UAB SCHOOL OF ENGINEERING
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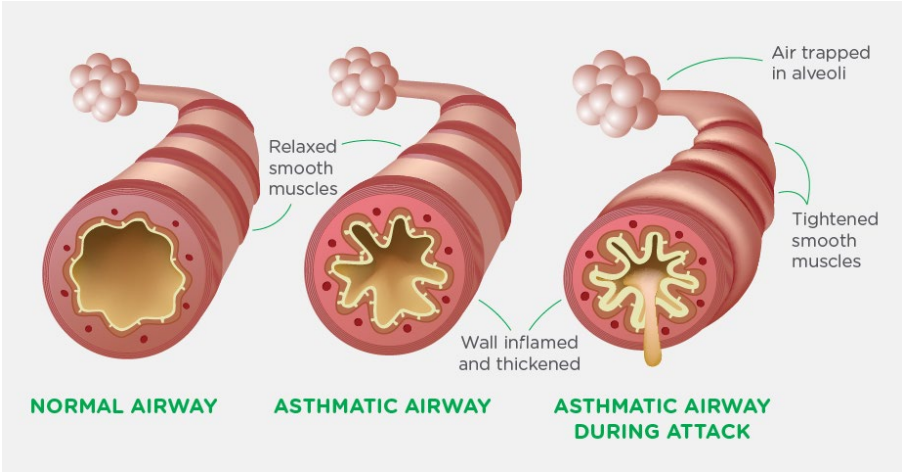


UAB HEERSINK SCHOOL OF MEDICINE
The University of Alabama at Birmingham

Quantitative Morphological Analysis of Multiparticle Aggregates

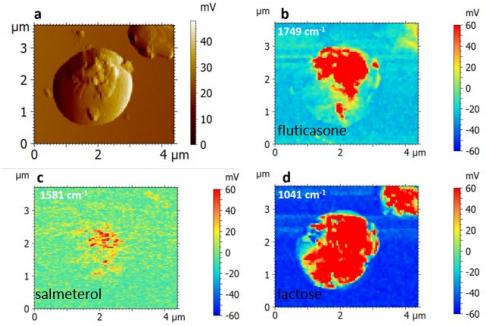
Mark Banaszak Holl

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

SciX October 2025

Banaszak Holl Lab Photothermal Infrared Applications

Drug Delivery



Analytical Chemistry **2025**, *97,* 16421.
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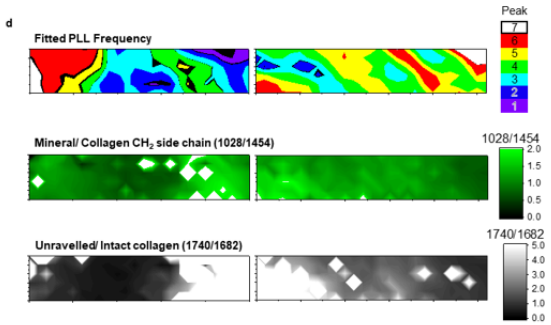
International Journal of Pharmaceutics **2023**, *646,* 123505
Advanced Drug Delivery Reviews **2023** *192*, 114646
International Journal of Pharmaceutics **2023** *632*, 122563
Analytical Chemistry **2020** *92*, 8328-8322

Bone and Ligament



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Journal of Structural Biology **2022**, 3, 107878
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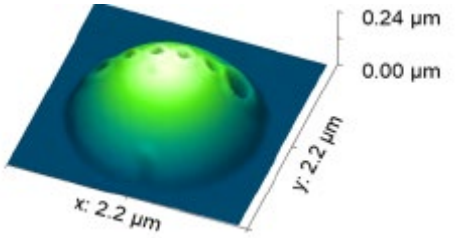

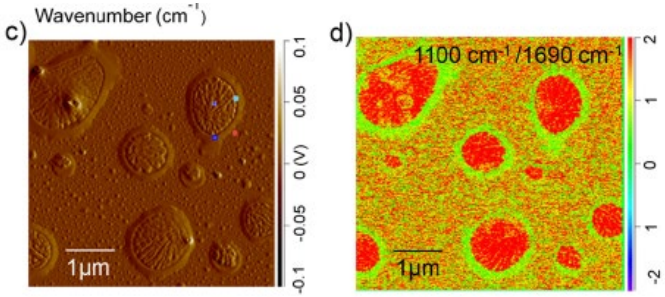

Cellulose **2024**, 31, 765
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Analytical Chemistry **2017** *89*, 8594

Bruker IconIR
AFM-IR

Photothermal mIRage-LS
O-PTIR

Engineering for the Human Process - Aerosols for Drug Delivery

University of Alabama at Birmingham

School of Engineering

Department of Mechanical and Materials Engineering

Division of Pulmonology, Allergy, and Critical Care Medicine

Lung Health Center

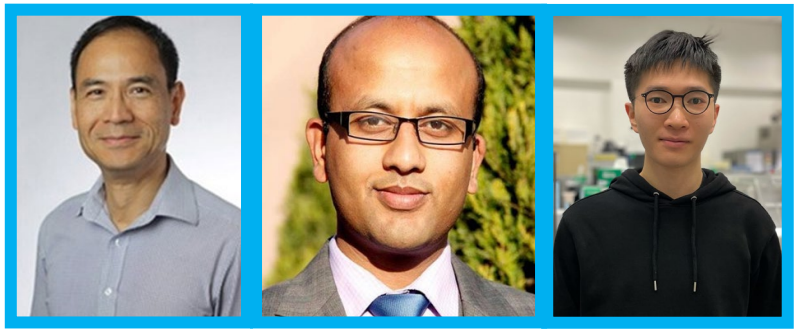
Heersink School of Medicine

Mark Banaszak Holl | Sheikh Tanzina Haque | Blessy Joseph | Terri Ross | Thanh Kien Ta

U.S. Food and Drug Administration

Elizabeth Bielski | Bryan Newman | Huzeyfe Yilmaz | Snober Ahmed

University of Sydney School of Pharmacy



Hak-Kim Chan | Dipesh Khanal | Eric Cao

$$$ U.S. FDA Contract 75F40122C00202

Identification of Drug Distribution in Aerosols: A Nanospectroscopy and NanoThermal Analysis

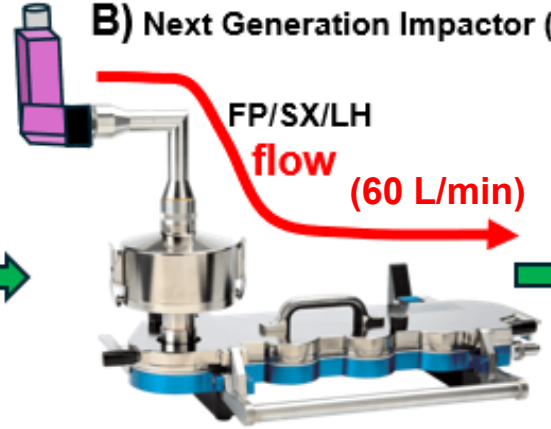
O-PTIR with Quantitative Morphology Analysis Evaluation of Dry Powder Inhalers Delivering Aerosolized Particles to the Human Lung

A) Formulation Methods

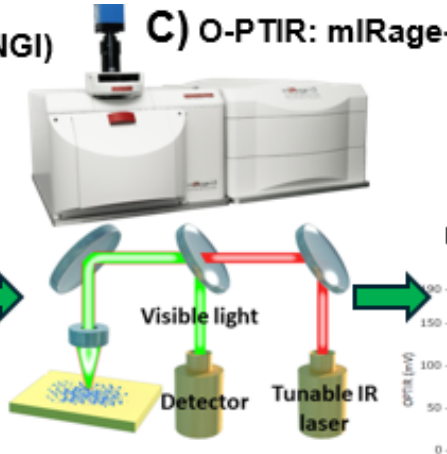
Turbula Mixer

Buchi S-300 Spray dryer

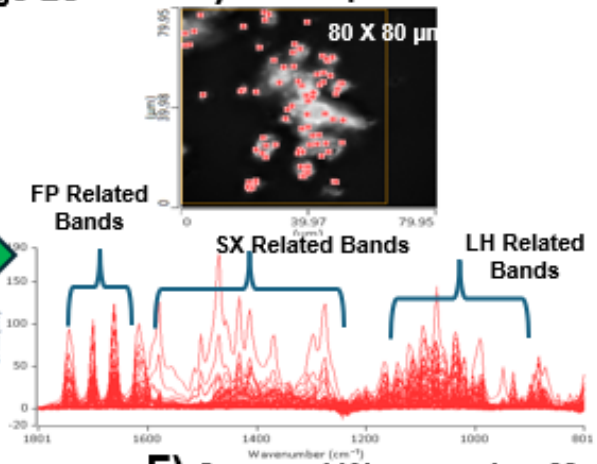
B) Next Generation Impactor (NGI)



C) O-PTIR: mIRage-LS

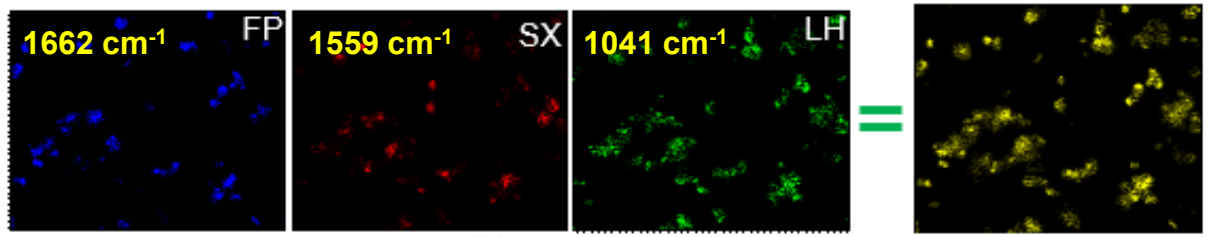


D) Point Spectra

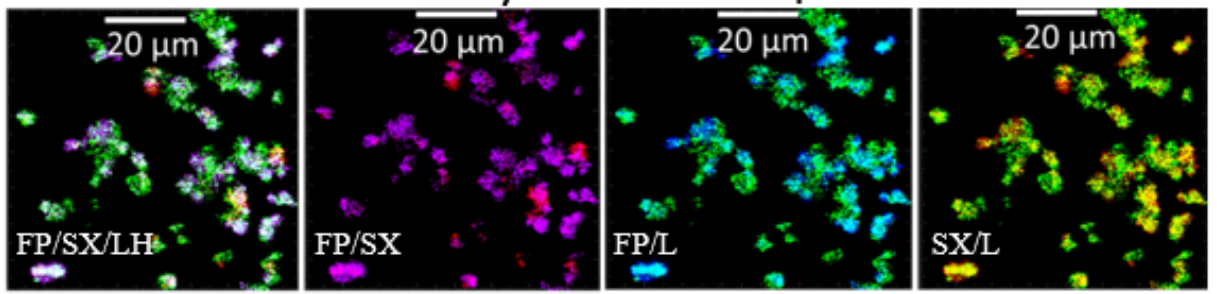


E) Single Wavenumber Maps

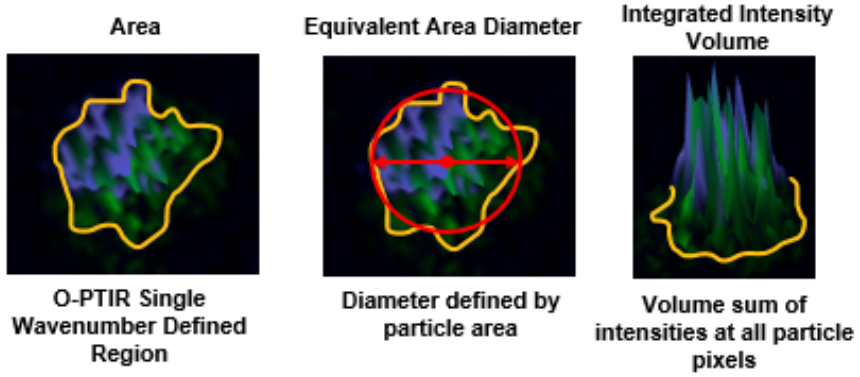
F) Summed Wavenumber Map



G) Colocalization Maps



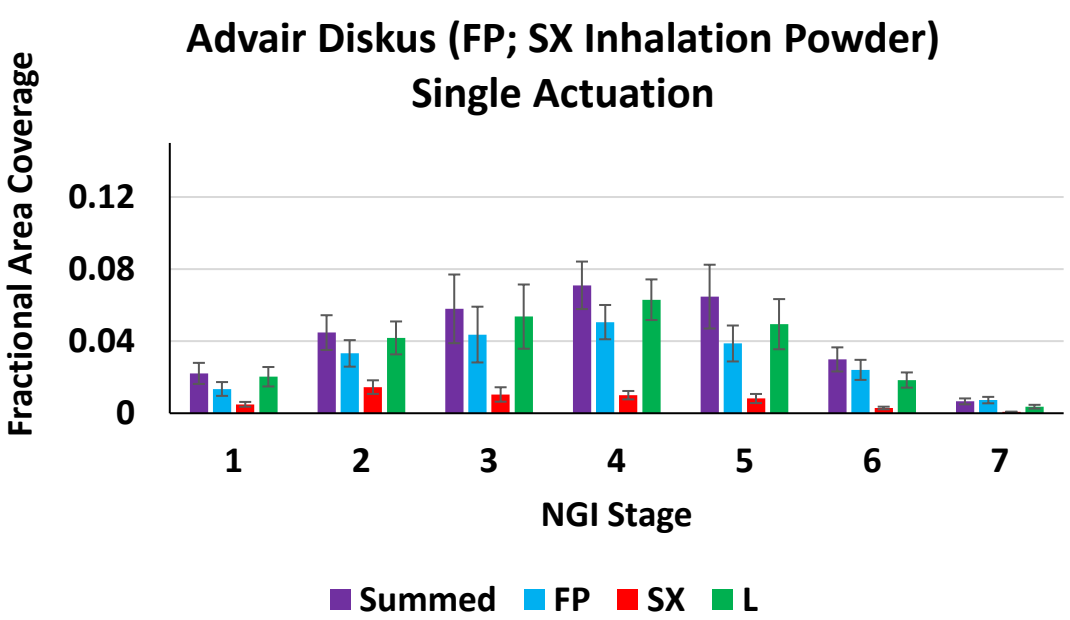
H) Key Morphological Parameters

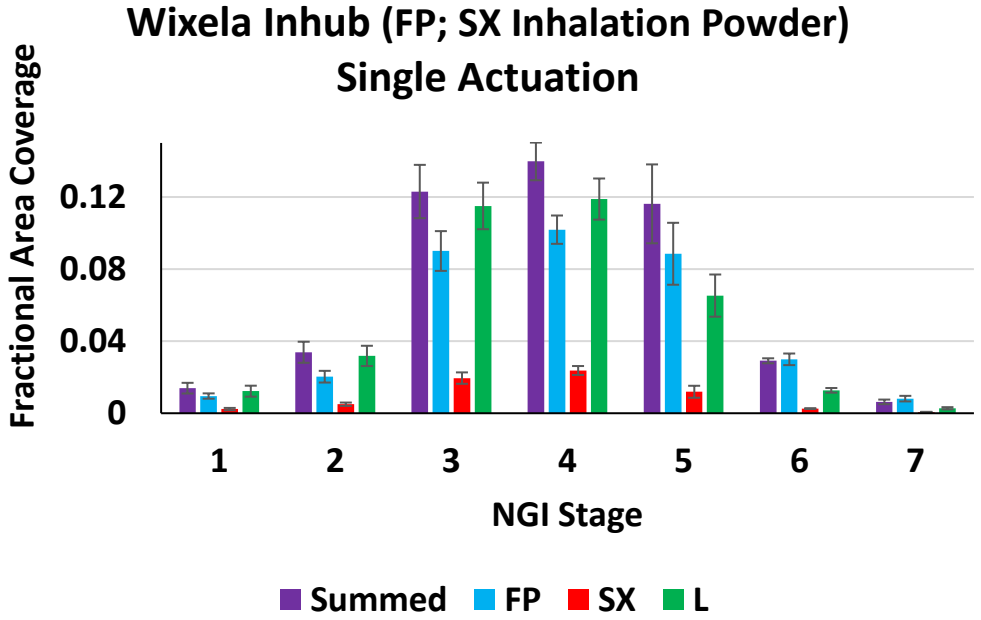


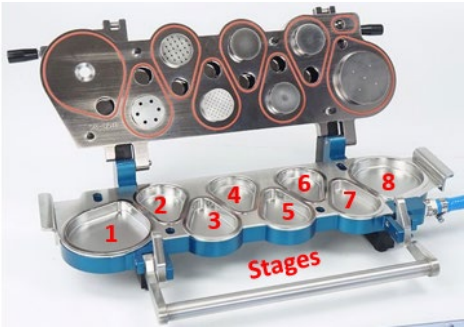
FP: fluticasone propionate; SX: salmeterol xinafoate; LH: lactose monohydrate

Fractional Area Coverage of Particles on Calcium Fluoride (CaF_2) Surface

Strength: 0.1 mg/inh; EQ 0.05 mg base/inh (FP; SX)





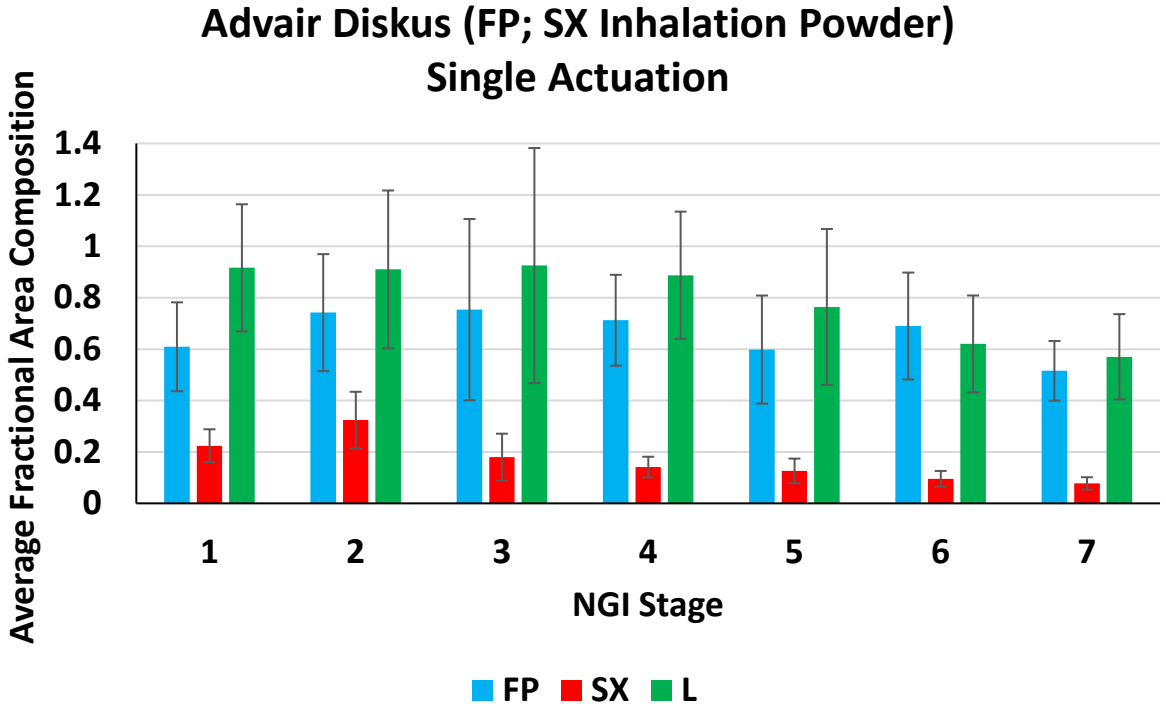


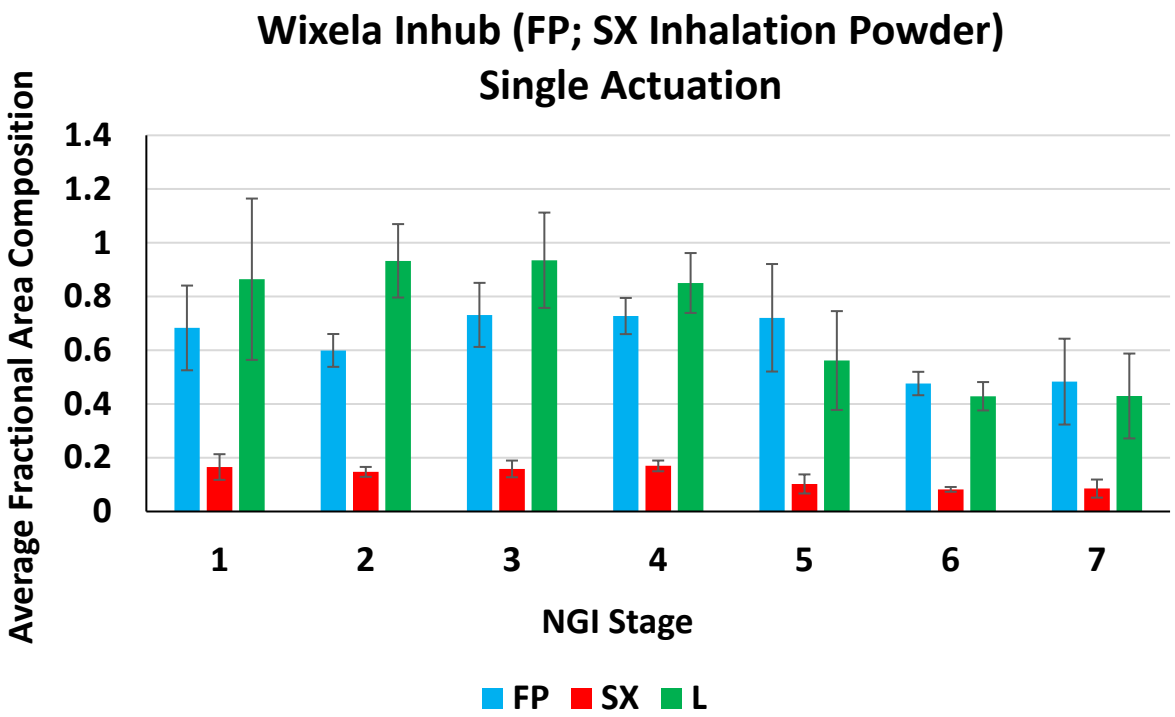
FP; SX inhalation powders, Advair Diskus and Wixela Inhub, at 0.1 mg/inh; EQ 0.05 mg base/inh strength show similar total material deposited on the CaF_2 surface as measured by total surface area of material

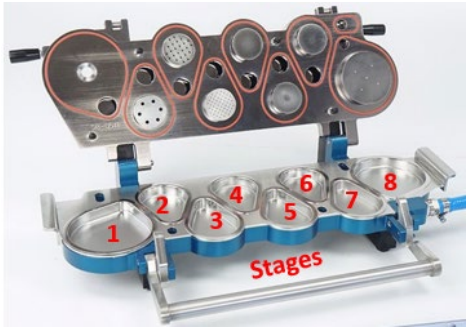
FP: fluticasone propionate; SX: salmeterol xinafoate; L: lactose monohydrate

Average Fractional Area Composition of Particles on CaF_2 Surface

Strength: 0.1 mg/inh; EQ 0.05 mg base/inh (FP; SX)





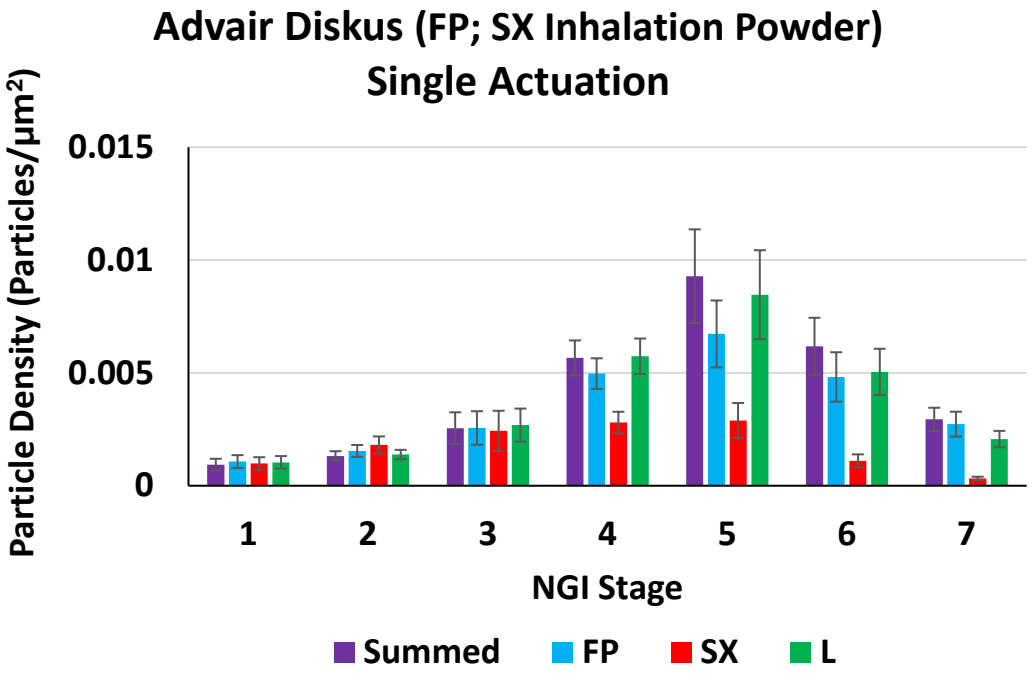


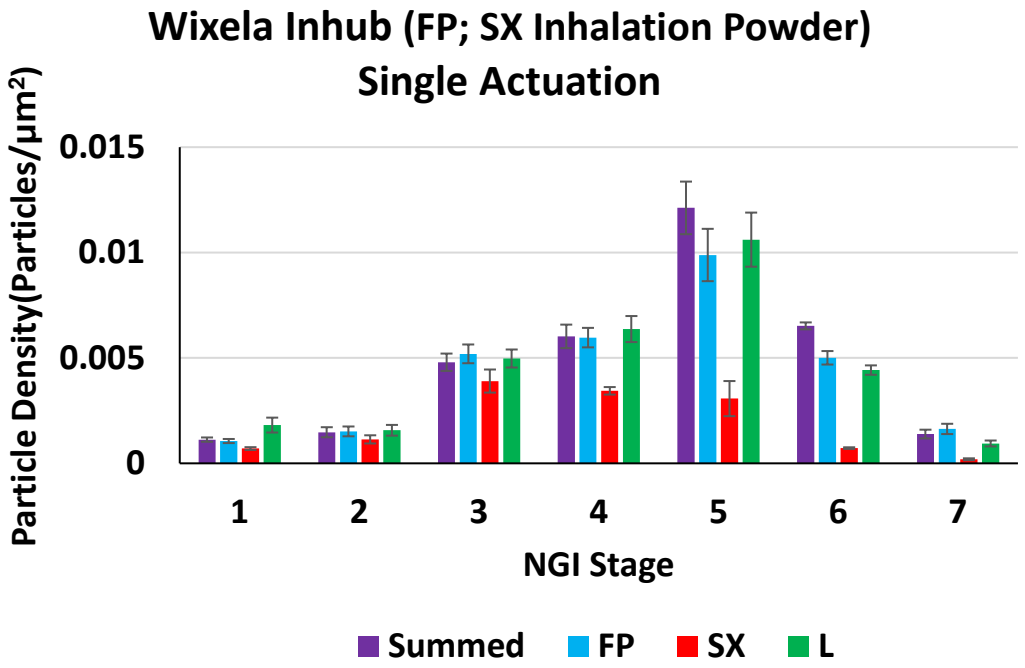
FP; SX inhalation powders, Advair Diskus and Wixela Inhub, at 0.1 mg/inh; EQ 0.05 mg base/inh strength are quite similar in fractional composition of the particles for both Fluticasone and Lactose

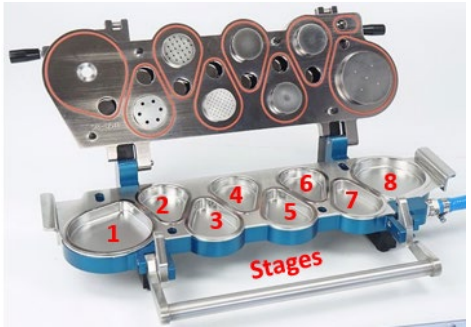
FP: fluticasone propionate; SX: salmeterol xinafoate; L: lactose monohydrate

Density of Particles on CaF_2 Surface

Strength: 0.1 mg/inh; EQ 0.05 mg base/inh (FP; SX)



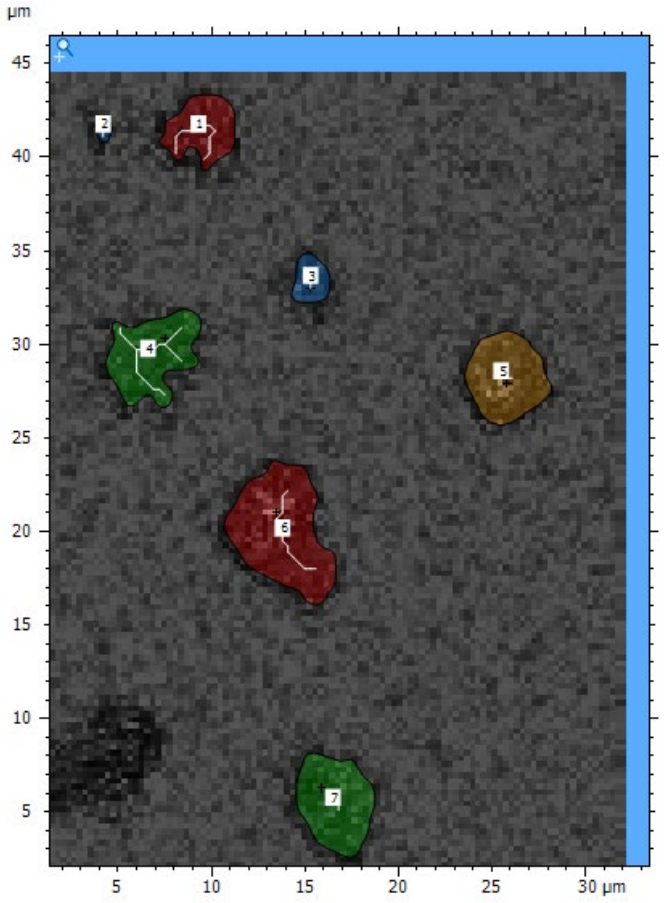




FP; SX inhalation powders, Advair Diskus and Wixela Inhub, at 0.1 mg/inh; EQ 0.05 mg base/inh strength show similar trend in particle density as a function of NGI stage

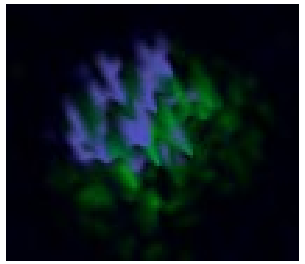
FP: fluticasone propionate; SX: salmeterol xinafoate; L: lactose monohydrate

Definitions of Particle Area and Diameter



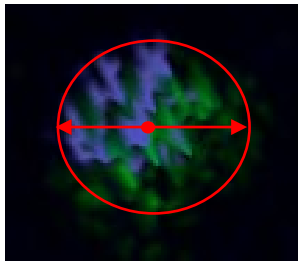
Area and Diameter Analysis for 7 Particle Case (1662 cm^{-1} image of Advair Diskus [fluticasone propionate; salmeterol xinafoate inhalation powder])

Spectroscopic Area



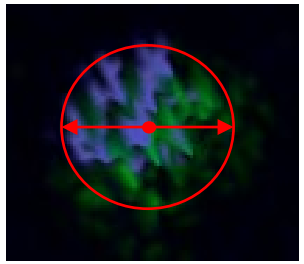
O-PTIR Single Wavenumber Defined Region

Spectroscopic Equivalent Area Diameter



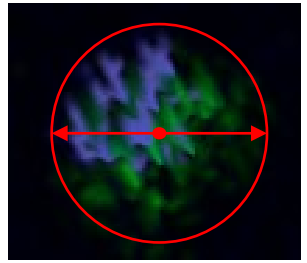
Diameter circle of equiv. area of particle

Spectroscopic Mean Diameter



Diameter defined by particle center of gravity

Spectroscopic Maximum Diameter



Diameter defined by longest rotation axis.

Individual results

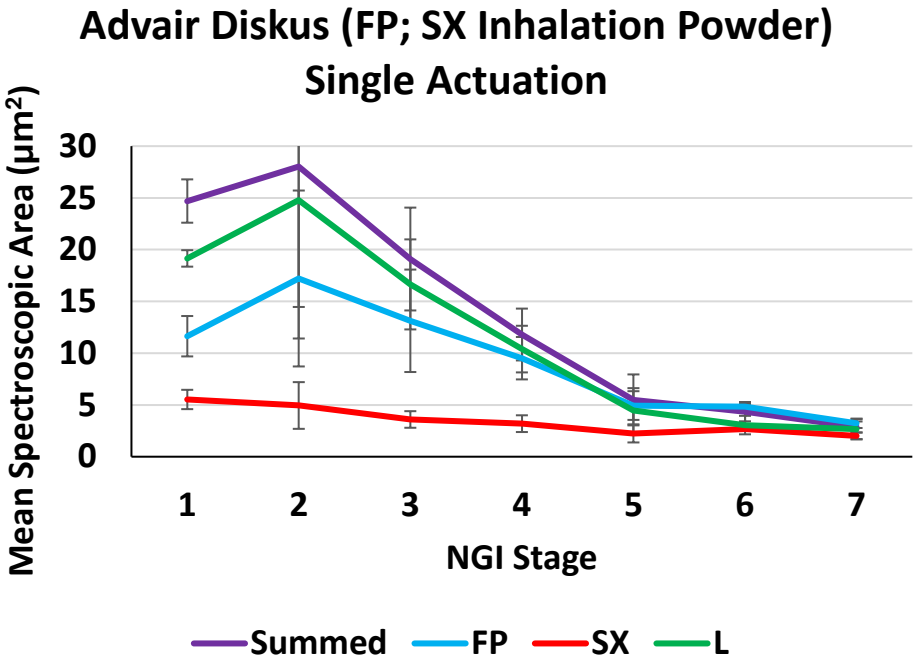
Parameters	Area	Equivalent diameter	Mean diameter	Maximum diameter
Unit	µm²	µm	µm	µm
Particle #1	10.78	3.705	3.525	4.216
Particle #2	0.8480	1.039	0.8963	1.315
Particle #3	4.150	2.299	2.157	2.645
Particle #4	17.30	4.694	4.502	5.787
Particle #5	16.41	4.571	4.404	5.023
Particle #6	27.92	5.962	5.739	7.568
Particle #7	17.13	4.670	4.503	5.859

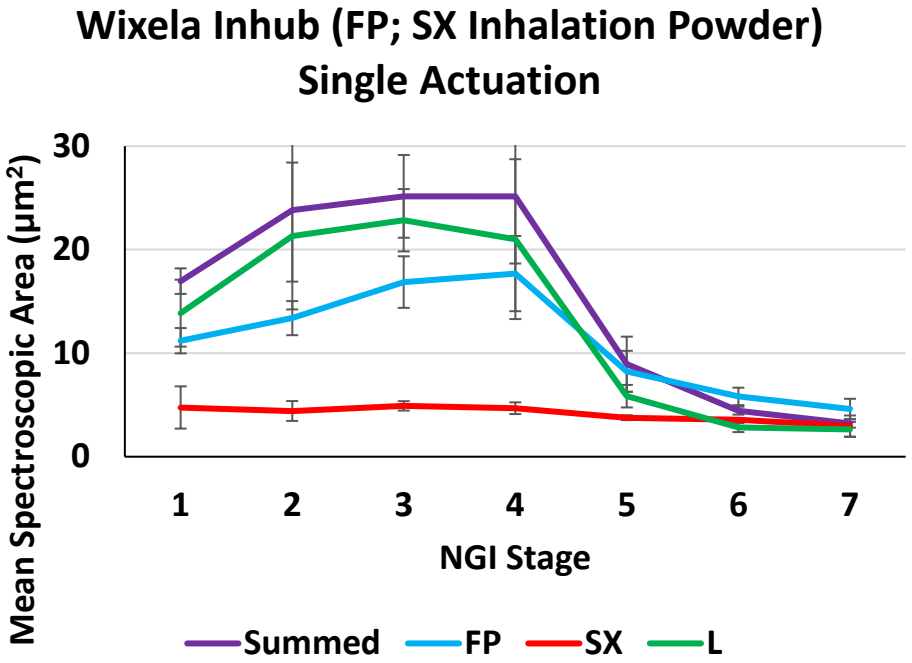
Global statistics

Mean	13.51	3.849	3.675	4.630

Mean Particle Area as Function of NGI Stage

Strength: 0.1 mg/inh; EQ 0.05 mg base/inh (FP; SX)

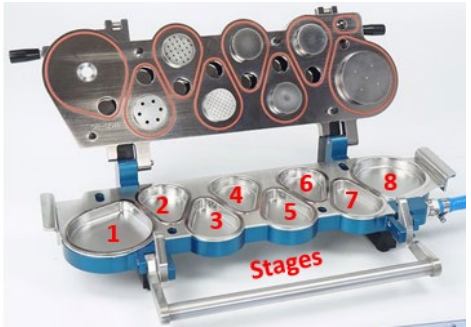


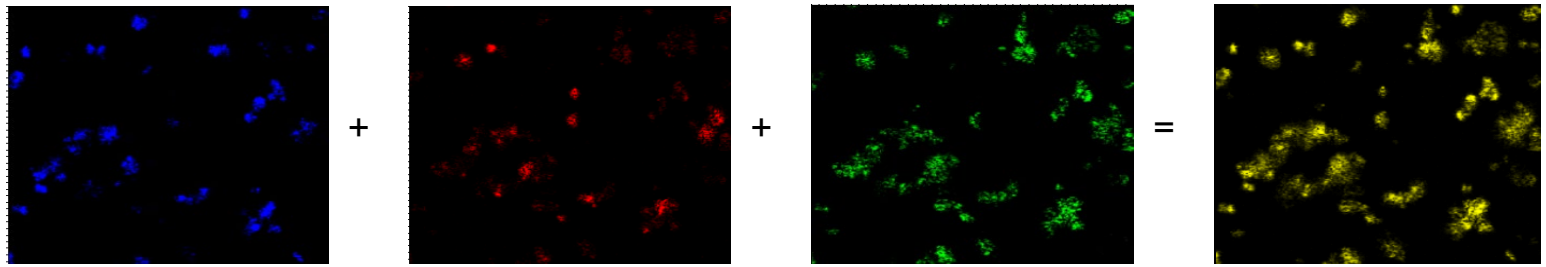


Aggregate particle size decreases with NGI Stage

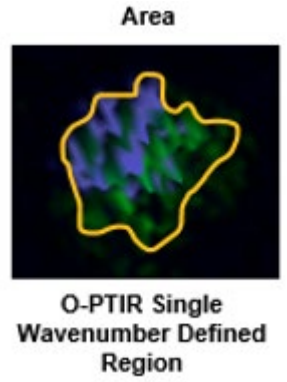
Fluticasone and Lactose also decrease with NGI Stage

Salmeterol particle size constant!





FP SX L Summed

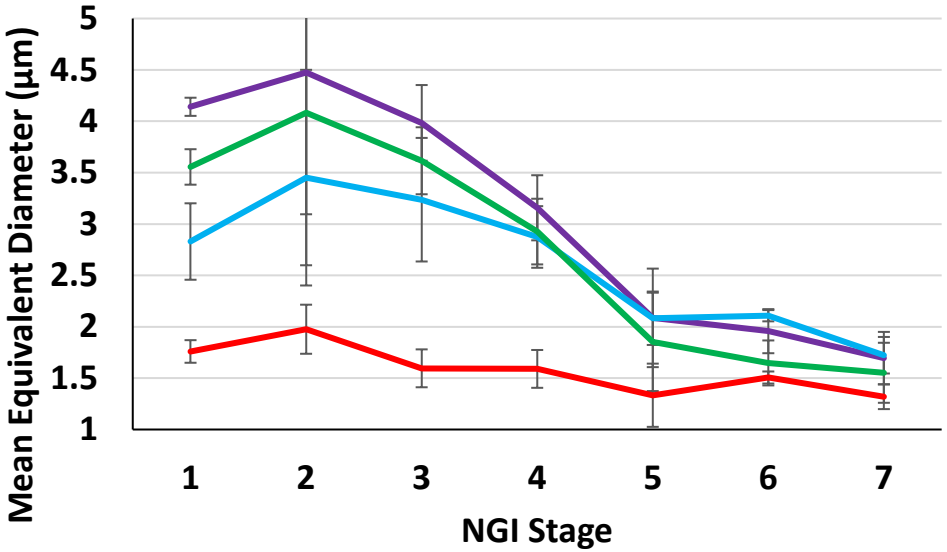


FP: fluticasone propionate; SX: salmeterol xinafoate; L: lactose monohydrate

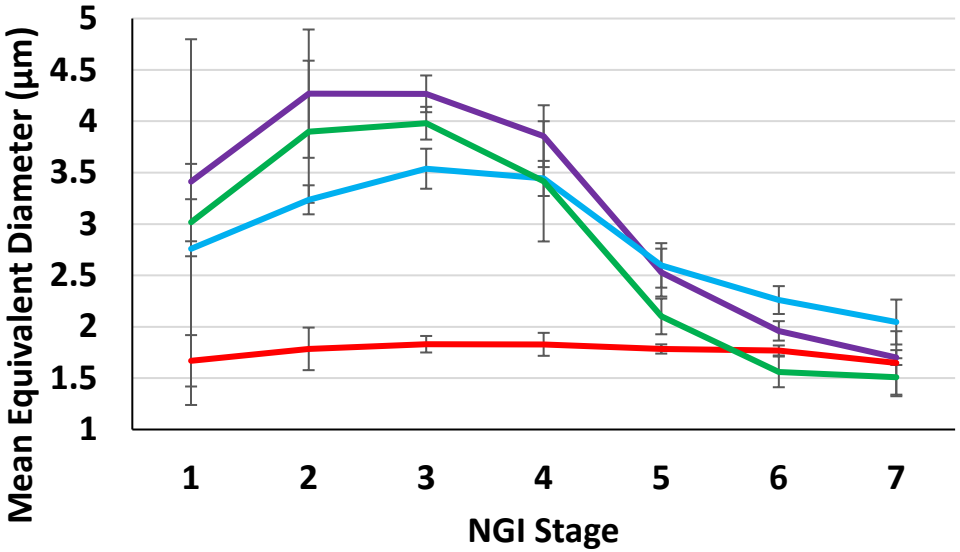
Mean Particle Equivalent Area Diameter as Function of NGI Stage

Strength: 0.1 mg/inh; EQ 0.05 mg base/inh (FP; SX)

Advair Diskus (FP; SX Inhalation Powder)
Single Actuation



Wixela Inhub (FP; SX Inhalation Powder)
Single Actuation

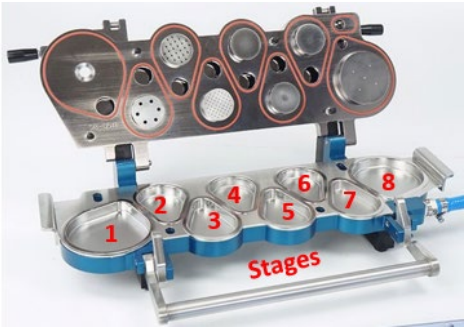


Aggregate particle size decreases with NGI Stage

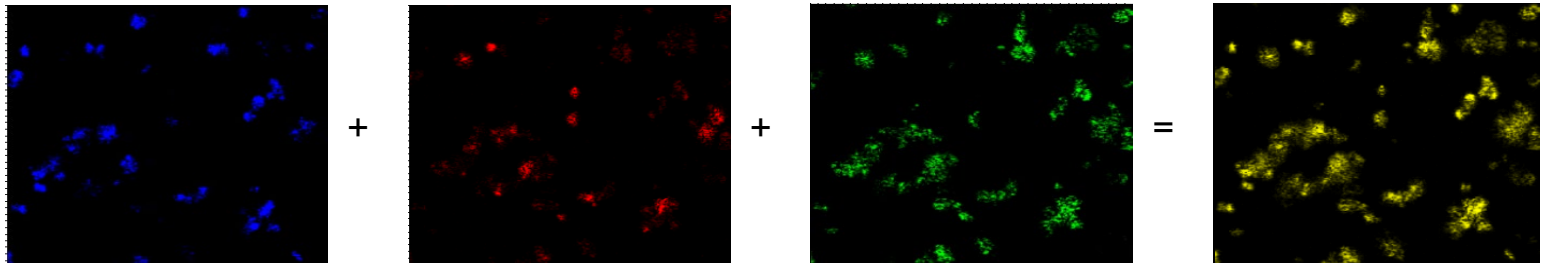
Fluticasone and Lactose also decrease with NGI Stage

Salmeterol particle diameter is constant!

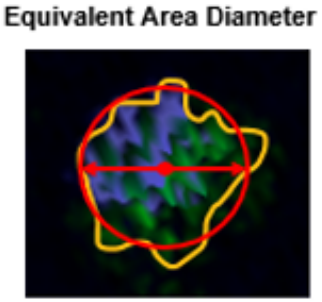
Summed FP SX L



Summed FP SX L



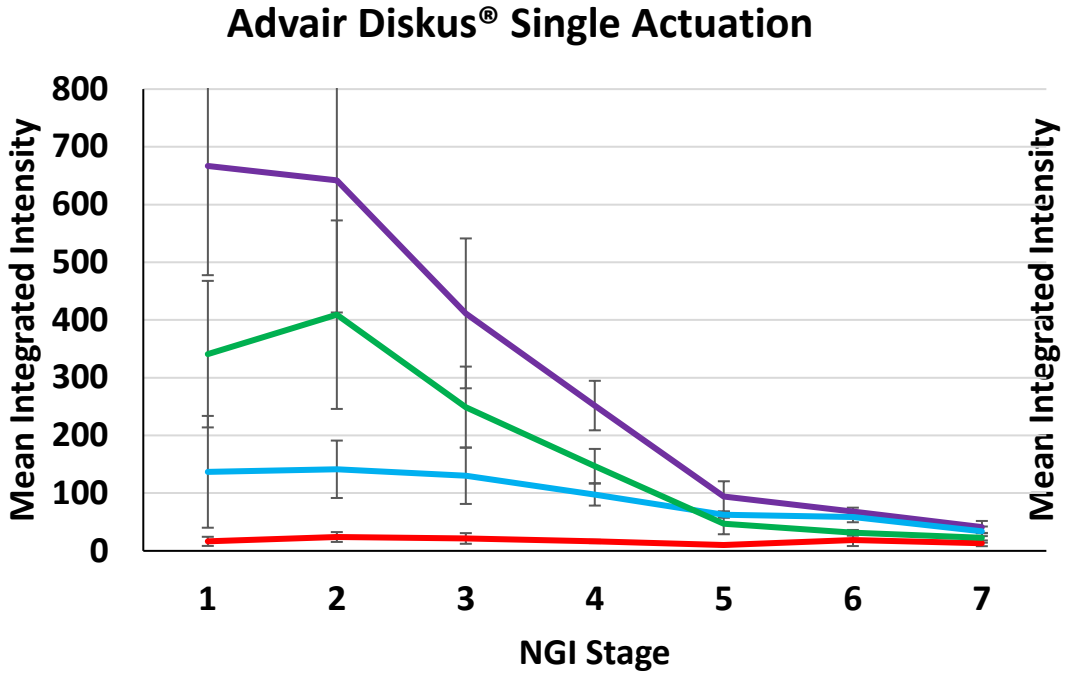
FP + SX + L = Summed

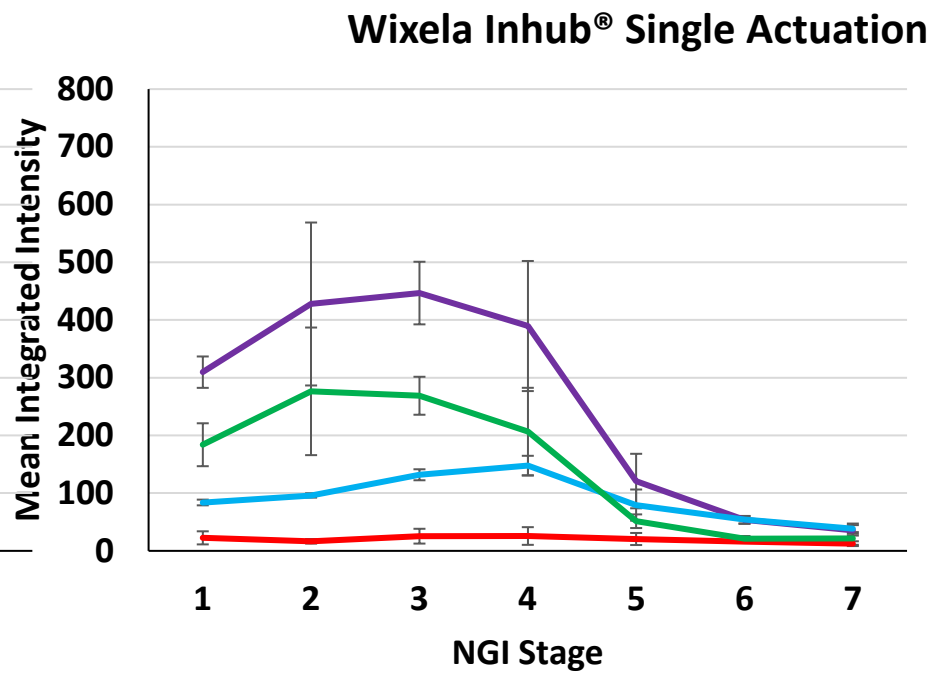


FP: fluticasone propionate; SX: salmeterol xinafoate; L: lactose monohydrate

Mean Integrated Intensity as Function of NGI Stage

Strength: 0.1 mg/inh; EQ 0.05 mg base/inh (FP; SX)

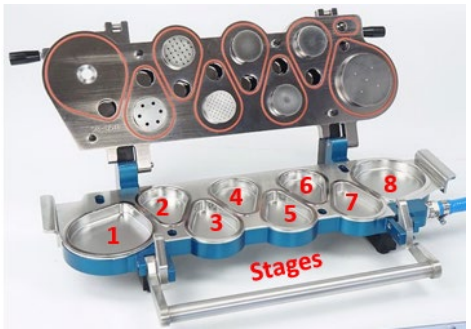


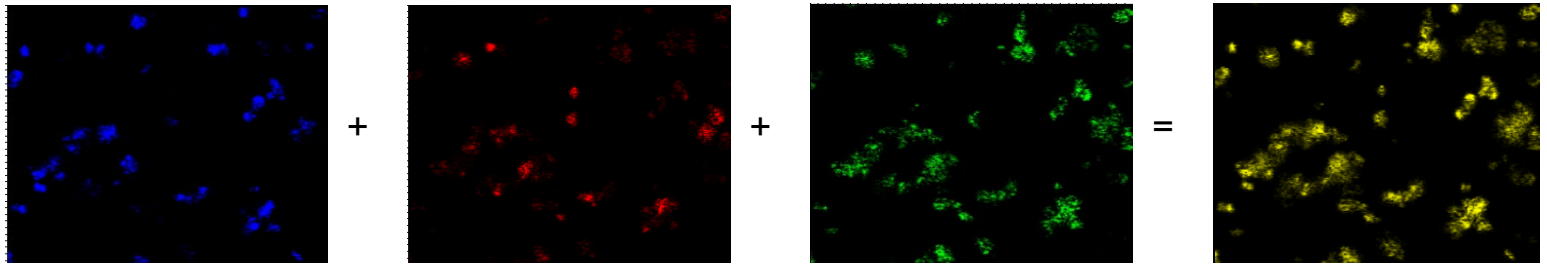


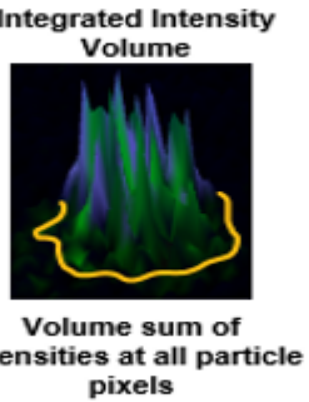
Aggregate particle volume decreases with NGI Stage. AD volume appears larger than WI for Stages 1-3.

Lactose and Fluticasone volume decrease with NGI stage

Salmeterol particle volume is constant!





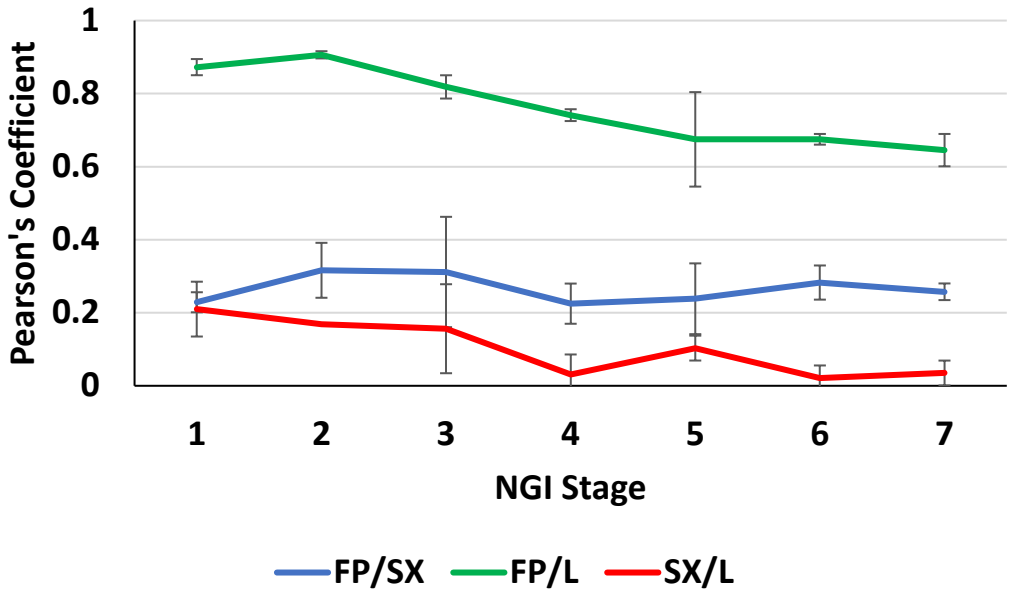


FP: fluticasone propionate; SX: salmeterol xinafoate; L: lactose monohydrate

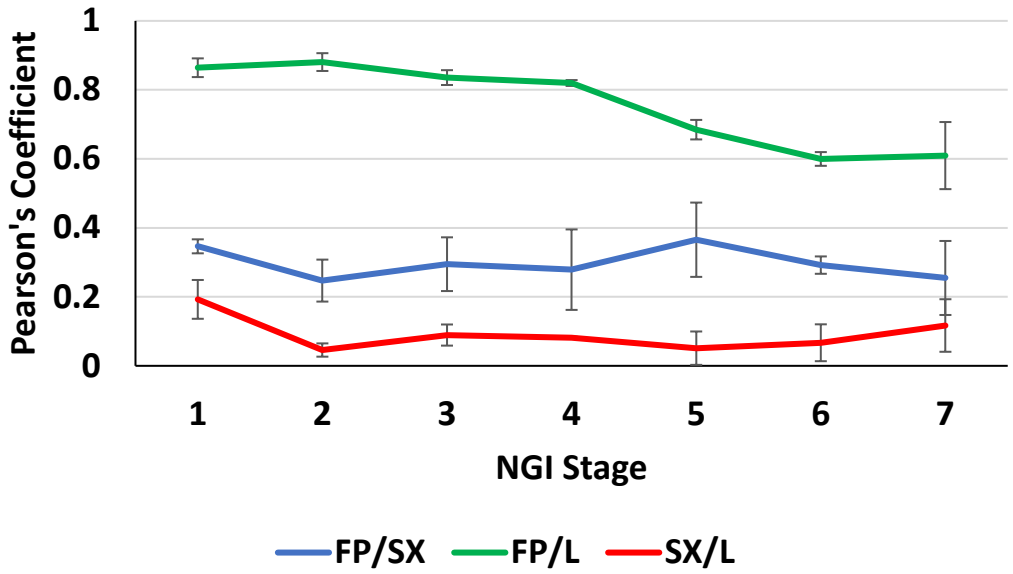
Pearson Coefficient Colocalization Analysis of FP; SX Inhalation Powders, Advair Diskus and Wixela Inhub as function of NGI stages 1-7

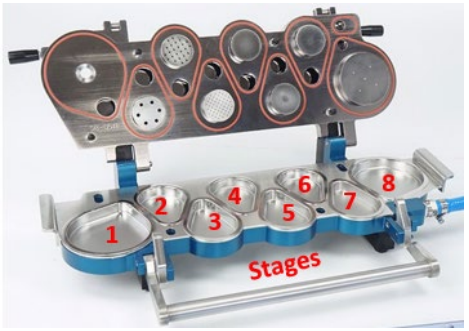
Strength: 0.1 mg/inh; EQ 0.05 mg base/inh (FP; SX)

Advair Diskus (FP; SX Inhalation Powder) Single Actuation



Wixela Inhub (FP; SX Inhalation Powder) Single Acuation





The Pearson correlation coefficient indicates the **strength and direction** of a linear relationship between two continuous variables. +1 Positive Linear Correlation, -1 negative linear correlation, 0 no correlation

- FP and L exhibit a strong, positive correlation for both formulations
- FP and SX exhibit a weak, positive correlation for both formulations
- There is little correlation of SX and L

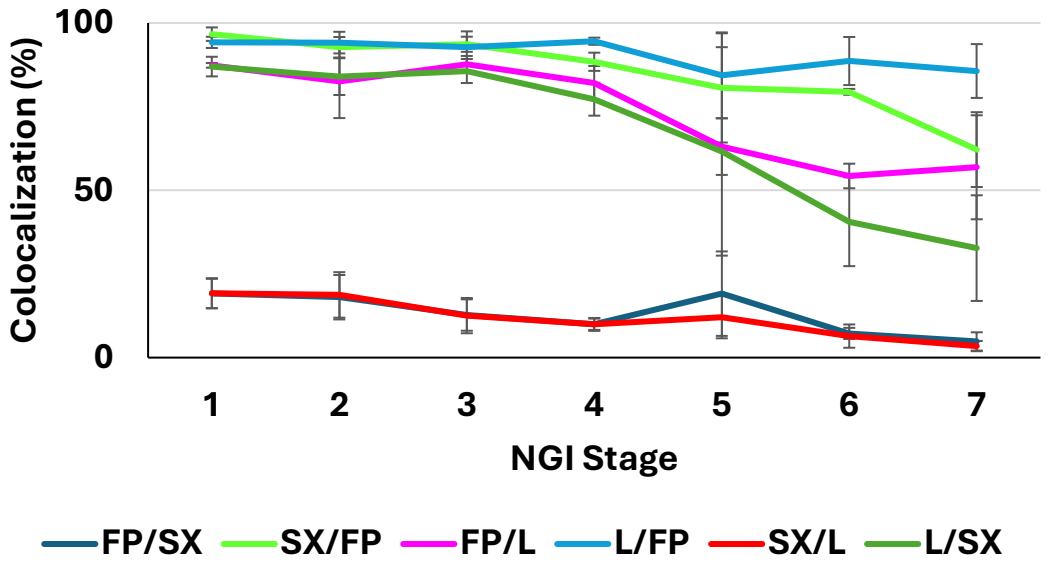
FP; SX inhalation powders, Advair Diskus and Wixela Inhub, show similar drug/drug and drug/excipient colocalization properties

FP: fluticasone propionate; SX: salmeterol xinafoate; L: lactose monohydrate

Manders Coefficient Colocalization Analysis of FP; SX Inhalation Powders, Advair Diskus and Wixela Inhub as function of NGI stages 1-7

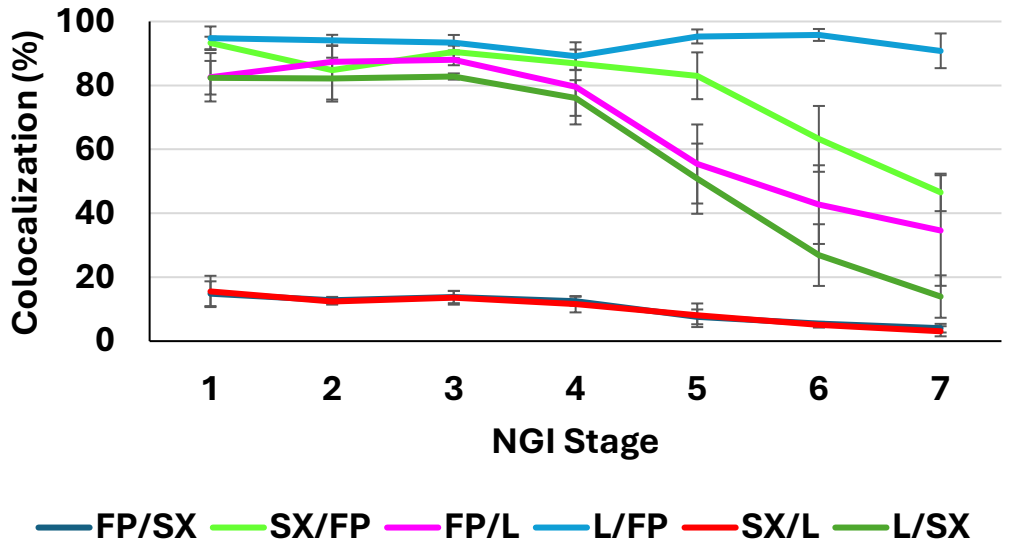
Strength: 0.1 mg/inh; EQ 0.05 mg base/inh (FP; SX)

Advair Diskus (FP; SX Inhalation Powder) Single Actuation



FP/SX SX/FP FP/L L/FP SX/L L/SX

Wixela Inhub (FP; SX Inhalation Powder) Single Actuation

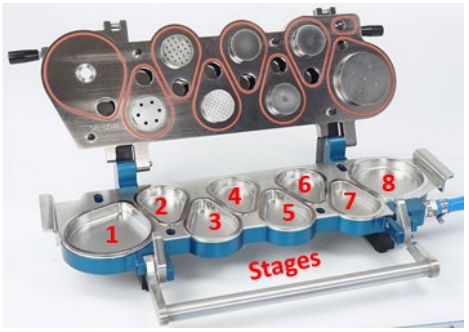


FP/SX SX/FP FP/L L/FP SX/L L/SX

The Manders correlation coefficient indicates the degree of spatial colocalization between a pair of Infrared signals. A value of 1 indicates complete colocalization and a value of 0 indicates no colocalization.

- L is highly spatially localized with FP
- FP is highly spatially localized with L for larger particles and less so for smaller particles
- SX is highly spatially localized with FP
- FP is not spatially localized with SX (there is far more FP in formulation)
- SX is not spatially localized with L

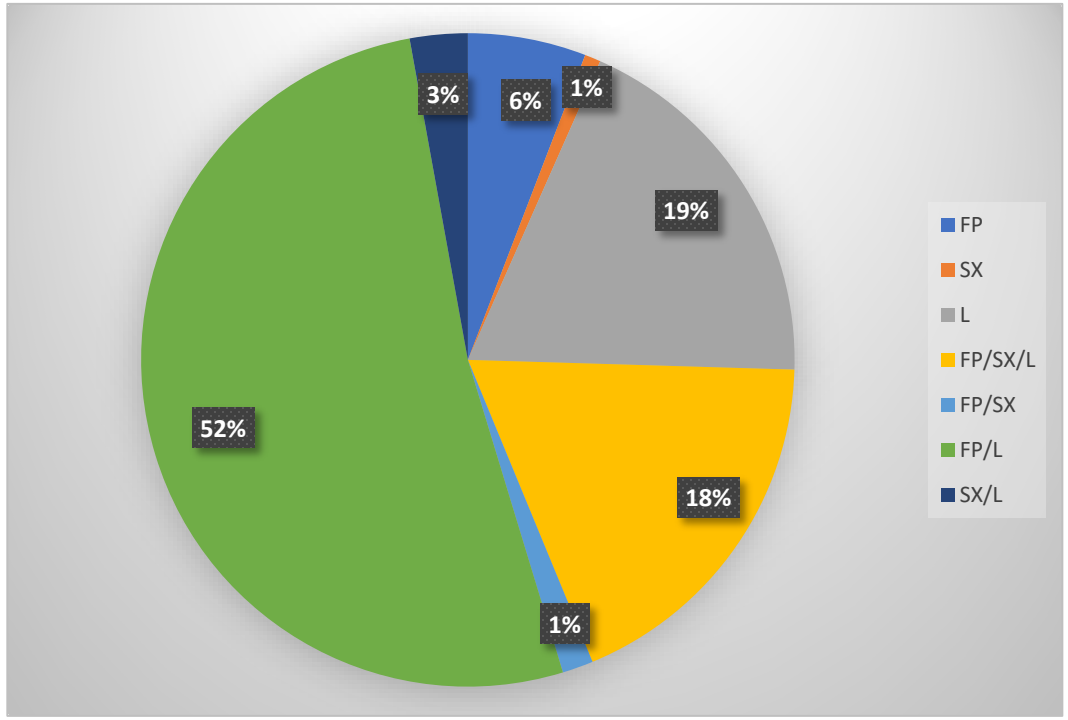
FP; SX inhalation powders, Advair Diskus and Wixela Inhub (0.1 mg/inh; EQ 0.05 mg base/inh), show similar drug/drug and drug/excipient spatial colocalization properties



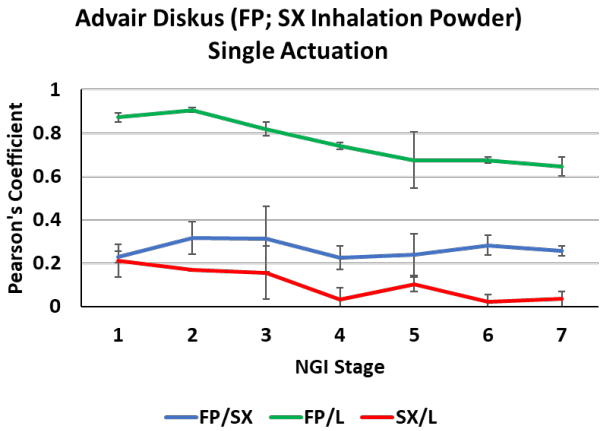
FP: fluticasone propionate; SX: salmeterol xinafoate; L: lactose monohydrate

MDRS and O-PTIR Comparison: NGI Stage 2

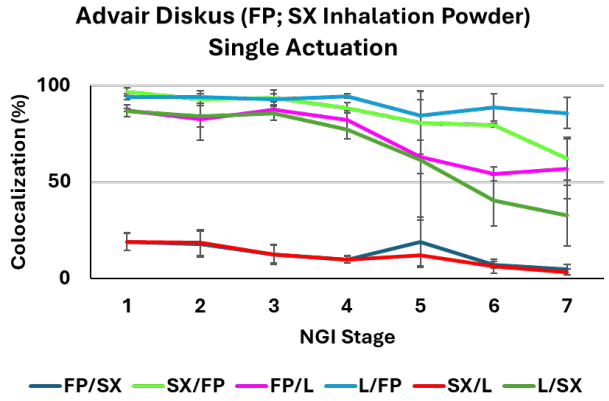
Single Actuation, NGI Stage 2



Pearson Coefficient



Manders Coefficient



Consistency:

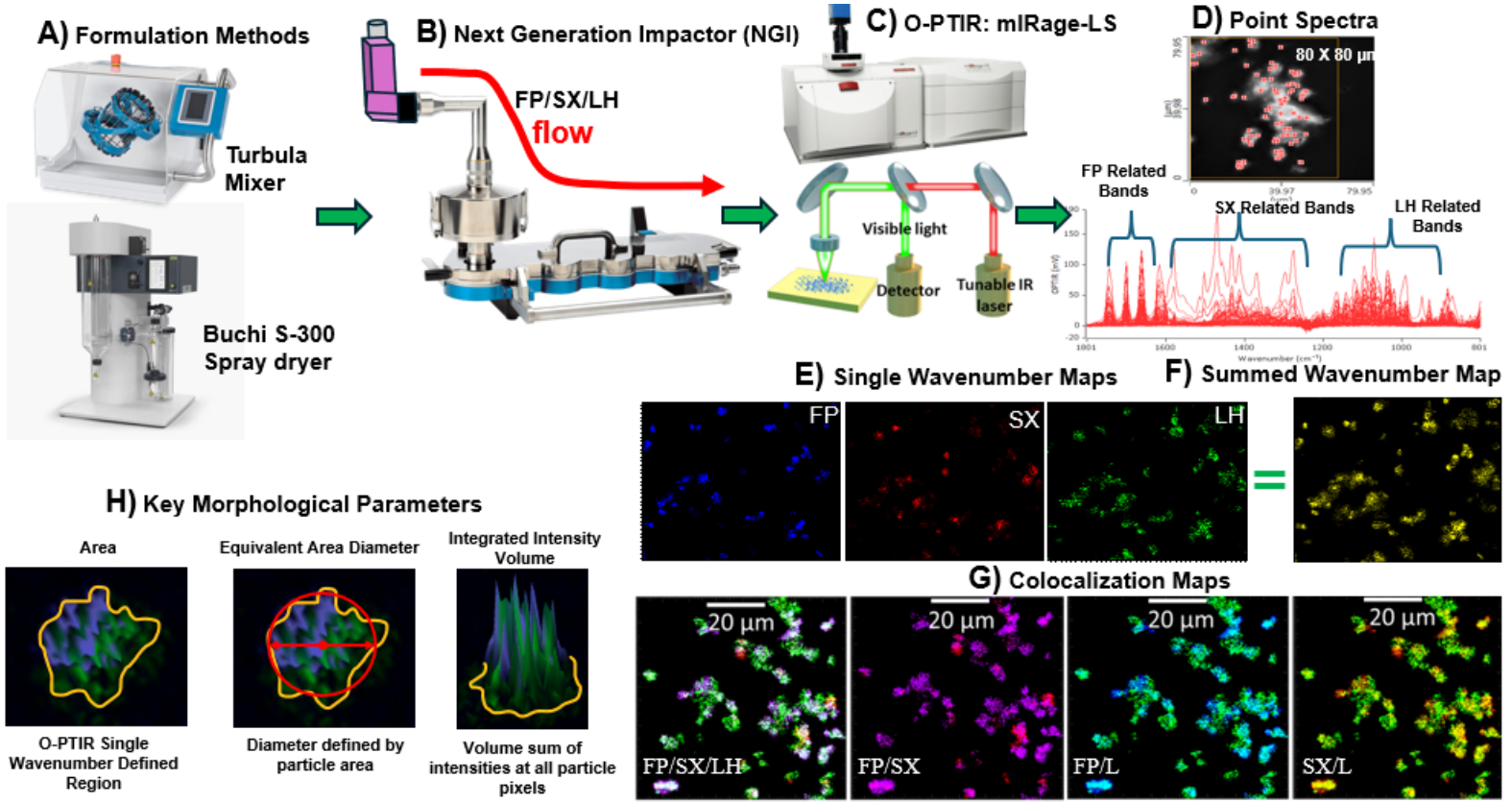
FP/L as major component of aggregates
SX/L as minor component of aggregates
FP/SX as minor component of aggregates

Note that O-PTIR also indicates that SX most often accompanied by FP consistent with Mass Spectroscopy reports

FP: fluticasone propionate; SX: salmeterol xinafoate; L: lactose monohydrate

Farias G, Ganley WJ, Price R, Conti DS, Mangal S, Bielski E, Newman B, Shur J., *Pharmaceutical Research*. **2024** Oct;41(10):2015-29.

O-PTIR with Quantitative Morphology Analysis



- Quantitative evaluation of area and diameter at the particle level for every component and the aggregate particles
- Quantitative evaluation of component colocalization
- Detailed morphological comparison of Brand Name and Generic Formulations

All for a Single Inhaler Actuation and Measured on Lung Relevant Emitted Dose