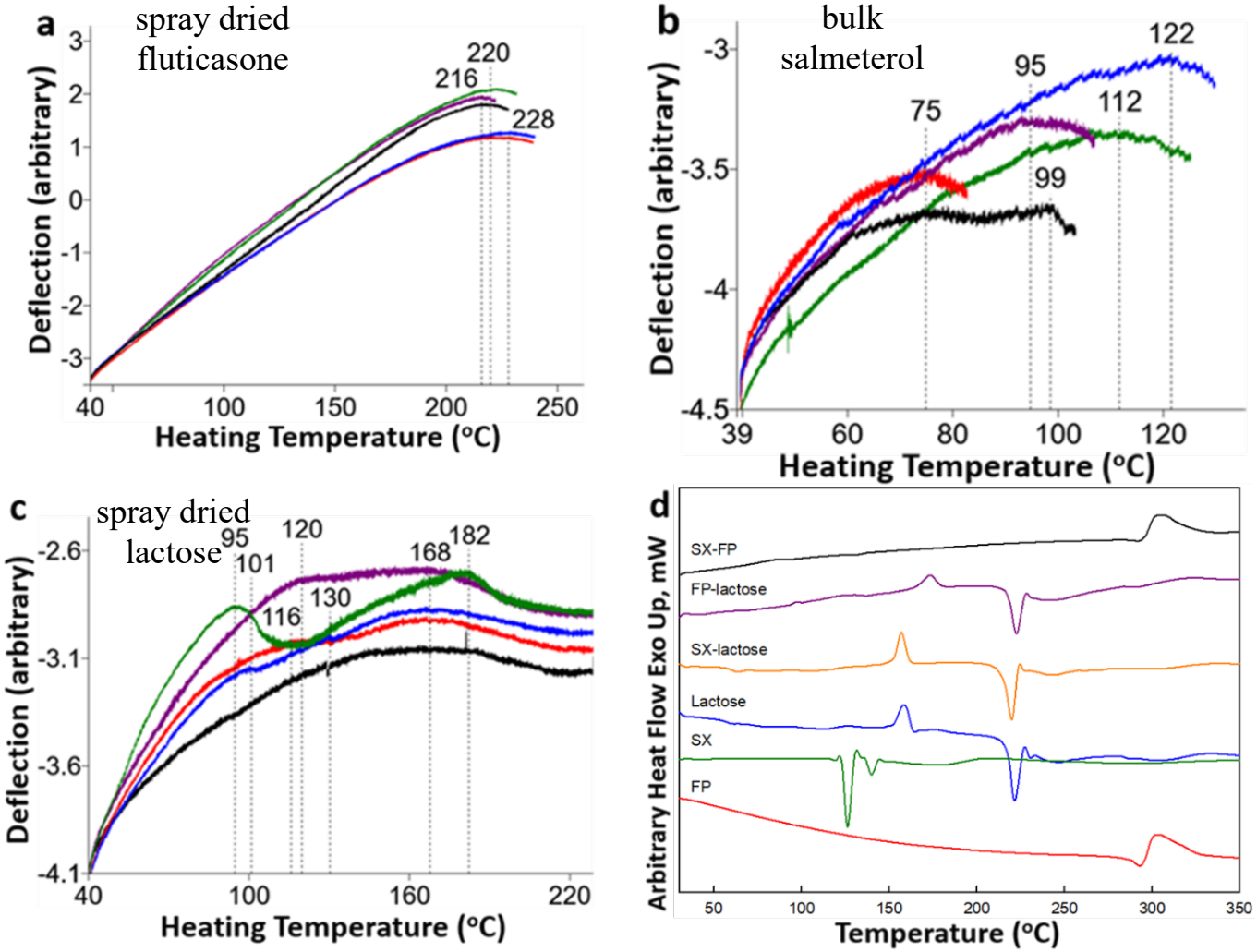
- Aerodynamics
- Dissolution Rate
- Bulk composition



Progress on nanoThermal (nanoTA) analysis

$$$ US FDA Contract 75F40123C00001
Identification of Drug Distribution in Aerosols A
Nanospectroscopy and NanoThermal Analysis

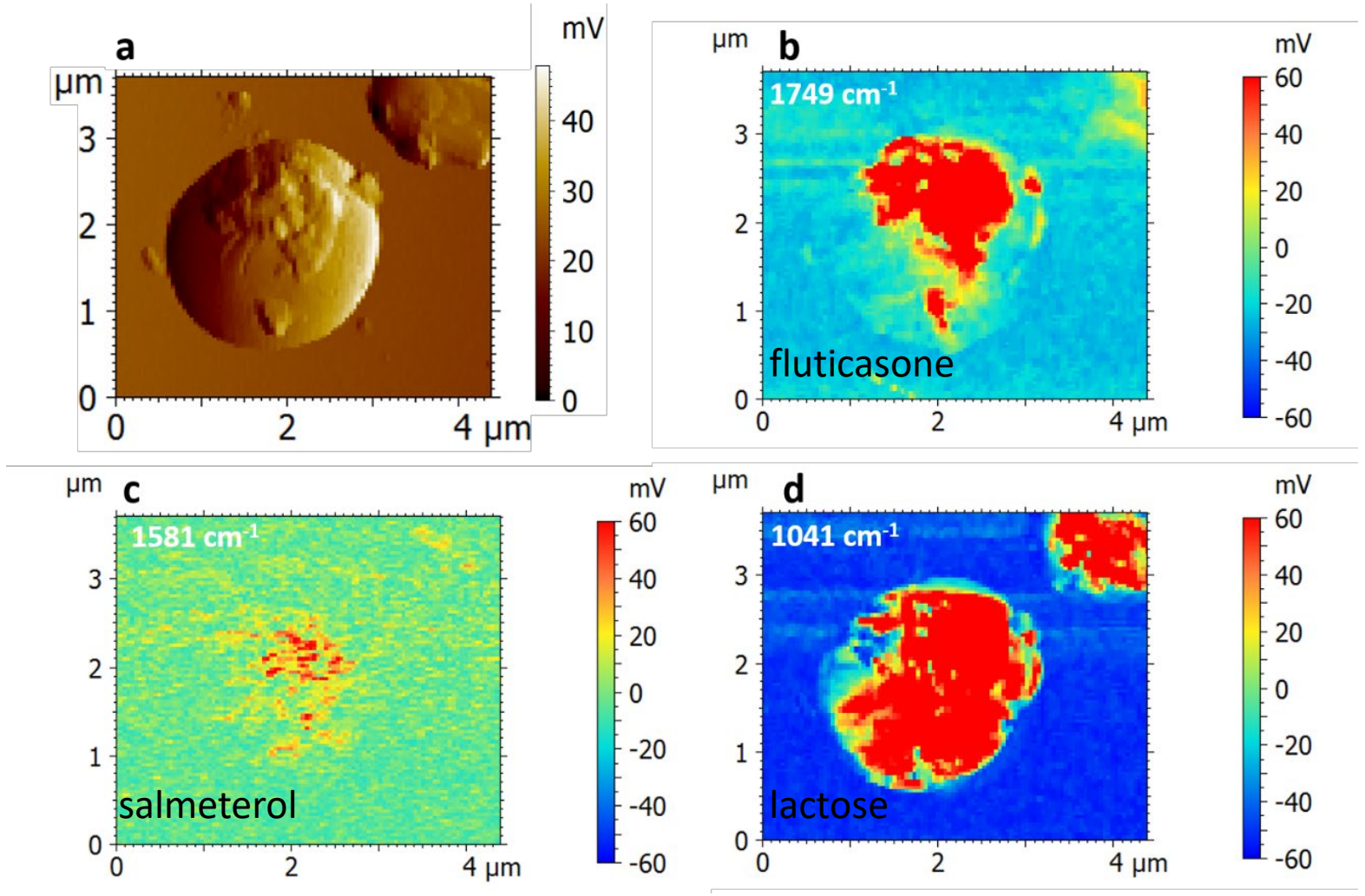
NanoTA thermal ramps and DSC thermograms of drugs and lactose excipient



Differential Scanning Calorimetry (DSC)

- Thermal properties of spray dried fluticasone provide narrow MP range
- Thermal properties of bulk, as-received salmeterol show substantial MP variation including presence of two known crystalline forms
- Thermal properties of lactose show evidence for glass transition (first peak 95-130 °C) and MP (168-182 °C)

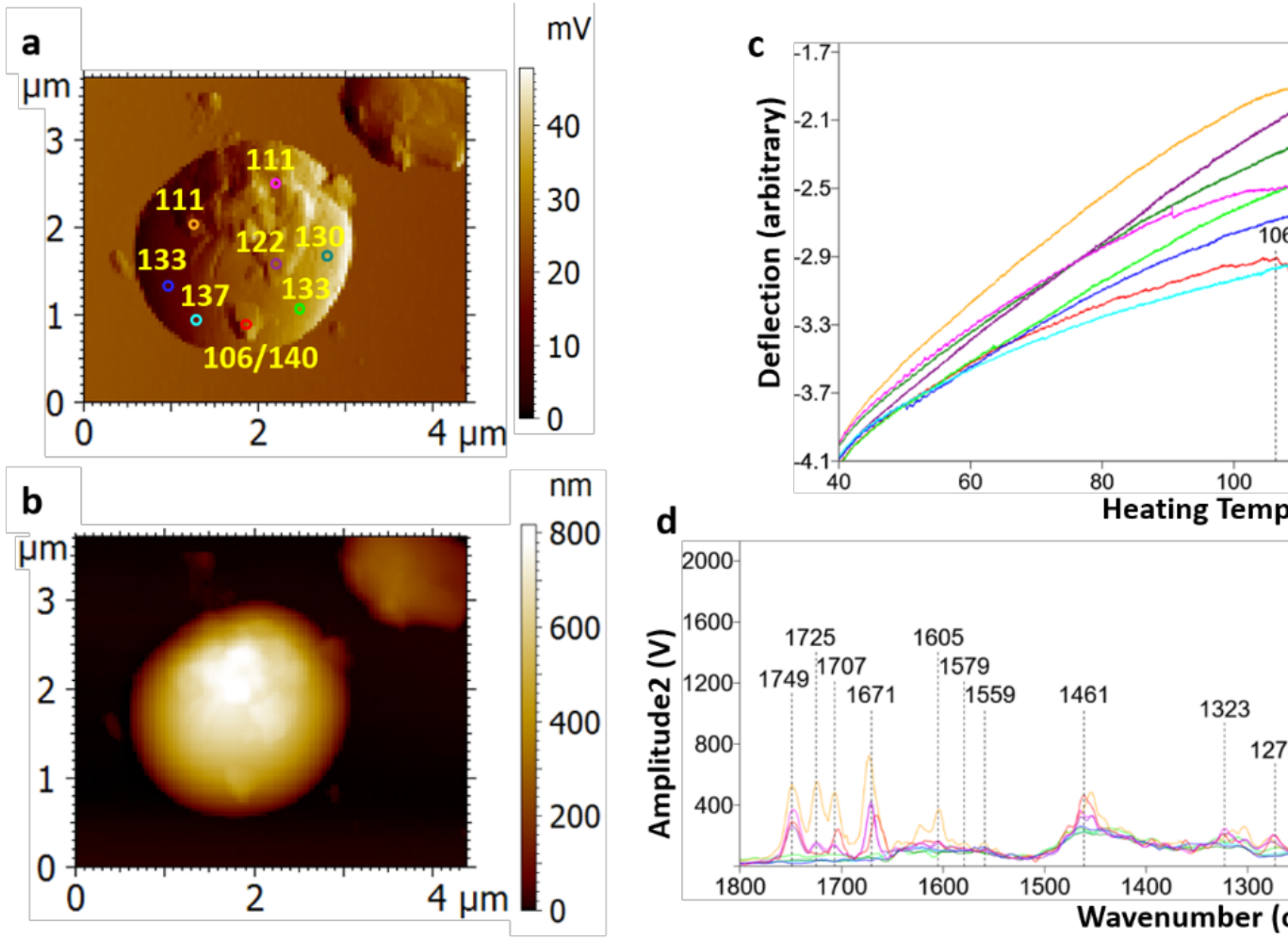
AFM-IR chemical maps acquired from an individual aerosol particle of a spray dried powder containing fluticasone propionate, salmeterol xinafoate and lactose collected on stage 5 of NGI.



1:7:70 by weight
salmeterol:fluticasone:lactose

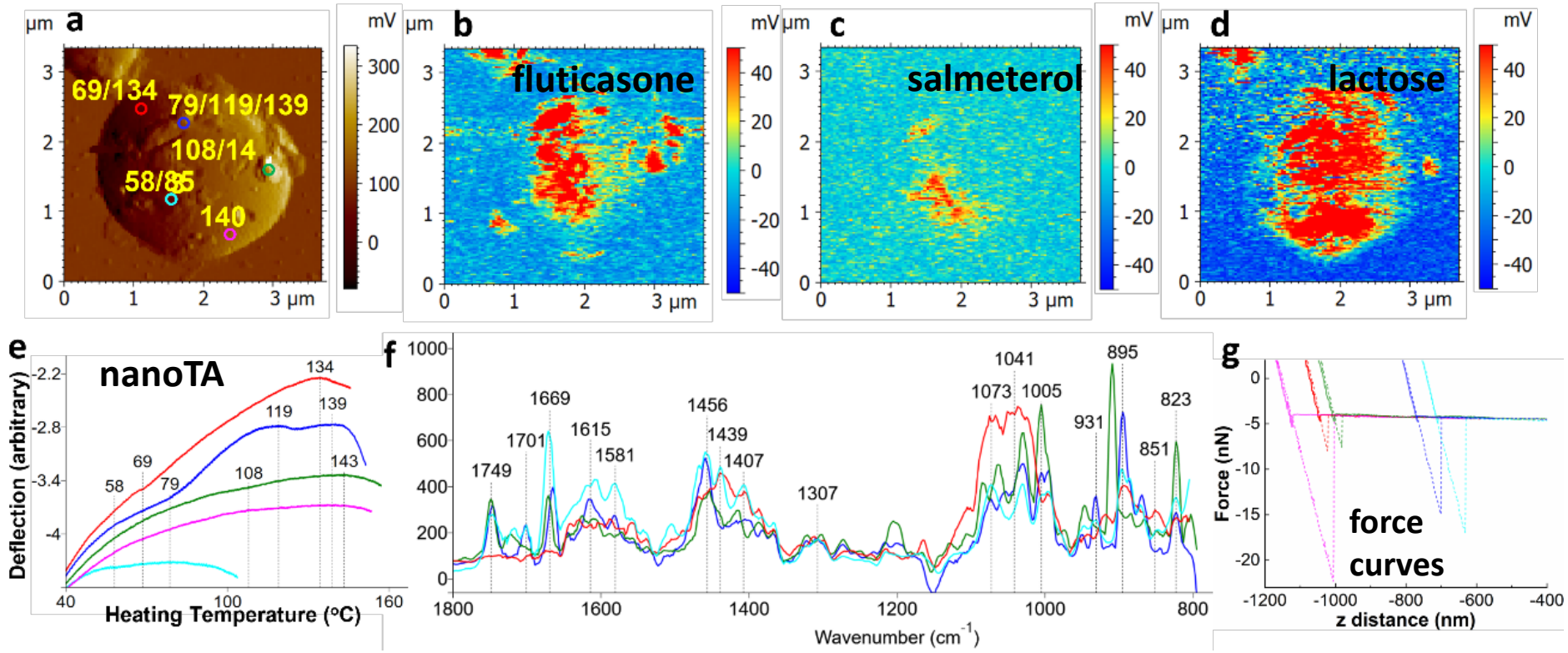
similar to Seretide®

NanoTA thermal ramps and AFM-IR spectra acquired from different locations of an individual aerosol particle of a spray dried powder containing fluticasone propionate, salmeterol xinafoate and lactose collected on stage 5 of NGI.



- T_m varies with local composition in particle
- Presence of drug lowers T_m as compared to lactose alone
- Smooth regions are lactose as indicated by spectra and T_m
- Rough regions are drugs as indicated by spectra and T_m

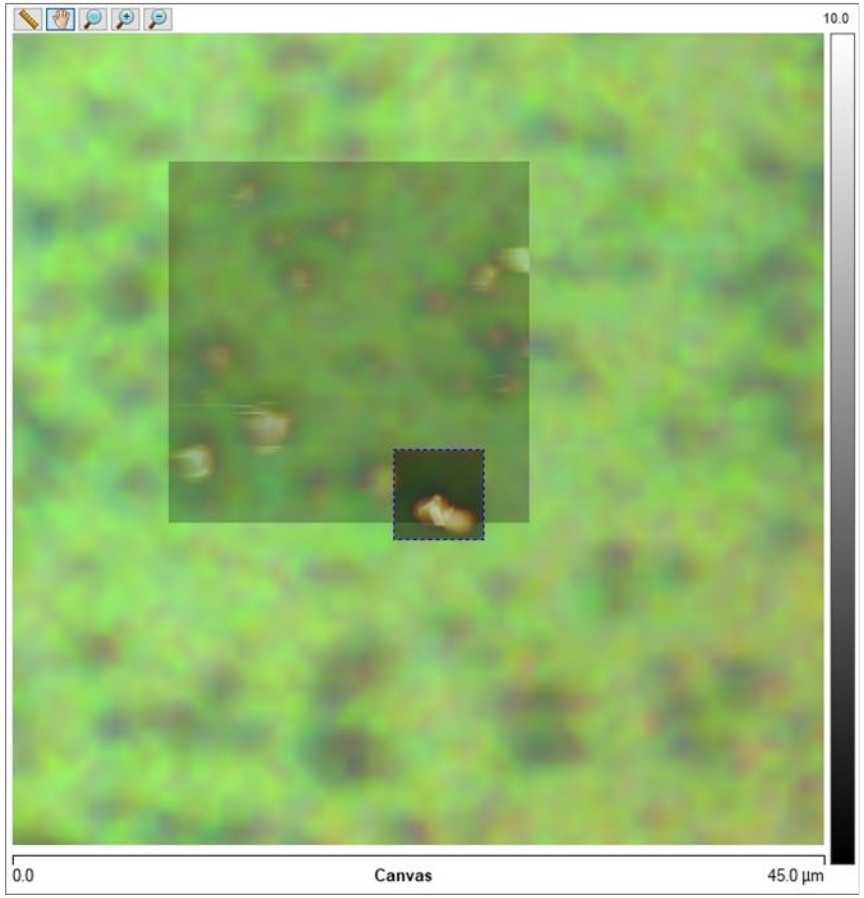
NanoTA thermal ramps, AFM-IR chemical maps, AFM-IR spectra and force curves acquired from an individual aerosol particle of a spray dried powder containing increased SX content collected on stage 5 of NGI (FP:SX:lactose = 1:2:23 by weight).

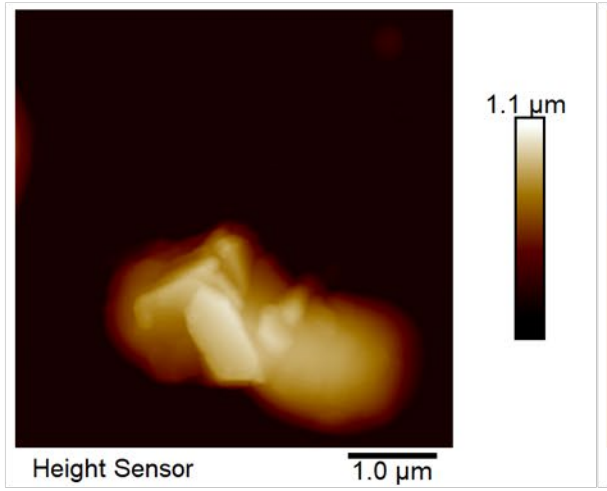


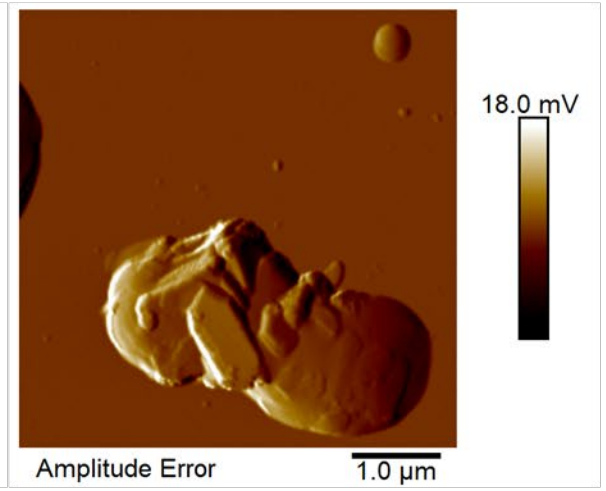
- Substantial decrease in MP for regions containing fluticasone, salmeterol, and lactose
- Lactose only regions of particle often exhibit MP for pure lactose
- Some “Lactose only” region do show decrease in MP – limited spectroscopic sensitivity?
- Greatest adhesion shown for lactose only regions, Less adhesion shown for drug regions
  - Unmodified gold-coated tip

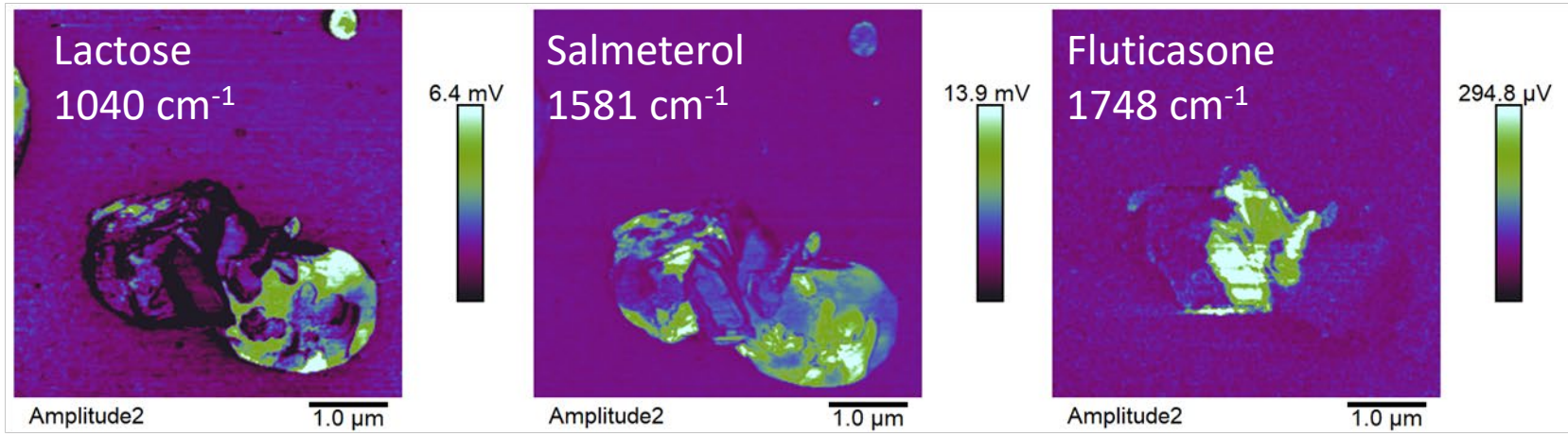
Recent Data with Bruker IconIR

Optical/AFM-overlay & photothermal AFM-IR imaging

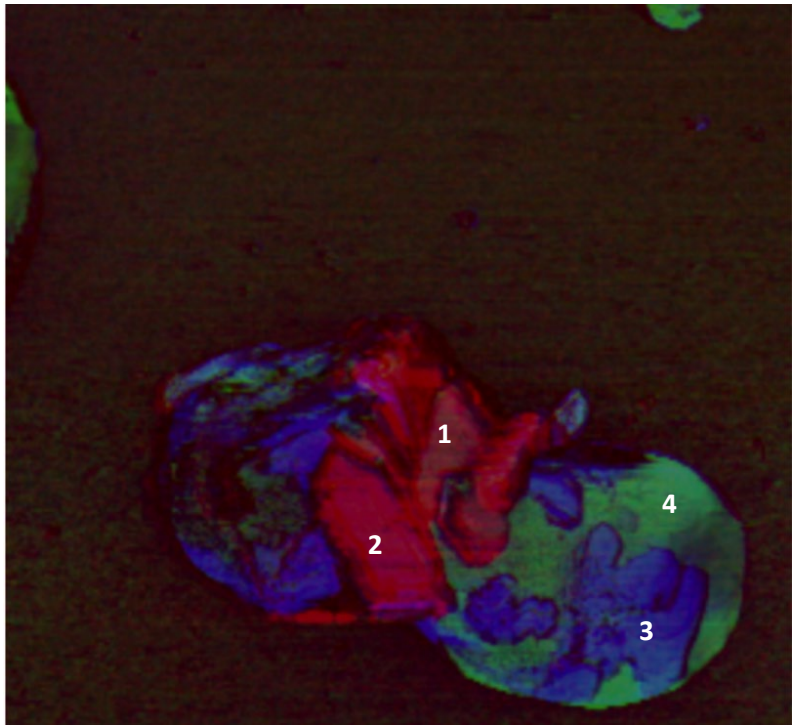








Improved Chemical Identification in Single Particle

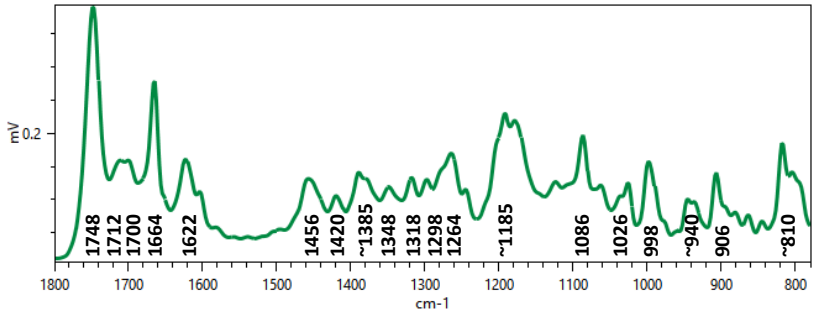


1040cm^{-1} (Lactose)

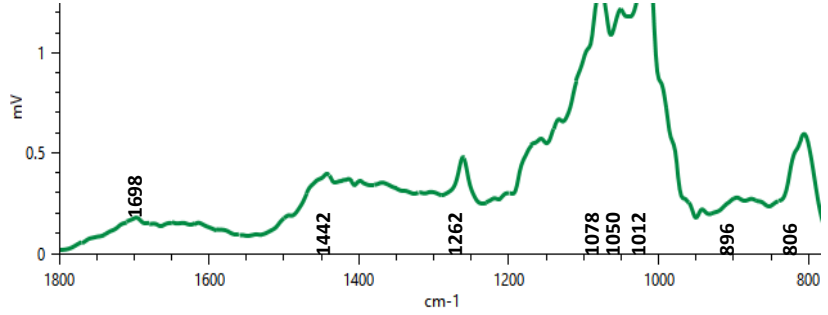
1581cm^{-1} (Salmeterol)

1748cm^{-1} (Fluticasone)

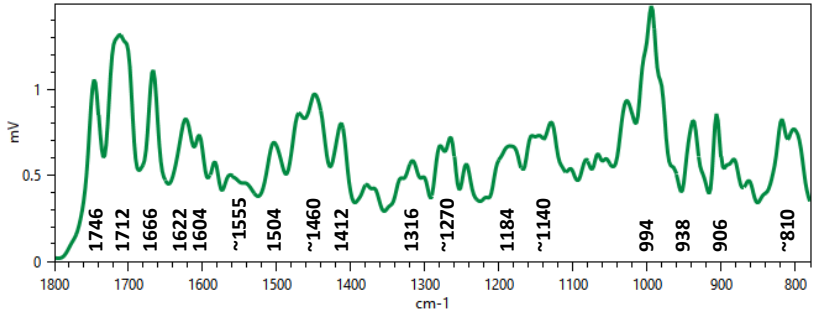
Pos. 1 — Fluticasone region



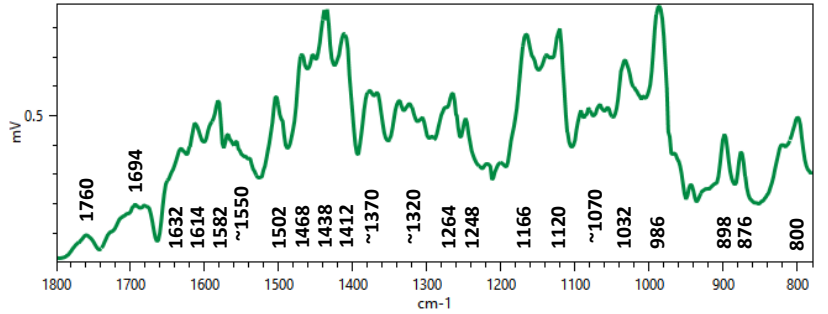
Pos. 4 — Lactose region

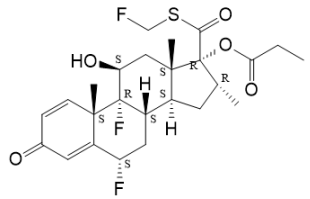


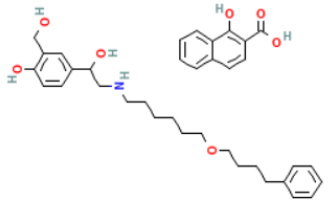
Pos. 2 — Fluticasone region



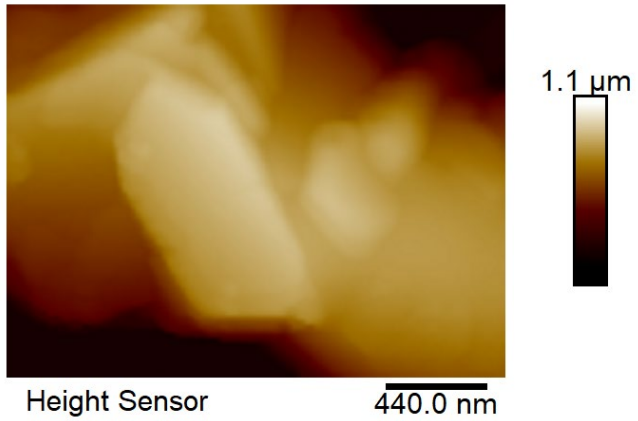
Pos. 3 — Salmeterol region

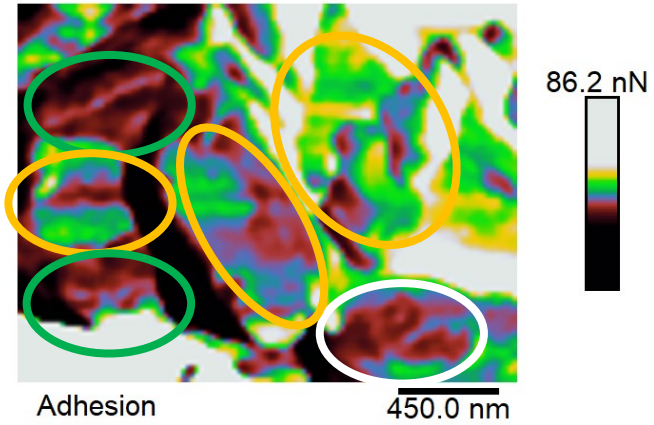




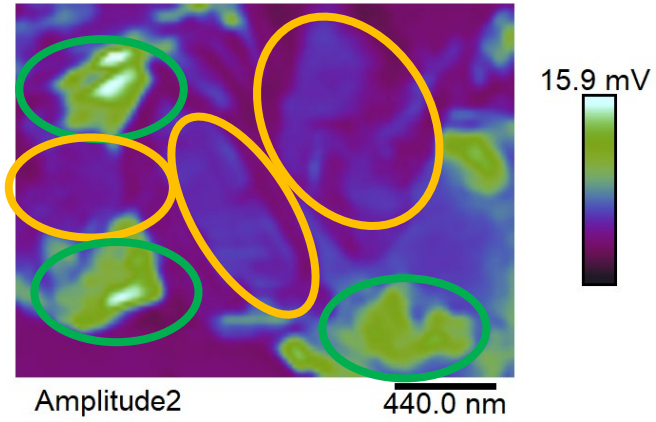


Colocalized Photothermal AFM-IR and QNM Mechanical Data on Single Particle

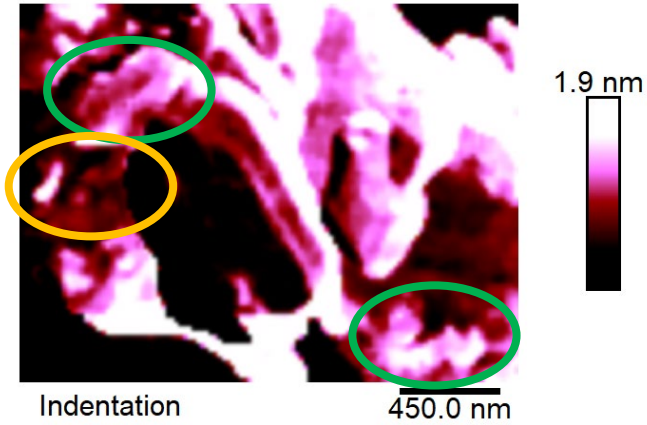




Mechanical (adhesion)



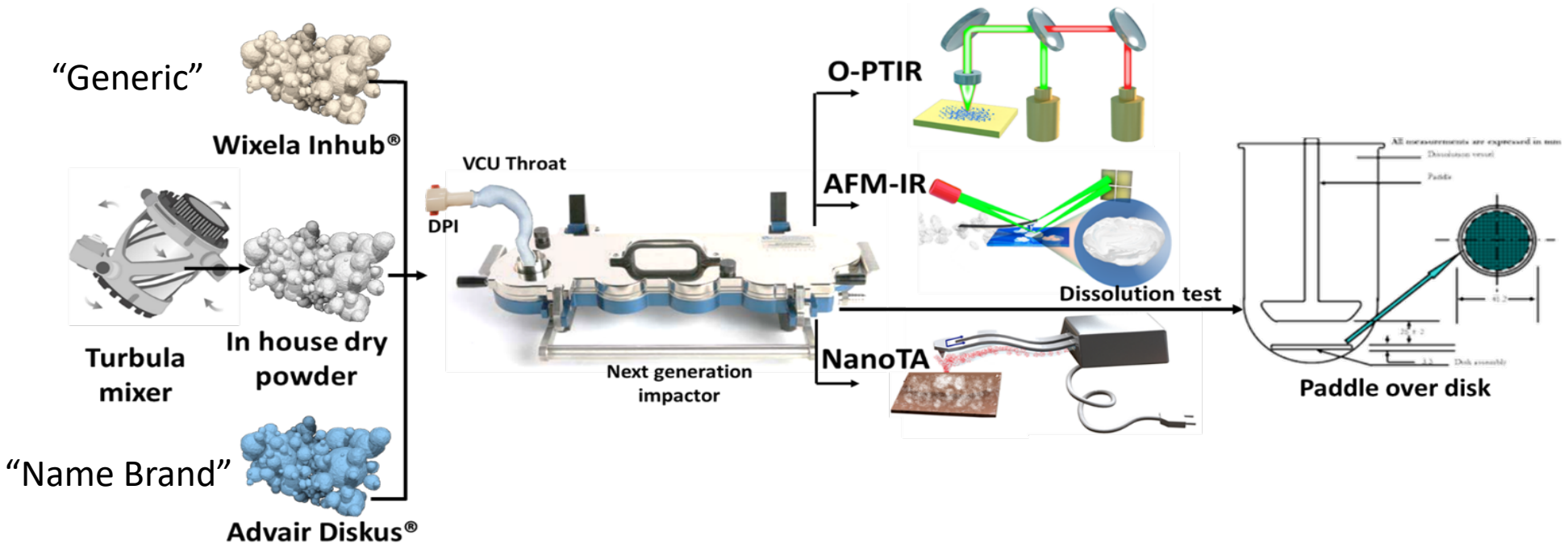
IR @ 1581cm^{-1} (Salmeterol)



Mechanical (indentation)

SCHOOL OF ENGINEERING
The University of Alabama at Birmingham
Division of Pulmonology, Allergy, and Critical Care Medicine
THE UNIVERSITY OF SYDNEY

Summary

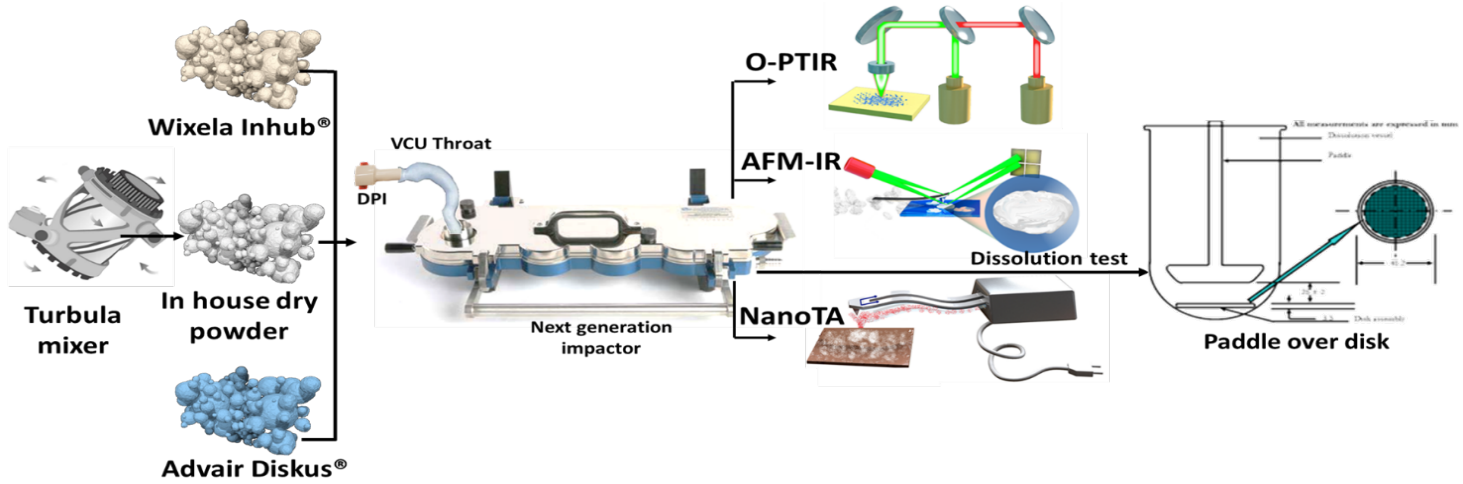


- Ongoing study for FDA using combination of Spectroscopic, Thermal, and Mechanical data to evaluate Generic vs Name Brand aerosol drug formulations
- Ongoing evaluation and optimization of Bacteriophage formulations for use as antibiotics
- Future Studies optimizing new formulations by evaluating therapeutic index as a function of Spectroscopic, Thermal, and Mechanical data

Future Directions and Possible Collaborations

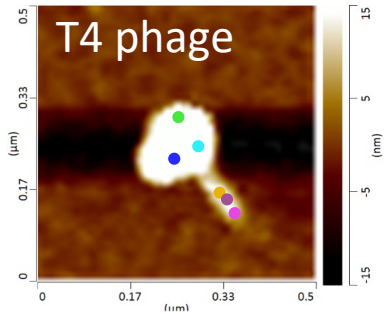
- Studies connecting detailed characterization of important parameters such as drug distribution in aerosol particles and important physical characteristics such as mechanical properties and melting point with therapeutic index as measured in cell culture, organoid, and animal models.
  - Fluticasone, salmeterol for asthma, COPD, and/or CF models
  - Other drug combinations were dry powder inhaler delivery of interest

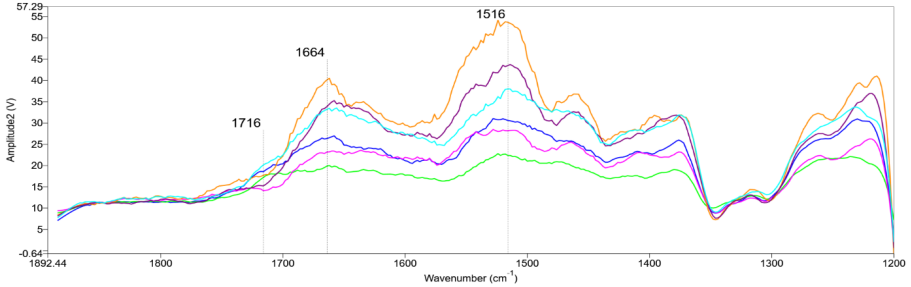
- Clinical studies using currently approved formulations to relate properties of drug delivery systems to patient efficacy and therapeutic index.



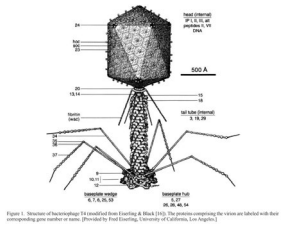
Future Directions and Possible Collaborations

- Bacteriophage formulation for antibiotic treatment
  - Active ARC grant with Prof. Chan at University of Sydney on bacteriophage formulation
  - Connections with Jeremy Barr and Trevor Lithgow (just began clinical trials at The Alfred Hospital) at the Centre for AMR at Monash University





Spectra from capsid and tail region of phage



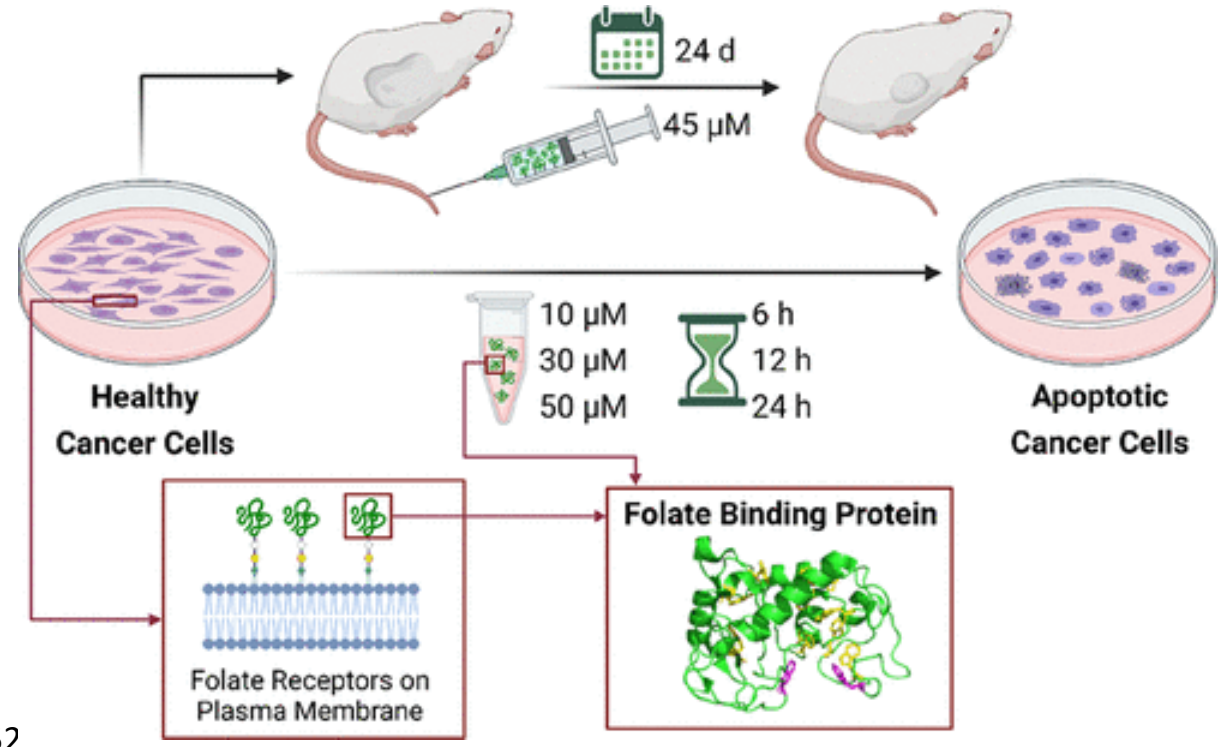
NIH-supported clinical trial of phage therapy for cystic fibrosis

ARLG
Antibacterial Resistance Leadership Group

The trial is evaluating whether the bacteriophage, or “phage,” therapy is safe and able to reduce the amount of bacteria in the lungs of volunteers. Investigators aim to enroll up to 72 adults at 16 CF centers across the United States.

Future Directions and Possible Collaborations

- Folate Binding Protein (FBP) as therapeutic
  - FBP alone slowed tumor growth in mouse xenograft model (KB cells)
  - Test possible use for lung cancer?
  - Other applications in CF?



Molecular Pharmaceutics **2022**, *19*, 843-852

Nanoscale **2017**, *9*, 2603-2615