PMI SCIENCE
PHILIP MORRIS INTERNATIONAL

ORIGINAL PAPERS

bit.ly/PMI-VC1 bit.ly/PMI-VC2 bit.ly/PMI-VC3
bit.ly/PMI-VC4 bit.ly/PMI-VC5 bit.ly/PMI-VC6
bit.ly/PMI-VC7

aerosol exposure system · air–liquid interface · computer fluid dynamics · deposition efficiency
deposition uniformity · online monitoring

Characterization of aerosol delivery in the Vitrocell® 24/48 aerosol exposure system

- An online method for characterizing the delivery of aerosols containing liquid particles in the Vitrocell® 24/48 system.
- A robust engineering model that can be applied to various exposure well chamber geometries and conditions without the need for fitting parameters.

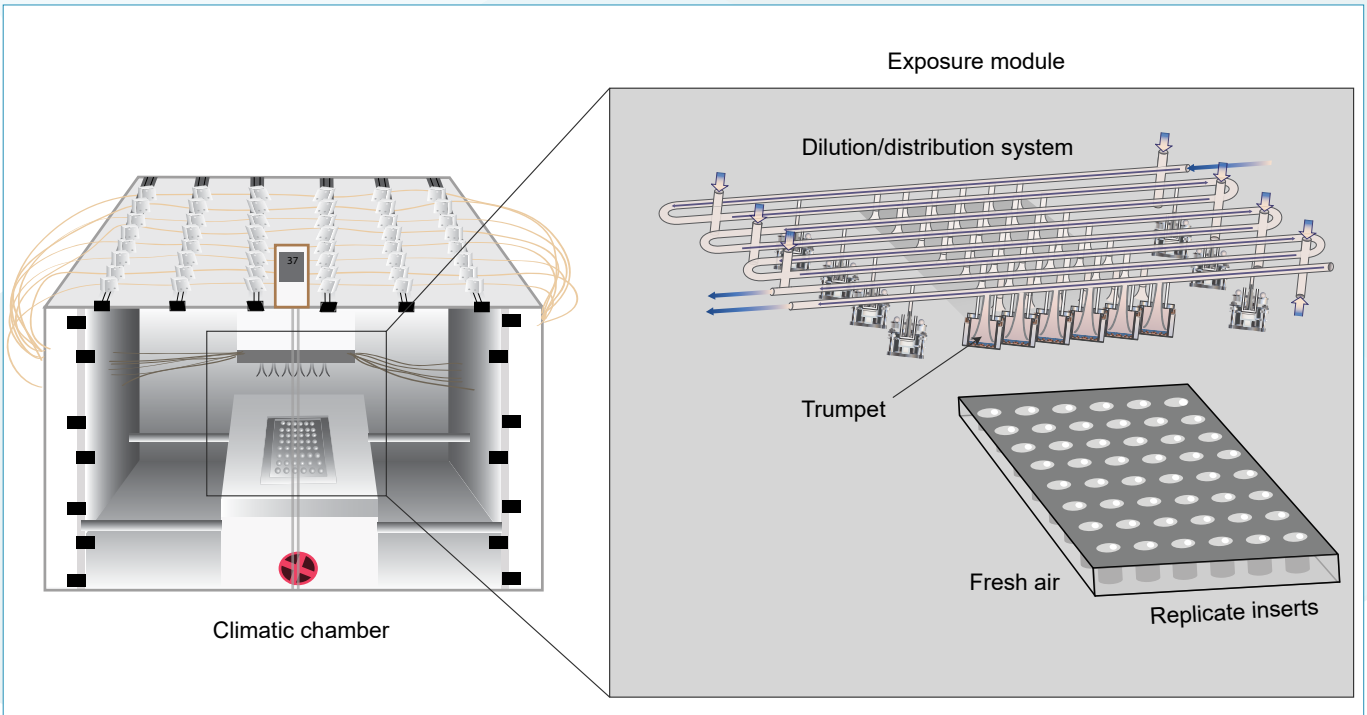

Figure 1 Schematic representation of the Vitrocell® 24/48 exposure system.

Chemical and physical characterization of transported, evolving aerosols in an in vitro system is complex. The challenges include appropriate sampling, measurement capabilities, and online measurement of constituents in flowing aerosol during exposure.

Tissue cultures grown on porous membranes allow exposure to aerosol at the air–liquid interface, providing a more realistic exposure method than traditional submerged *in vitro* cultures.

In vitro tissue culture studies have helped elucidate the potential adverse effects of individual components in cigarette smoke as well as those of smoke fractions and whole smoke. While testing of individual components and smoke fractions is well suited to submerged cell culture methods, testing of whole smoke is not feasible with this approach. Additionally, the effects on submerged cells are not representative of the effects in the respiratory tract, where cells are partly exposed to air. Tissue cultures grown on porous membranes allow exposure to whole smoke at the air–liquid interface (ALI), which provides a more realistic exposure method than traditional submerged in vitro cultures and has the potential to provide translational data.

High-throughput technologies for exposure of living cell cultures at the ALI are available and offer flexibility and efficiency. Vitrocell® 24/48 (Vitrocell Systems GmBH, Waldkirch, Germany) is a versatile exposure system that has a dilution and distribution system in a climactic chamber for up to eight different airflows (Fig. 1). Aerosols can be diluted by different airflows, and a fraction of each concentration can be simultaneously delivered to six replicate tissue cultures via delivery trumpets (Fig. 1; Fig. 3). However, the diluting air increases the volumetric flow rate, which affects velocity and aerosol sampling efficiency, thus necessitating controlled aerosol delivery. Evaporation, condensation, and coalescence can also change aerosol characteristics. Additionally, many of the tools developed for particle size-specific aerosol deposition have used solid particles, which are not fully representative of liquid aerosol characterization.

Because consistent, predictable aerosol dosimetry is crucial for understanding biological responses, robust characterization of aerosol delivery and deposition is necessary. Several studies have been performed to assess these characteristics for solid and liquid particles. Because physical characterization of aerosols with high concentrations of small-sized particles is very challenging, and there are limited experimental data to draw on for study design, the feasibility of computational fluid dynamics (CFD) for predicting deposition efficiency has also been assessed.

WHOLE SMOKE/AEROSOL ASSESSMENT

The Vitrocell® 24/48 system has been shown to be well suited for whole cigarette smoke exposure of cells growing at the ALI, on the basis of its overall performance in distributing smoke to inserts of an exposure model.1 A large set of delivery efficiencies that describe the conversion of an applied dose of cigarette smoke to a delivered dose during in vitro exposure has also been investigated.[2] These delivery efficiencies provide a simple dose metric tool that allows characterization and comparison of aerosol exposure experiments with respect to the composition of the aerosol presented to the biological system.

PARTICLE SIZE-SPECIFIC ASSESSMENT

Glycerol aerosols of different mean particle sizes and narrow size distributions were generated in a condensation monodisperse aerosol generator.[3] Detailed characterization confirmed their stability as well as the robustness and reproducibility of the generation process. Test exposure under relevant experimental settings in the Vitrocell® 24/48 aerosol exposure system further confirmed the feasibility of the system for simulating exposure and the high sensitivity of the method.

The efficiency and uniformity of deposition was measured experimentally in the Vitrocell® 24/48 system by using monodisperse solid fluorescent particles.[4] The experimental results were compared with predicted deposition and efficiency from Lagrangian and Eulerian CFD approaches. There was good agreement between the average experimentally measured and CFD-predicted results.

The physical characteristics of aerosols can be measured to determine the particle number density, mean mass particle size, and geometrical size distribution width, assuming log-normal distribution (Fig. 4A). The overall effective deposition efficiency was found to be influenced by particle size (Fig. 4B).[5]

SINGLE-PHOTON IONIZATION MASS SPECTROMETRY

When assessing exposure to liquid aerosols in the Vitrocell® 24/48 exposure system, it is necessary to consider the higher variability between delivery of the aerosols to individual exposure chambers in comparison with that observed in cigarette smoke exposure.[6]

A previous study used single-photon ionization mass

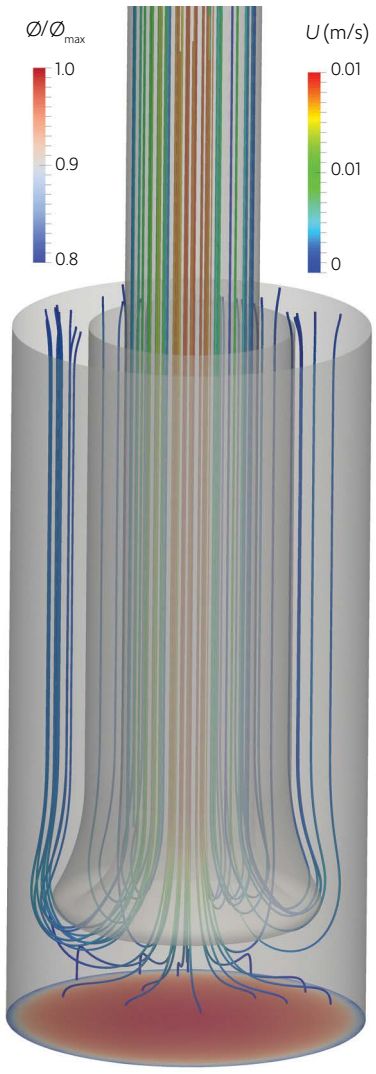

Figure 2 Vitrocell® 24/28 aerosol trumpet and exposure well chamber.

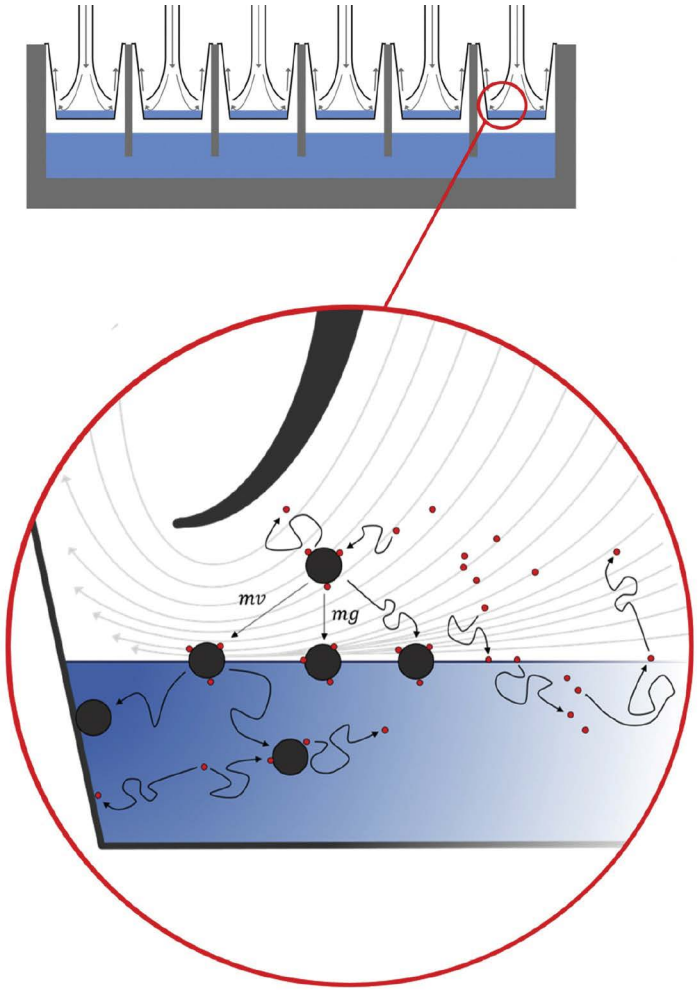

Figure 3 Processes by which aerosol constituents are transferred into an exposed liquid.

Red spheres represent (semi-) volatile aerosol constituents, and black spheres represent particles. The flow pattern in the exposure chamber is indicated by light gray, curved arrows. Particles deposit on the liquid surface by sedimentation (mg), inertial impact (mv), or diffusion (irregularly shaped arrows), and, depending on their hydrophilicity and density, they may subsequently enter the bulk liquid.

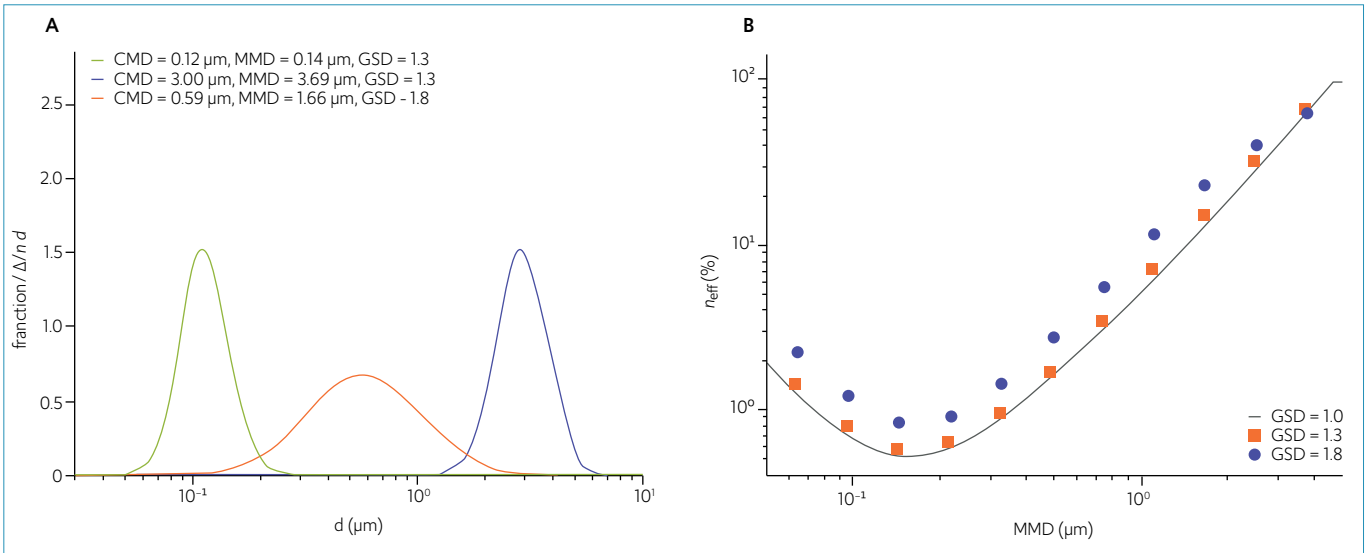

Figure 4 (A) Various log-normal size distributions; (B) Effective deposition efficiency calculated for varying mass median diameters at selected distribution widths.

CMD: count median diameters; **GSD:** geometric size distribution; **MMD:** mass median diameters.

Tobacco Harm Reduction

Cigarette smoke (CS) is the leading modifiable risk factor for many human diseases. Complete smoking cessation is the best approach to reduce the risks of smoking-related diseases. However, while the prevalence of cigarette smoking has been steadily declining over the years, millions of individuals across the globe continue to smoke. Smoking cessation has proven difficult for many smokers, who might benefit from using alternative products that have the potential to reduce the harm caused by CS.

For smokers who would otherwise continue smoking cigarettes, PMI's goal is to offer reduced-risk products (RRPs),* that have the potential to reduce the risk of developing smoking-related diseases as compared to continued smoking.

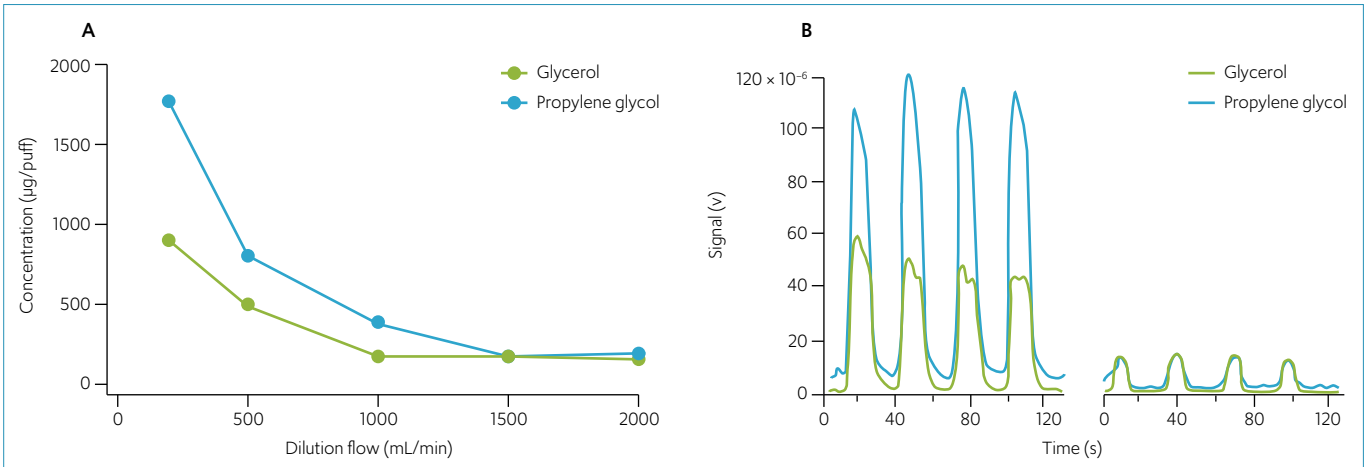

Figure 5 Comparison of glycerol and propylene glycol concentrations at different dilutions: (A) Concentrations decrease as a function of dilution; (B) Signal intensity for four puffs at 200 mL/min dilution (*left*) and 2 L/min (*right*).

spectrometry (SPI-MS) to measure the properties of the aerosol generated by the MESH e-cigarette.[7] After generation, the test aerosol was characterized in terms of aerodynamic particle size distribution and chemical composition. With a time resolution of one second, the SPI-MS measured the full spectrum of the aerosol, although the study focused on its main constituents: propylene glycol, glycerin, and nicotine. The sampling efficiency of the mass spectrometer was studied under three different dilution protocols to capture the evolving aerosol process inside the exposure system. This was evaluated by CFD modeling.

As expected, a decrease was observed in the concentrations of the monitored substances as dilution was applied. However, this decrease varied depending on the dilution protocol used. Furthermore, the concentration of the targeted substances was not reduced proportionally with dilution, implying changes in particle size distribution in the aerosol (Fig. 5).

The sampling efficiency of the SPI-MS showed dependency on aerosol particle size and aerosol flow rate, which was demonstrated not only by CFD modeling but also by experimental observations.

CONCLUSION

In aerosols containing cigarette smoke, the concentrations of some constituents differ notably in applied and delivered aerosols. Thus, where possible, dosimetry should be based on the delivered aerosol and not on the applied aerosol. Results show that the experimental design of in vitro studies should report not only dilution flow rates but also details on the applied dilution protocol, adding a layer of complexity to the design and comparison of studies.

Engineering considerations led to the development of a simple and robust size-dependent theoretical model of aerosol deposition efficiency that can be applied to various exposure well chamber geometries under different operating conditions. □

REFERENCES

1. Majeed S, et al. Characterization of the Vitrocell® 24/48 *in vitro* aerosol exposure system using mainstream cigarette smoke. *Chem Cent J* 2014; **8:** 62.
2. Steiner S, et al. Delivery efficiencies of constituents of combustion-derived aerosols across the air-liquid interface during *in vitro* exposures. *Toxicol In Vitro* 2018; **58:** 384–98.
3. Steiner S, et al. A new fluorescence-based method for characterizing *in vitro* aerosol exposure systems. *Toxicol In Vitro* 2017; **38:** 150–58.
4. Oldham MJ, et al. 2020. Deposition efficiency and uniformity of monodisperse solid particle deposition in the Vitrocell® 24/48 Air-Liquid-Interface *in vitro* exposure system. *Aerosol Sci and Tech* 2020; **54:** 52–65.
5. Lucci R, et al. Characterization and modeling of aerosol deposition in Vitrocell® exposure systems: exposure well chamber deposition efficiency. *J Aerosol Sci* 2018; **123:** 141–60.
6. Steiner S, et al. Characterization of the Vitrocell® 24/48 aerosol exposure system for its use in exposures to liquid aerosols. *Toxicol In Vitro* 2017; **42:** 263–72.
7. Frege C, et al. 2020. Assessment of single-photon ionization mass spectrometry for on-line monitoring of *in vitro* aerosol exposure experiments. *Chem. Res. Toxicol.* 2020, **33**(2): 505-514.

FULL STUDY DATA AVAILABLE

https://doi.org/10.26126/intervals.aosi3a.1