Development of an Image Analysis Algorithm to Evaluate Dissolution of Respirable Particles

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Introduction

- **Airway mucus** protects the lung tissue and serves as a barrier in the transport of drugs to the underlying epithelium[1,2].
- **While dissolution** acts as a **rate limiting step** in the pulmonary bioavailability of poorly water-soluble inhaled drug products, the impact of mucus diffusion rate on bioavailability is less understood.

Objective

- **Develop image analysis method** for dissolution rate of respirable particles in airway fluids, following substrate deposition.
- Coupled approach with published and commercialized methods for dissolution, utilizing flow-through cells and inverted microscopes.

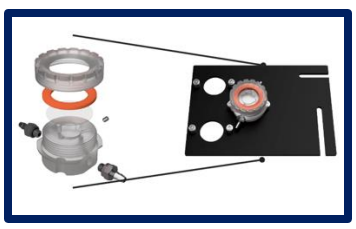
Methods

▼ Fig. 1: Aerosol particles deposited on a glass slide using a laser diffractor inverted into a mucus membrane in a custom-flow through cell to image & measure its rate of immersion & dissolution

A) Laser Diffractor for Dispersion

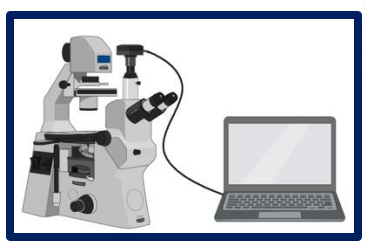
1) Modular adapters for existing lab equipment
2) Preliminary data indicates size separation capabilities of the plume
3) Simultaneous measurement of aerosol particle size distribution (q_0 & q_3)

B) Custom flow-through cell



1) Developing biorelevant artificial mucus model that reduce dependency on *ex vivo* samples
2) Incorporates existing equipment found in an aerosol lab

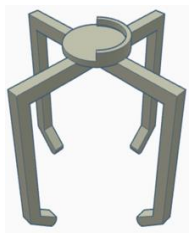
C) Microscope Image Analysis



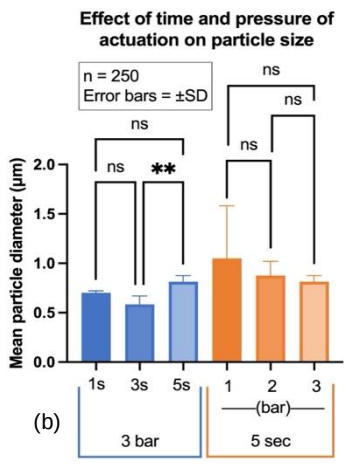
1) Surface-level insights of drug immersion through image analysis
2) Cost-effective and easy to set-up

Results

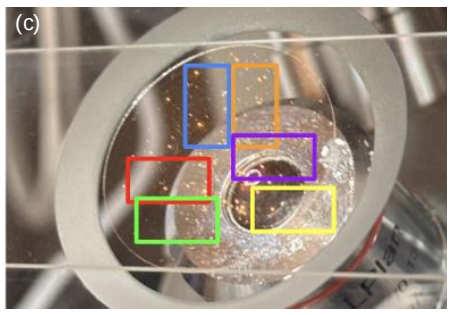
Aerosolization and Dispersion of Microparticles

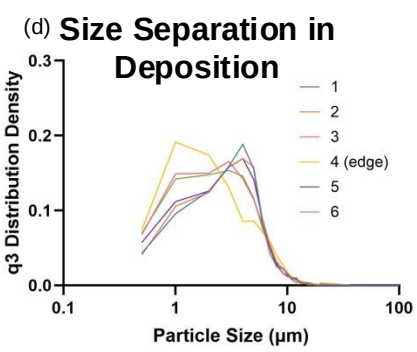


(a)



(b)

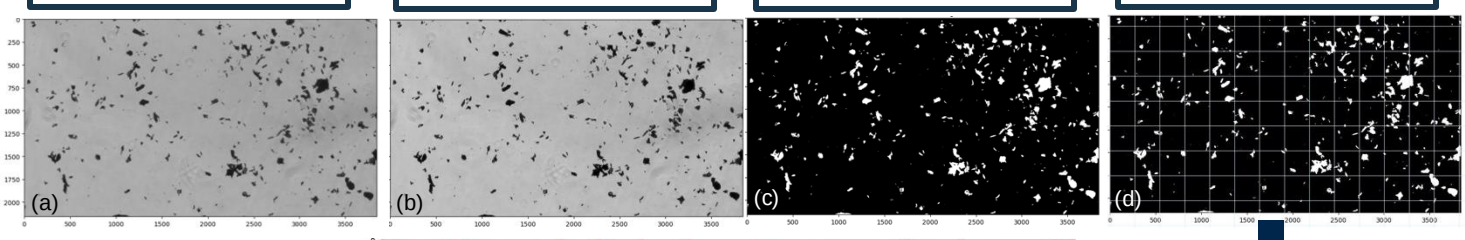


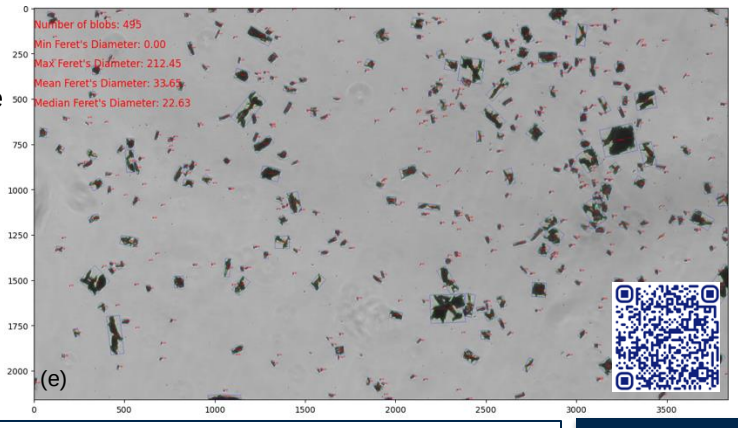


▲ Fig. 2: Particle dispersion conditions: a) 3-D printed attachments for vacuum & dispenser, b) Effect of time & pressure of actuation on size of particle deposited on the glass slide, c) & d) Post-actuation particle size separation once deposited on glass slide

Designed-for-purpose Image Analysis Algorithm

Original Image via Microscope Camera → Grayscale Conversion & Gaussian Blur → Image Binarization & Morphological Transformation → Contouring and Detection of Edges on 'Blobs'

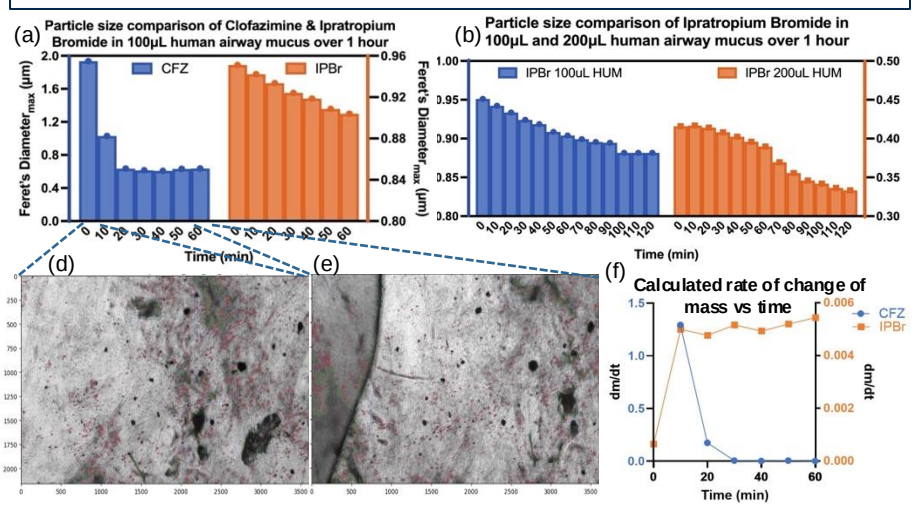




▲ Fig. 3: Image processing for particle detection: a) Original Image, b) Grayscale & blurring, c) Binarizing and morphologically transformed image, d) Contouring & detecting particles, e) Edge (green), fitted rectangles (blue) & analyzed statistical parameters eg. Feret's diameter (red), overlayed on the original image

Final Overlayed Image depicting:
1. Number of edge-detected particles
2. Fitted rectangle
3. Calculated Feret's diameter

Rate of Drug Immersion & Dissolution



▲Fig. 4: a) Comparing particle size changes of model drugs Clofazimine (CFZ) & Ipratropium Bromide (IPBr) over 1h in 100µL of HUM b) Particle size changes of IPBr over 2h in 100µL & 200µL of HUM, c) & d) Rep. microscope images analyzed at t=0 and 60min for CFZ, e) Indirect release rate of CFZ & IPBr calculated from rate of particle disappearance from image data, f) Particle size & surface energy values of CFZ & IPBr

(g)

Particle Parameter	CFZ	IPBr
Size: x50 (µm)	0.8	0.8
Size: x90 (µm)	1.57	1.59
S. Energy (mJ/m²)	44.1	40.94

Conclusion

1) The geometry of the particle plume created by our 3-D printed attachment to the disperser can cause size-dependent distribution. (Fig. 2)

2) The results for IPBr may indicate surface dissolution followed by immersion, while for CFZ, particle immersion occurs first followed by disaggregation or fragmentation which could be explained by their surface energy difference (Fig. 4)

References

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