Characterization of an Air-Liquid-Interface (ALI) in vitro Exposure System (VITROCELL® VC1/7 and Ames 48) Using a Prototype E-vapor Product

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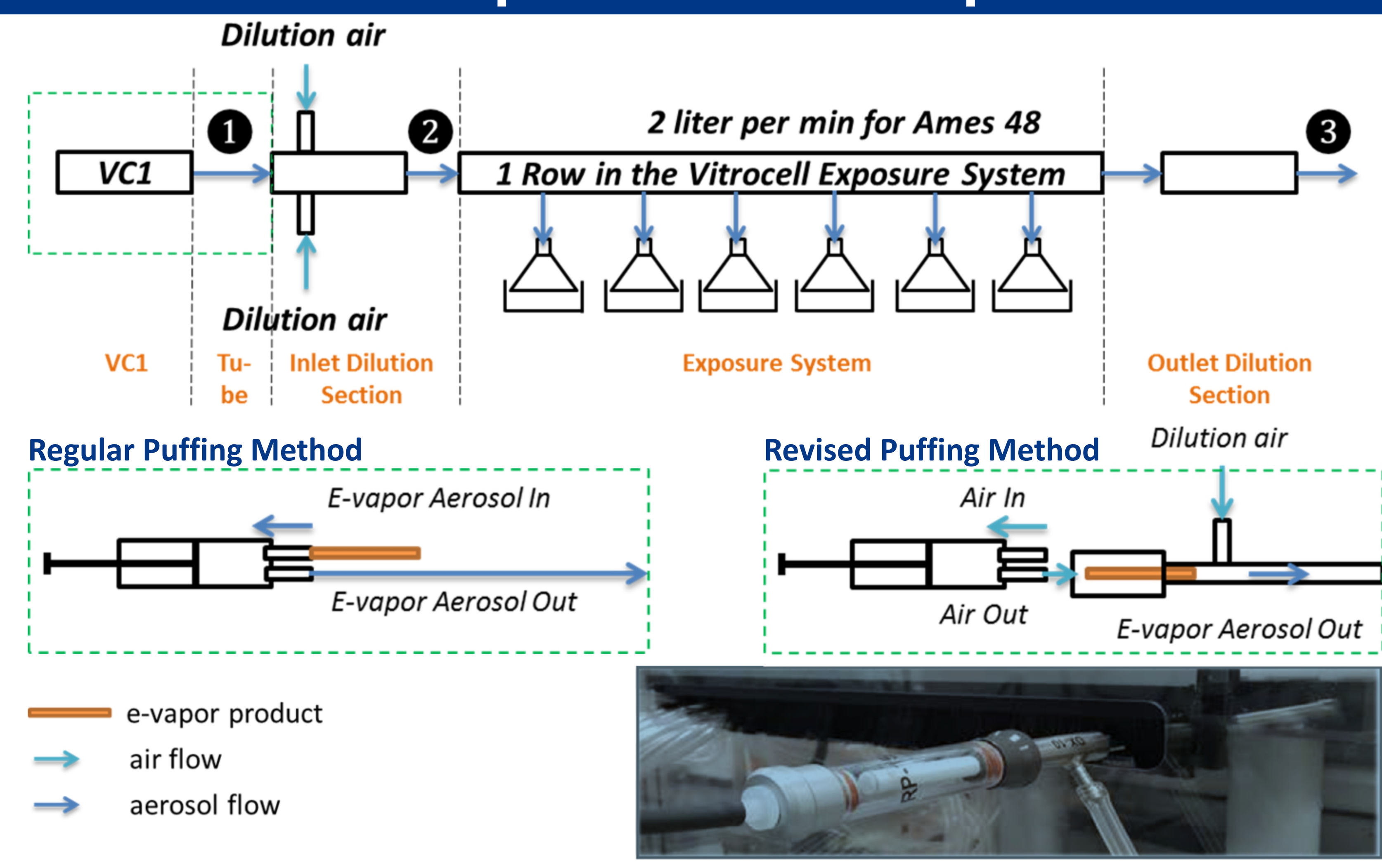
Abstract

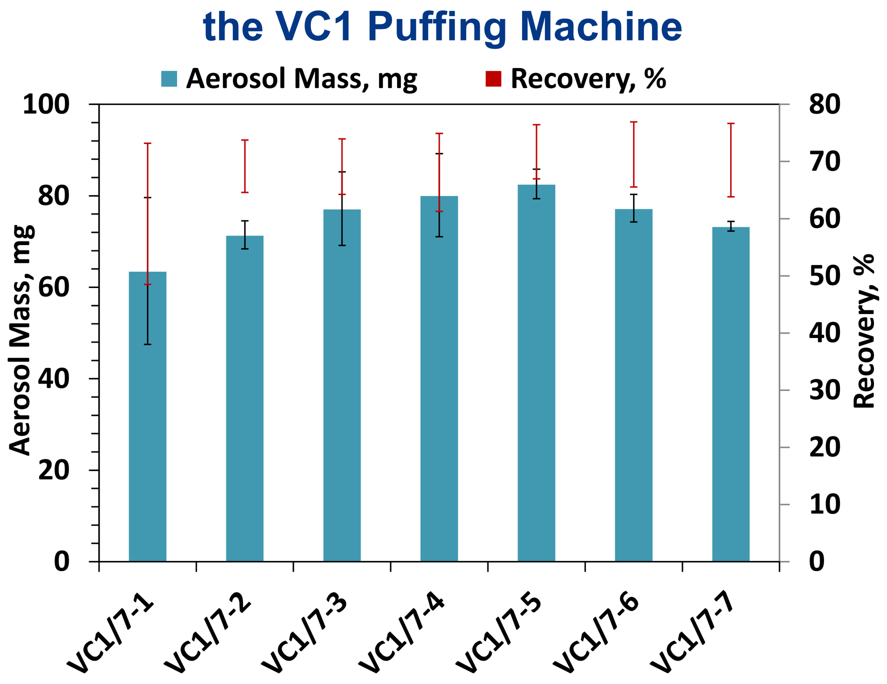
Direct delivery of aerosol or vapor to the apical surface of cells (ALI) allows more relevant exposure for in vitro toxicological evaluation of inhalable chemicals. In this study, we quantitatively characterized the aerosol delivery in a commercially available ALI in vitro exposure system (VITROCELL® VC1/7 puffing machine and Vitrocell® Ames 48 (Ames 48)) using a prototype e-vapor product (MarkTen® e-cigarette with a prototype e-liquid containing propylene glycol, glycerin, nicotine, and water). The e-vapor product, with a fully-charged battery, was puffed using a 55 ml puff over 5 seconds, with a 30 second inter puff period, by a VC1 puffing machine. As specified by the manufacturer, e-vapor aerosol was pulled into the VC1 puffing machine and then pushed into the exposure system over 8 seconds. Aerosol mass was collected and measured gravimetrically following the first 20 puffs at the exit of each puffing unit (7 VC1s) (position 1) and the inlet (position 2) and outlet (position 3) of the Ames 48. The average aerosol mass delivery (calculated as measured mass/total product weight loss × 100%) was 68.6%, 49.1%, and 46.6%, respectively, with about 0.39–0.46% of aerosol mass delivered to the exposure inserts. Results suggested about 30% aerosol loss in the aerosol transportation path (VC1 and tubing) prior to entry into the exposure system. To minimize the aerosol loss and consequently increase the aerosol delivery to the inserts, we revised the aerosol delivery method by shortening the aerosol transportation path. With the revised puffing method, the VC1 pushed 55 ml of air through the e-cigarette over 5 seconds; the resulted aerosol delivery at the inlet of the in vitro exposure system was about 93.5–95.3%, with increased aerosol delivery to 1.0–1.2% in the exposure inserts.

Experimental Setup

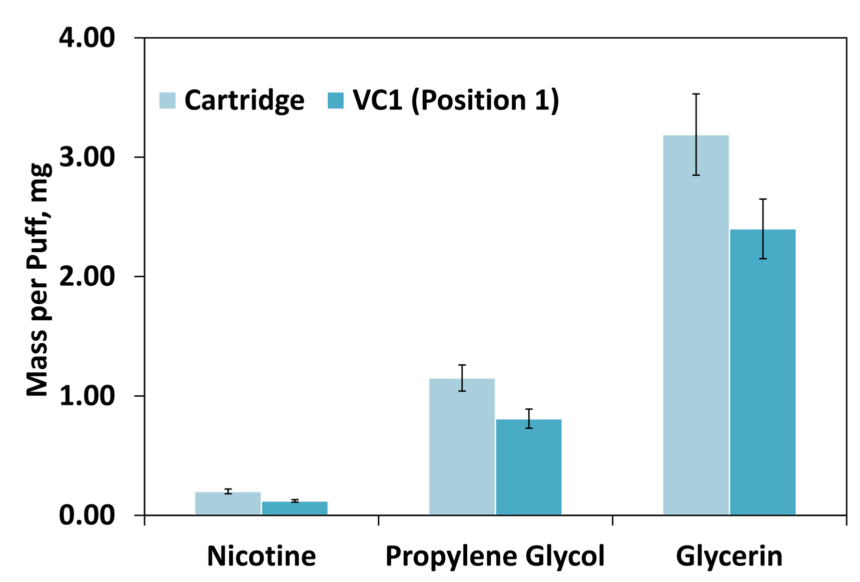


Qualification of VC1/7 Smoking Machine with the Regular Puffing Method

Aerosol Mass at the Exit of the VC1 Puffing Machine



Nicotine, Propylene Glycol (PG), and Glycerin Analysis



Aerosol Size Distribution at the Exit of the VC1 Puffing Machine (Position 1)

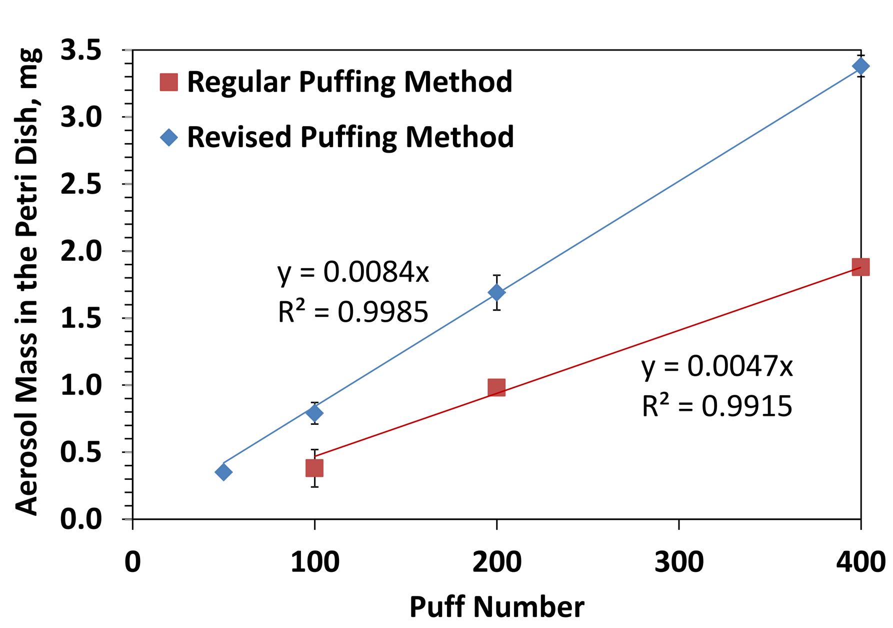
Position 1 (Mean ± SD; N = 3)		
	MMAD (um)	GSD
VC1/7 - 1	1.20 ± 0.09	2.13 ± 0.11
VC1/7 - 2	1.29 ± 0.13	2.19 ± 0.12
VC1/7 - 3	1.36 ± 0.05	2.01 ± 0.07
VC1/7 - 4	1.31 ± 0.28	1.99 ± 0.41
VC1/7 - 5	1.15 ± 0.16	2.15 ± 0.05
VC1/7 - 6	1.42 ± 0.34	2.11 ± 0.19
VC1/7 - 7	1.23 ± 0.09	2.05 ± 0.15
Overall Mean	1.28	2.09
Overall SD	0.18	0.18
Overall %RSD	14.4	8.5

Method

- **Aerosol Mass** (mg) was gravimetrically measured for the first 20 puffs of a cartridge which was puffed by a VC1 pump at the programmed regimen. Results are mean of 3 measurements for each pump; the error bars is the standard deviation (SD).
- **Recovery (%)** = $\frac{\text{Aerosol Mass Collected at the Exit of VC1 (mg)}}{\text{E-vapor Cartridge Weight Loss (mg)}} \times 100\%$.
- **Nicotine, Propylene Glycol, and Glycerin Analysis:** For VC1/7, 20-puff aerosol was collected at Position 1. Results are mean of 3 replicates of 7 units (N = 21). For cartridge, 20-puff aerosol was directly collected at the e-cigarette mouth piece (N = 3).
- **Aerosol Size Distribution** was measured with a cascade impactor (MSP 135-8). The first 3 puffs of each cartridge were collected for 3 cartridges (N = 3). Assuming a lognormal distribution, the mass median aerodynamic diameter (MMAD, μm) and the geometric standard deviation (GSD) were obtained by using a linear data reduction scheme (O'Shaughnessy and Raabe, 2003).

Aerosol Mass Delivery: Regular vs. Revised Puffing Method

Aerosol Mass in the Petri Dish (Trumpet Flowrate = 10 cc / min)



Percent Aerosol Mass Deposition on Ames48 System Sections Prior to Position 2 (%)

(Mean ± SD; N = 3)	Regular Puffing (Trumpet Flow 5 cc/min)		Revised Puffing (Trumpet Flow 10 cc/min)	
	200 puffs	400 puffs	200 puffs	400 puffs
Prior to Position 2 (Total)	33.5	33.8	6.0	6.0
VC1 Puffing Machine	15.6 ± 0.5	15.1 ± 2.3	NA	NA
Connecting Tube	12.0 ± 0.4	12.8 ± 1.0	NA	NA
Custom-built Dilution Tube	NA	NA	5.5 ± 0.8	5.1 ± 0.3
Inlet Dilution Section	5.9 ± 2.8	5.9 ± 1.0	0.5 ± 0.1	0.8 ± 0.1

Percent Aerosol Mass Deposition in All the Petri Dish (%) (Trumpet Flow 10 cc/min)

(Mean ± SD; N = 3)	Regular Puffing		Revised Puffing	
	200 puffs	400 puffs	200 puffs	400 puffs
ALL 6 Petri Dish	0.64 ± 0.03	0.55 ± 0.01	1.05 ± 0.06	1.13 ± 0.05
Estimated Target [a]	~2.2%		~2.2%	

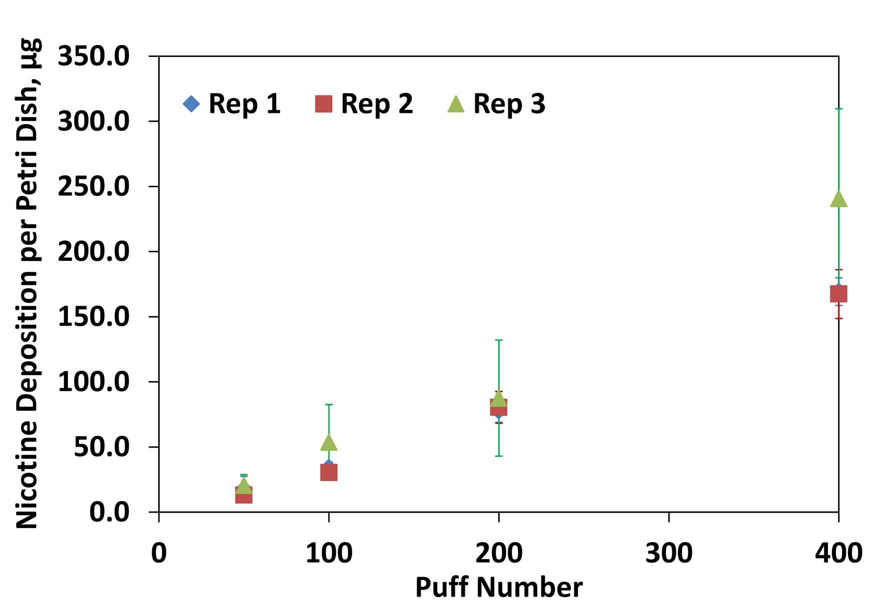
[a] The target was estimated based on the flowrate ratio, assuming all aerosol drawn into the trumpet deposit in the petri dish. The total flowrate through the exposure system during puffing was 2.660 L/min, while the total trumpet flowrate for all 6 petri dish was 60 mL/min.

Method

- **Aerosol Mass per Petri Dish (mg):**
  - Aerosol mass was gravimetrically measured in each of 6 petri dishes in a row with pre-weighed filter pads of an appropriate size and averaged over the 6 wells.
  - Results are the mean of 3 rows with independent aerosol generation. The error bar in the figure is the standard deviation (SD).
- **Percent Aerosol Mass Deposition on All System Sections (%)**
  - The aerosol mass deposited on various parts throughout the system was measured gravimetrically in 3 rows with independent aerosol generation (200 and 400 puffs from MarkTen® e-cigarettes).
  - For parts that were too heavy or too large to weigh directly, aerosol mass was removed by wiping with a pre-weighed filter pad or a swab, then the pad or swab were weighed to determine the mass.
  - The data in the table were normalized by the cartridge weight loss.
  - **Percent Aerosol Mass on All System Sections (%)** = $\frac{\text{Aerosol Mass on Each Section (mg)}}{\text{E-vapor Cartridge Weight Loss (mg)}} \times 100\%$.

Nicotine, PG, and Glycerin Analysis in the Petri Dish: Revised Puffing Method

Nicotine Deposition per Petri Dish, μg



Nicotine, PG, and Glycerin per Petri Dish, μg (400 puffs)

(3 Replicates for Each Measurement)	Measurement 1[b]			Measurement 2[b]		
	Nicotine	PG	Glycerin	Nicotine	PG	Glycerin
Overall Average (μg)	192.45	1396.0	3620.0	190.1	1538.7	2484.1[a]
Overall RSD (μg)	36.02	227.6	144.0	5.8	20.4	182.4
Overall %RSD	18.7%	16.3%	4.0%	3.1%	1.3%	7.3%
% of Sum of 3 Constituents (Measured)	3.7%	26.8%	69.5%	4.5%	36.5%	59.0%
% of Sum of 3 Constituents (Theoretical)	4.7%	28.6%	66.7%	4.7%	28.6%	66.7%

Nicotine, PG, and glycerin in all air lines were below the LOQ. LOQ for nicotine, PG, and glycerin is 2.56, 804, and 2500 μg, respectively.
[a] The result of one run was below the LOQ.
[b] Measurement 1 and 2 were conducted on different days using the same experimental method.

Method

- **Experimental Setting:** trumpet flowrate = 10 cc / min
- **Nicotine, PG, and Glycerin per Petri Dish (μg)**
  - 50, 100, 200, and 400 puffs of aerosol was collected in the petri dish containing 4 mL of 0.2 M sodium phosphate buffer (PBS). After exposure, the buffer was analyzed for nicotine with a GC-MS method. PG and glycerin were analyzed by a GC-FID method.
  - For each measurement, there were 3 replicates for each measurement; and at least 1 row in the Ames48 system served as the concurrent air control with 400 puffs of air. The results are the mean of 3 replicates with independent aerosol generation.
- **% of Sum of 3 Compounds (Nicotine, PG, and Glycerin)**
  - Mass of each compound was normalized to the sum of mass of the 3 measured compounds (nicotine, PG, and glycerin).
  - The theoretical values were calculated based on the formulation composition (4% nicotine, 24.3% PG, 56.7% glycerin, and 10% water; by weight).

Conclusions

- Consistent recovery ratio of aerosol mass and aerosol size distribution demonstrated that the 7 units of the VC1 puffing machine functioned.
- Despite consistency, >27% loss of aerosol mass was observed in the VC1 and the connecting tube with the regular puffing method.
- For all methods, the aerosol mass deposited in the petri dish (the exposure insert) increased linearly with the puff numbers.
- The revised puffing method delivered about twice as much aerosol mass to the petri dish (the exposure insert) as the regular puffing method. The revised puffing method reduced aerosol loss in the transportation line prior to the exposure system from >30% to ~6%.
- Nicotine, PG, and glycerin were measured with the revised puffing method. Nicotine delivered to the petri dish increased with the puff number. PG and glycerin were quantified at the highest 400 puffs due to the limitation of the analytical method. After normalization (% sum of 3 measured constituents), the composition of the deposited aerosol (measured) was in general comparable with that of the formulation (theoretical).

Reference

Patrick T. O'Shaughnessy & Otto G. Raabe (2003). A comparison of cascade impactor data reduction methods. Journal of Aerosol Science and Technology. Volume 37 (2): 187. https://doi.org/10.1080/02786820300956

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