AAPS PharmSciTech, Vol. 15, No. 5, October 2014 (© 2014)
DOI: 10.1208/s12249-014-0111-1

Research Article

Investigation of Dry Powder Inhaler (DPI) Resistance and Aerosol Dispersion Timing on Emitted Aerosol Aerodynamic Particle Sizing by Multistage Cascade Impactor when Sampled Volume Is Reduced from Compendial Value of 4 L

Hlack Mohammed,[1] Jan Arp,[2] Frank Chambers,[3] Mark Copley,[4] Volker Glaab,[5] Mark Hammond,[6] Derek Solomon,[6] Kerry Bradford,[6] Theresa Russell,[6] Yvonne Sizer,[6] Steven C. Nichols,[7] Daryl L. Roberts,[8] Christopher Shelton,[9] Roland Greguletz,[10] and Jolyon P. Mitchell[11,12]

Received 29 October 2013; accepted 13 March 2014; published online 29 May 2014

Abstract. Compendial methods determining dry powder inhaler (DPI)-emitted aerosol aerodynamic particle size distribution (APSD) collect a 4-L air sample containing the aerosol bolus, where the flow, which propagates through the cascade impactor (CI) measurement system from the vacuum source, is used to actuate the inhaler. A previous article described outcomes with two CIs (Andersen eight-stage cascade impactor (ACI) and Next-Generation Pharmaceutical Impactor (NGI)) when the air sample volume was ≤4 L with moderate-resistance DPIs. This article extends that work, examining the hypothesis that DPI flow resistance may be a factor in determining outcomes. APSD measurements were made using the same CI systems with inhalers representing low and high flow resistance extremes (Cyclohaler® and HandiHaler® DPIs, respectively). The ratio of sample volume to internal dead space (normalized volume (V^*)) was varied from 0.25 to 1.98 (NGI) and from 0.43 to 3.46 (ACI). Inhaler resistance was a contributing factor to the rate of bolus transfer; the higher resistance DPI completing bolus relocation to the NGI pre-separator *via* the inlet when V^* was as small as 0.25, whereas only ca. 50% of the bolus mass was collected at this condition with the Cyclohaler® DPI. Size fractionation of the bolus from either DPI was completed within the ACI at smaller values of V^* than within the NGI. Bolus transfer from the Cyclohaler® capsule and from the HandiHaler® to the ACI system were unaffected by the different flow rise time observed in the two different flow controller systems, and the effects the ACI-based on APSD measurements were marginal.

KEY WORDS: cascade impactor; compendial method; dry powder inhaler; inhaler resistance; sample volume.

INTRODUCTION

The compendial methodologies for determining emitted aerosol aerodynamic particle size distribution (APSD) from a dry powder inhaler (DPI) collect a 4-L sample containing the aerosol bolus, where the flow, which propagates through the cascade impactor (CI) measurement system from the vacuum source, is used as the means of actuating the inhaler (1,2). This system typically comprises a European Pharmacopeia (Ph.Eur.)/United States Pharmacopeia (USP) induction port and a pre-separator, connected at the inlet of the CI, which is in turn connected to a vacuum pump *via* a flow controller containing a critical orifice that eliminates the impact of fluctuations caused by variations in pump performance. A control valve is normally also employed to adjust the flow rate through the system to provide a 4-kPa pressure drop across the device, which is the approach described in the compendia to standardize energy input into the DPI upon actuation. However, the entire measurement system contains significant internal dead space; in the case of the Andersen eight-stage CI (ACI) and Next-Generation Pharmaceutical Impactor (NGI), this volume can be as much as 1.155 and 2.025 L, respectively, when the internal volume of each pre-separator is included (3).

A previous article developed by members of the Impactor Sub-Team of the European Pharmaceutical Aerosol Group (EPAG) described the outcome of experiments to investigate how the APSDs of medium-resistance DPI products might change as the result of reducing the

[1] GSK plc, Park Road, Ware, Hertfordshire SG12 0DP, UK.
[2] TEVA-Pharmachemie, 2003 RN, Haarlem, Netherlands.
[3] Covance, Otley Road, Harrogate, North Yorkshire HG3 1PY, UK.
[4] Copley Scientific Ltd, Nottingham, NG4 2JY, UK.
[5] Boehringer-Ingelheim Pharma GmbH & Co. KG, Binger Straße 173, 55216, Ingelheim, Germany.
[6] Intertek Pharmaceutical Services Melbourn, Cambridgeshire, SG8 6DN, UK.
[7] OINDP Consultancy, Smallwood, Cheshire, CW11 2UN, UK.
[8] MSP Corporation, Shoreview, Minnesota 55126, USA.
[9] Boehringer-Ingelheim Roxane Inc, 1809 Wilson Rd, Columbus, Ohio 43228, USA.
[10] Almirall-Sofotec GmbH, Benzstraße 1-3, 61352, Bad Homburg, Germany.
[11] Jolyon Mitchell Inhaler Consulting Services Inc, London, Ontario N6H 2R1, Canada.
[12] To whom correspondence should be addressed. (e-mail: mitchelljolyon@gmail.com)

sample volume below 4 L (4). The quest underlying the original investigation was driven by a suggestion that the sample of DPI-derived aerosol assessed by the CI method should ideally match more closely the volume of air inhaled by an adult in a single inspiratory maneuver, from those seeking greater clinical realism in the *in vitro* evaluation of inhalers (5). In the initial series of experiments undertaken by Mohammed *et al.* (4), the NGI behaved as would be expected when the ratio of sample volume (V_{sample}) to dead space ($V_{dead\ space}$), defined as the dimensionless parameter V^*, decreased below unity, in that incomplete transfer of the aerosol bolus and size fractionation were clearly evident. However, contrary to expectations, the corresponding ACI-measured data indicated that complete size fractionation took place earlier than would be expected from a model based on a steady progression of the bolus through the impactor from the stage separating the largest to that which size-fractionated the finest particle. The interpretation given to this finding was that the incoming flow containing the aerosol somehow circumvents the expected stage-by-stage progression through the ACI, possibly linked to the fact that the circular collection plates for the first two stages are annular rather than full plates. Under these circumstances, the flow profile across this CI from the central axis to the periphery of the internal flow pathway for these stages at least is more complex than that which exists within the NGI at start-up, and the radial profile may, in fact, be significantly maldistributed. It was recognized that further progress in understanding the problem would require theoretical assessment, modeling the flow field in both systems by means of computational fluid dynamics (CFD) under start-up (unsteady pressure field) conditions. However, the question whether or not the flow resistance of the DPI might be a contributory factor to the observed behavior differences between the two CI systems remained unanswered, and it was subsequently realized that further experimental work might be able to shed light on this important matter, given the wide range of flow resistance for currently marketed DPIs.

The purpose of the present investigation was therefore to test experimentally the hypothesis that inhaler resistance influences the flow rise time on the APSD measurement process. The Cyclohaler®, TEVA-Pharmachemie, Haarlem, Netherlands, and HandiHaler®, Boehringer Ingelheim, Ingelheim, Germany were chosen as DPIs representing the extremes of specific flow resistance for this class of inhaler.

The opportunity was also taken to examine in a more systematic way linked to DPI flow resistance, the influence of flow rise time on the APSD measurement process. In the previous article, two different flow controllers were used in collecting APSD measurements: a commercially available flow controller, the model TPK (Copley Scientific Ltd., Nottingham, UK), and proprietary designed flow controller that was based on the rather straightforward timer-solenoid valve arrangement described in the compendia and which had been used exclusively with the ACI system. There was concern whether the response time of the timer-solenoid valve operation based on the pharmacopeial specification of <100 ms for valve opening/closing time (1,2), which is significantly slower than the valve control arrangement of the TPK controller (valve opening/closing time of 25 ms; Product Brochure for 2012, Copley Scientific Ltd., Nottingham, UK), may have allowed significant volume of additional air to enter the ACI system following the elapsed time to acquire the sample from each DPI. Such a situation may falsely give the impression that this CI system is less sensitive to changes to sample volume when close to or less than the dead space of this measurement apparatus. Air flow rise time has been studied previously by both De Boer *et al.* (6) and Beron *et al.* (7) and was shown to affect the delivered mass from low- and medium-resistance DPI devices when acceleration rates were less than 5 L/s/s. However, the effect of this parameter on the resulting APSD measurement has not been explored.

MATERIALS AND METHODS

The methodology used in the present investigation was mostly the same as that described in the previous article (4), so only the details pertinent to the changes that were made to accommodate the different DPIs are described in detail. Two impactor systems were investigated. The first system comprised an ACI that was equipped with its pre-separator and a GSK-type induction port. This inlet is considered similar to the USP throat, and dimensions have been fully defined in the associated compendial literature (8). The ACI configuration normally used for measurements at 28.3 L/min (stages 0 to 7) was retained for these studies that were all undertaken at 60 L/min. The second system was a NGI, equipped with its pre-separator and the standard Ph.Eur./USP induction port, as described in the article providing the design for this CI (9). The collection plates for the ACI and corresponding cups for the NGI were each coated with silicone oil by a validated procedure, in order to avoid bias from particle bounce and re-entrainment.

All measurements were undertaken at a fixed flow rate of 60 L/min, rather than constraining the flow rate to that achievable (39 L/min in the case of the HandiHaler® DPI) if a fixed pressure drop of 4 kPa had been imposed as being broadly representative of the pressure drop generated during inhalation by patients using this DPI. Further, had the compendial procedure been followed exactly in this respect, the low resistance of the Cyclohaler® DPI would have meant that the flow rate would have had to be set at 100 L/min, which would have required a duration of 0.3 s, a value at the limit of the capability of the systems, to achieve the short sample volumes at low V^*. This deviation from the compendial methodology was deemed necessary so that a consistent comparison between the different CI systems could be made on the basis of timing the movement of an equivalent volume of air containing the aerosol bolus at different intervals from initiation of sampling after opening the valve to the vacuum pump.

Measurements of APSD ($n=1$ device, 3 replicates/device) were determined at each condition (Table I) using the Cyclohaler® DPI (10) and HandiHaler® DPI (11) representing low (0.018 $kPa^{0.5}$min/L) and high (0.049 $kPa^{0.5}$min/L) specific flow resistance DPIs, respectively. The Cyclohaler® DPIs were evaluated with capsules containing proprietary product (code "Y"), and likewise, the HandiHaler® DPIs were tested using a different proprietary product (code "Z") made up in capsules intended for use with this inhaler. Both formulations were carrier (lactose)-based. The baseline sample time (4 s) at the nominal flow rate under steady-state conditions therefore

Table I. Test Conditions (Each DPI) for Measurements with Low and High Flow Resistance Inhalers

Time air flow allowed to continue (s)	Use of TPK (for ACI system only)	Number of doses sampled by CI system	Number of determinations for each impactor	Normalized volume[a], V^* (dimensionless)	
				NGI	ACI
0.5	Y N	1	3	0.25	0.43
1.0	Y N	1	3	0.49	0.87
4.0	Y N	1	3	1.98	3.46

ACI Andersen eight-stage cascade impactor, *CI* cascade impactor, *NGI* Next-Generation Pharmaceutical Impactor, *Y* yes, *N* no
[a] V^* = sample volume/internal dead space of CI system including inlet and pre-separator

allowed a 4-L sample to be taken. This time was reduced in two steps to 1 s and then to 0.5 s. Overall, the number of complete volume changes assuming plug flow evaluated with each CI system (equivalent to V^*, where $V^* = V_{sample}/V_{dead\ space}$) ranged from approximately 3.46 to 0.43 with the ACI and from 1.98 to 0.25 using the NGI. The shortest duration (500 ms) was intentionally selected as being the minimum sample time that was achievable with the existing control options. This sample time was already known from the previous investigation to be adequately short that incomplete aerosol bolus transfer would likely be observable even with the ACI system that contained the smaller dead space (4).

One actuation of the DPI on test per determination was collected in the CI system under evaluation to ensure that there was no opportunity for material in the CI to become re-entrained. This approach provided adequate active pharmaceutical ingredient (API) from either DPI type for recovery and assay. The API was recovered from each component of the impactor system using a suitable solvent and subsequently assayed by means of validated HPLC-UV spectrophotometric procedures for product Y (Cyclohaler® DPI) and product Z (HandiHaler® DPI).

A supplementary study was also undertaken, whose objective was to establish comparative performance of the two flow controller systems that had been used in both the previous and present investigations. Flow controller performance, reported as time for air flow rate to rise from initiation of air flow (0 L/min) to 90% of the set value of 60 L/min, assessed the response time of the timer-solenoid component of the controller, as all other components within the test apparatus were held constant. Three replicate measurement of rise times from 0 to 90% of the set flow rate were made under standardized conditions without a DPI present. Each flow controller was evaluated with and without the ACI present in the sampling train by means of a sensitive calibrated mass flow meter (model 4040, TSI Inc., St Paul, MN, USA), whose electronic signal was processed by purpose-developed proprietary flow-time data-logging software (FlowMonitor v.1.2, Almirall-Sofotec GmbH, Bad Homburg, Germany), capable of 5-ms resolution. A critical orifice that generated 4 kPa pressure drop at 60 L/min was used at the inlet of the test system, and the flow meter was connected at the flow inlet of the orifice. Thus, all measurements were made at the distal point in the system. Initially, the two flow controllers were evaluated in isolation from a CI to determine their fastest rise times. Later, the measurements were repeated with each flow controller connected in series with an ACI in accordance with the compendial arrangement.

RESULTS

The additional dead volumes introduced to the CI systems investigated by the space occupied by the capsule chamber and air way inside the mouthpiece of the Cyclohaler® and HandiHaler® DPIs were estimated to be 6 and 2.2 mL, respectively. These values are three orders of magnitude smaller than the dead volumes associated with the CI measurement apparatus configurations that were evaluated (3) so that the contribution from either inhaler to the overall dead space under consideration can therefore be ignored.

Mass-based API depositions at each site in the CI systems under investigation are summarized in Tables II, III, and IV for the Cyclohaler® DPI-based evaluations, and corresponding data are provided in Tables V, VI, and VII for the measurements using the HandiHaler® DPIs. The ratio of sample volume to internal dead space was represented as the normalized volume (V^*) to facilitate comparison between the two CI systems. The measurements of total mass recovered obtained at the smallest

Table II. API Deposition Data (μg Product Y/Capsule; Mean±SD) for Cyclohaler® DPI Evaluations: NGI (the Compendial Sample Volume (4 L at 60 L/min) Is Shaded)

Location	[a]Normalized Volume, V^* (dimensionless)		
	0.25	0.49	1.98
DPI mouthpiece and inlet	34.9 ± 14.0	62.1 ± 3.6	65.4 ± 2.6
Pre-separator	78.8 ± 33.4	145.5 ± 2.6	118.2 ± 15.3
Stage 1	4.2 ± 1.3	14.6 ± 2.3	9.4 ± 1.7
Stage 2	1.0 ± 0.3	12.1 ± 4.5	22.2 ± 5.1
Stage 3	0.0 ± 0.0	11.2 ± 5.9	28.5 ± 4.5
Stage 4	0.0 ± 0.0	6.2 ± 3.9	24.0 ± 2.1
Stage 5	0.0 ± 0.0	0.8 ± 0.9	7.7 ± 1.0
Stage 6	0.0 ± 0.0	0.0 ± 0.0	1.5 ± 0.3
Stage 7	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Micro-orifice collector (MOC)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
TOTAL	119.0 ± 49.0	252.5 ± 20.9	277.0 ± 26.1

[a] V^* = sample volume/internal dead space of CI system including inlet and pre-separator

Table III. API Deposition Data (μg Product Y/Capsule; Mean±SD) for Cyclohaler® DPI Evaluations: ACI with TPK Controller (the Compendial Sample Volume (4 L at 60 L/min) Is Shaded)

Location	[a]Normalized Volume, *V** (dimensionless)		
	0.43	0.87	3.46
DPI mouthpiece and inlet	48.0 ± 7.8	58.9 ± 2.7	60.5 ± 3.9
Pre-separator	116.0 ± 43.6	124.1 ± 14.3	133.1 ± 4.4
Stage 1	15.5 ± 6.2	17.0 ± 3.8	18.0 ± 2.5
Stage 2	15.5 ± 6.6	19.0 ± 5.8	23.9 ± 3.4
Stage 3	12.1 ± 4.8	21.1 ± 7.8	34.1 ± 4.6
Stage 4	3.1 ± 1.3	7.2± 2.6	12.6 ± 1.0
Stage 5	0.0 ± 0.0	0.9 ± 0.8	2.4 ± 0.3
Stage 6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Stage 7	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Back-up Filter	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
TOTAL	**210.2 ± 69.3**	**248.3 ± 37.1**	**284.6 ± 16.9**

[a]*V** = sample volume/internal dead space of CI system including inlet and pre-separator

values of *V** were generally, but not always, more variable compared with those at higher values, an outcome that might be anticipated given the short sample time (0.5 s) that was close to the lower limit of capability for the flow control systems.

Total API mass from the Cyclohaler® DPI when tested under compendial conditions with a 4-L sample volume (*V**=1.98 for the NGI and 3.46 for the ACI systems) was close to 280 μg/capsule product Y, irrespective of CI system. The same measure decreased slightly to about 250 μg/capsule at the intermediate values of sample volume (*V**=0.49 for the NGI and 0.87 for the ACI). There was evidence of incomplete capsule emptying determined at the smallest sample volume (*V**=0.25 for the NGI and 0.43 for the ACI), demonstrated by the further reduction in mean values of total mass (119 μg for the NGI, 210 μg for the ACI-TPK controller, and 212 μg for the ACI-timer/solenoid valve) together with large coefficients of variation associated with total mass recovered (41%—NGI; 33%—ACI with TPK; 46%—ACI-timer/solenoid) at this condition. These low and relatively variable mass recovery data were retained because they likely represent a potentially severe consequence of making APSD measurements utilizing such a short sample time with this particular inhaler.

Conversely, the corresponding mean values of absolute API mass from the HandiHaler® DPI were almost all in the range from 2.3 to 2.4 mg of product Z/capsule, irrespective of *V**, except for the NGI measurements at the two lower settings (*V** of 0.25 and 0.49), where values of total API mass were 2.15 and 2.20 mg/capsule,

Table IV. API Deposition Data (μg Product Y/Capsule; Mean±SD) for Cyclohaler® DPI Evaluations: ACI with Timer-Solenoid Flow Control (the Compendial Sample Volume (4 L at 60 L/min) Is Shaded)

Location	[a]Normalized Volume, *V** (dimensionless)		
	0.43	0.87	3.46
DPI mouthpiece and inlet	54.2 ± 23.8	54.7 ± 1.5	63.8 ± 1.1
Pre-separator	98.9 ± 48.1	131.7 ± 20.2	130.3 ± 6.9
Stage 1	15.0 ± 6.2	19.0 ± 5.4	17.8 ± 1.7
Stage 2	16.9 ± 8.8	20.1 ± 5.1	21.0 ± 2.3
Stage 3	18.8 ± 10.3	22.6 ± 4.8	28.4 ± 1.5
Stage 4	6.9± 4.5	7.2 ± 1.7	11.9 ± 0.9
Stage 5	1.5 ± 1.4	0.6 ± 1.0	2.4 ± 0.3
Stage 6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Stage 7	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Back-up Filter	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
TOTAL	**212.2 ± 98.1**	**255.9 ± 34.4**	**275.7 ± 11.3**

[a]*V** = sample volume/internal dead space of CI system including inlet and pre-separator

Table V. API Deposition Data (mg Product Z/Capsule; Mean±SD) for HandiHaler® DPI Evaluations: NGI (the Compendial Sample Volume (4 L at 60 L/min) Is Shaded)

Location	[a]Normalized Volume, *V** (dimensionless)		
	0.25	0.49	1.98
Powder capsule	0.20 ± 0.20	0.09 ± 0.03	0.11 ± 0.03
DPI mouthpiece and inlet	0.71 ± 0.08	0.77 ± 0.03	0.75 ± 0.03
Pre-separator	1.19 ± 0.09	0.59 ± 0.01	0.16 ± 0.00
Stage 1	0.20 ± 0.05	0.23 ± 0.01	0.10 ± 0.00
Stage 2	0.05 ± 0.01	0.28 ± 0.02	0.41 ± 0.02
Stage 3	0.01 ± 0.00	0.21 ±0.01	0.45 ± 0.02
Stage 4	0.00 ± 0.00	0.10 ± 0.00	0.35 ± 0.02
Stage 5	0.00 ± 0.00	0.02 ± 0.00	0.14 ± 0.01
Stage 6	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.02
Stage 7	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Micro-orifice collector (MOC)	0.00 ± 0.00	0.00 ± 0.00	0.00± 0.00
TOTAL	**2.15 ± 0.21**	**2.20 ± 0.08**	**2.40 ± 0.09**

[a]*V** = sample volume/internal dead space of CI system including inlet and pre-separator

Table VI. API Deposition Data (mg Product Z/Capsule; Mean±SD) for HandiHaler® DPI Evaluations: ACI with TPK Controller (the Compendial Sample Volume (4 L at 60 L/min) Is Shaded)

Location	[a]Normalized Volume, *V** (dimensionless)		
	0.43	0.87	3.46
Powder capsule	0.15 ± 0.03	0.21 ± 0.07	0.12 ± 0.01
DPI mouthpiece and inlet	0.91 ± 0.03	0.88 ± 0.01	0.85 ± 0.02
Pre-separator	0.50 ± 0.02	0.35 ± 0.00	0.37 ± 0.02
Stage 1	0.28 ± 0.01	0.23 ± 0.01	0.26 ± 0.02
Stage 2	0.28 ± 0.00	0.28 ± 0.00	0.30 ± 0.01
Stage 3	0.23 ± 0.01	0.35 ± 0.01	0.38 ± 0.02
Stage 4	0.09 ± 0.00	0.16 ± 0.00	0.17 ± 0.01
Stage 5	0.03 ± 0.00	0.05 ± 0.00	0.06 ± 0.00
Stage 6	0.00 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Stage 7	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Back-up Filter	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
TOTAL	**2.31 ± 0.06**	**2.32 ± 0.03**	**2.40 ± 0.06**

[a]*V** = sample volume/internal dead space of CI system including inlet and pre-separator

Table VII. API Deposition Data (mg Product Z/Capsule; Mean±SD) for HandiHaler® DPI Evaluations: ACI with Timer-Solenoid Flow Control (the Compendial Sample Volume (4 L at 60 L/min) Is Shaded)

Location	[a]Normalized Volume, *V** (dimensionless)		
	0.43	0.87	3.46
Powder capsule	0.16 ± 0.08	0.11 ± 0.04	0.12 ± 0.06
DPI mouthpiece and inlet	0.79 ± 0.02	0.82 ± 0.02	0.81 ± 0.03
Pre-separator	0.46 ± 0.02	0.39 ± 0.03	0.39 ± 0.04
Stage 1	0.30 ± 0.02	0.29 ± 0.02	0.29 ± 0.02
Stage 2	0.30 ± 0.02	0.30 ± 0.01	0.30 ±0.01
Stage 3	0.29 ± 0.02	0.34 ± 0.00	0.35 ± 0.02
Stage 4	0.12 ± 0.00	0.15 ± 0.01	0.16 ± 0.01
Stage 5	0.03 ± 0.00	0.04 ± 0.00	0.04 ± 0.01
Stage 6	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Stage 7	0.00 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Back-up Filter	0.00 ±0.00	0.00 ± 0.00	0.00 ± 0.00
TOTAL	**2.31 ± 0.10**	**2.34 ± 0.02**	**2.36 ± 0.03**

[a]*V** = sample volume/internal dead space of CI system including inlet and pre-separator

respectively. Capsule emptying from this DPI was therefore relatively unaffected by *V**, as confirmed by less API mass retention in the capsules (10% of total recovered mass with the NGI; 3% for the ACI with TPK; 3% with the ACI-timer/solenoid valve). This outcome is indicative that emptying of the HandiHaler® DPI capsule and transfer of the powder at least as far as the inlet of the CI system was completed earlier after starting flow to the CI.

The nominal fill weights of powder per capsule were 8 and 25 mg from the HandiHaler® and Cyclohaler® DPIs, respectively. However, the values of absolute mass of API for product Z associated with measurements at each location in the CI system using the HandiHaler® DPI were between one and two orders of magnitude greater than the corresponding values with product Y-loaded capsules in the Cyclohaler® DPI tests. The disposition of API at each location within the CI system under evaluation was therefore normalized with respect to the total mass emitted from the DPI on test at the specified value of *V**. Thus, these calculations excluded the mass of API retained in the powder capsule for the measurements with the HandiHaler® DPI (corresponding data were not obtained for the Cyclohaler® DPI capsules). These normalized deposition profiles for the measurements made with the NGI, ACI with TPK controller, and ACI with timer/solenoid valve flow control are illustrated in Figs. 1, 2, and 3, respectively. Profiles obtained using the NGI clearly show the progression of the aerosol bolus, based on API mass, from either DPI into this CI system as *V** was increased; thus, almost all the API mass was recovered from the pre-separator and stage 1 of this CI when *V** was lowest (0.25), with increasing mass transfer of API to lower CI stages corresponding with finer particle sizes as *V** was increased to 0.49. This progression continued when *V** was increased again to reach the compendial value of 1.98, at which point detectable amounts of API had reached the more distal size-fractionating components of this apparatus (stages 5 and 6). This trend is in agreement with the behavior observed with the NGI in the original study (4). It is also compatible with the expected model for aerosol dispersion from the powder capsule that describes (a) the steady progression of the aerosol bolus

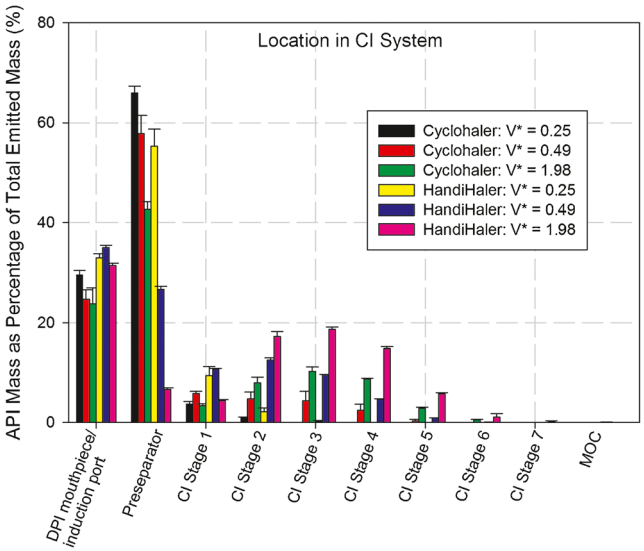

Fig. 1. Disposition of API as percent of total emitted mass: NGI at 60 L/min

released from the inhaler to the induction port; (b) subsequent transfer of the bolus to the pre-separator where deposition of API attached to over-size carrier particles takes place; (c) and ensuing movement of the inhalable aerosol through the CI with consequent size fractionation into coarse and fine particle components (11). On the other hand, the API disposition profiles obtained using the ACI with either flow control option indicated that penetration of some particles containing API had reached the most distal stages of this impactor at the smallest value of *V** (0.43) that could be observed with this CI system. Increases in *V** to 0.87 and 3.46 resulted in some redistribution of the API mass associated with the HandiHaler® DPI more distally within the size-fractionating stages. However, the effect was much smaller than that already described with the corresponding NGI data and was barely apparent from changes to the mass of API recovered from

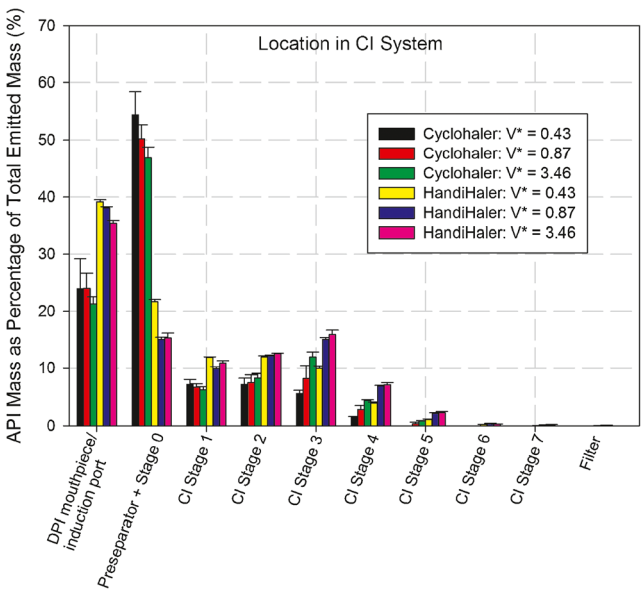

Fig. 2. Disposition of API as percent of total emitted mass: ACI with TPK controller at 60 L/min

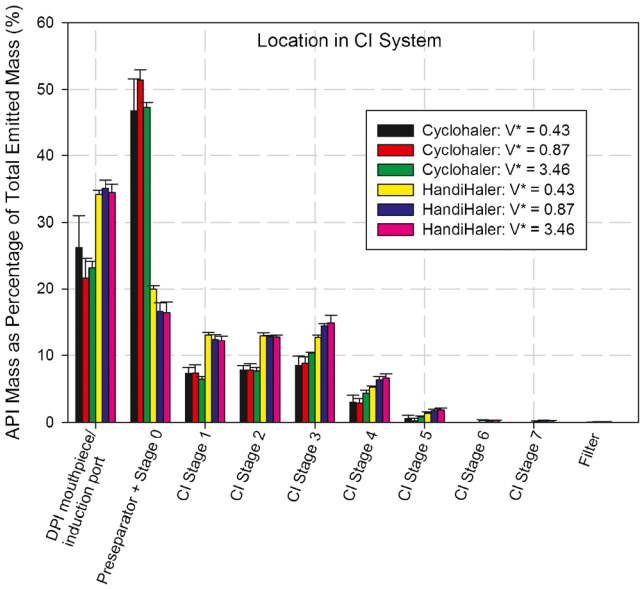

Fig. 3. Disposition of API as percent of total emitted mass: ACI with timer-solenoid valve flow control at 60 L/min

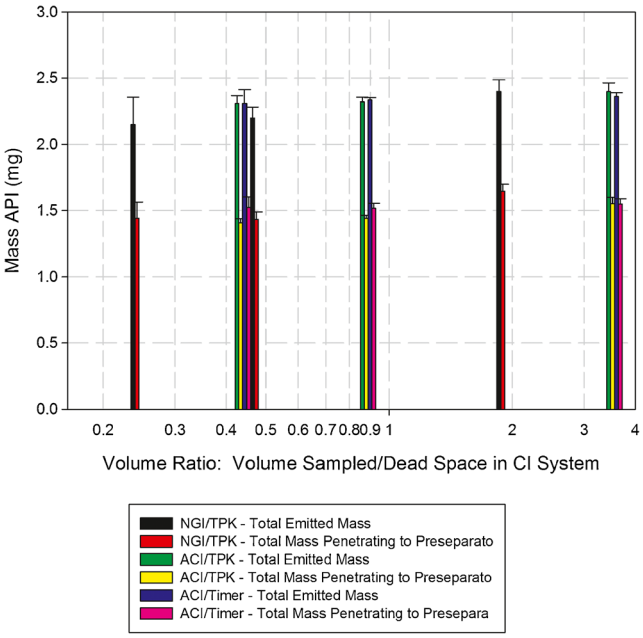

Fig. 5. Product Z mass transfer from inhaler to the CI system for measurements made at different values of V^* with the HandiHaler® DPI

stages 1 to 5 with the corresponding ACI-measured profiles obtained using the Cyclohaler® DPI.

The API transport kinetics from either DPI were examined in more detail by reducing these API disposition profiles so that only the relationships between the variables representing total mass penetrating as far as the pre-separator ($T_{CI\ system}$) and total emitted mass ex inhaler (TEM) at the different values of V^* needed to be considered. The difference between these values represents the mass collected in the induction port. Comparatively low values of total emitted mass represent more API retained within the inhaler. Figures 4 and 5 contain this information for the Cyclohaler® and HandiHaler® DPIs, respectively; the ordinate scale (mass of API) is expressed in terms of mean±SD at each condition, and since V^* is a ratio, the abscissa is scaled logarithmically.

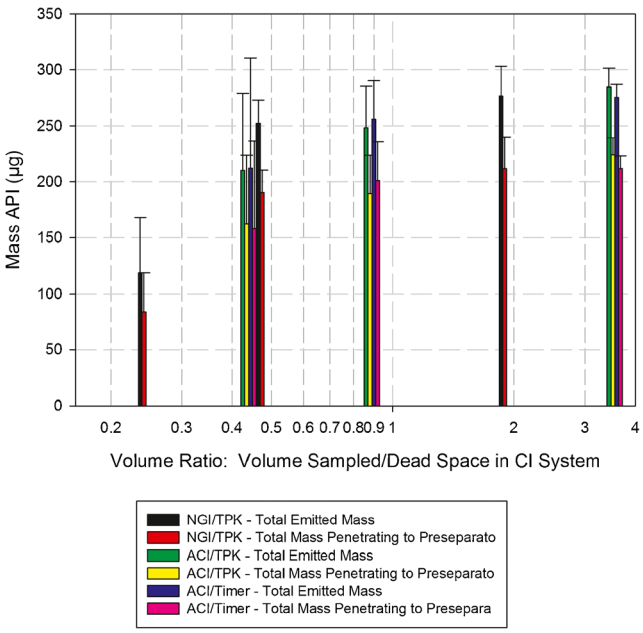

Fig. 4. Product Y mass transfer from inhaler to the CI system for measurements made at different values of V^* with the Cyclohaler® DPI

Taking the Cyclohaler®-based data with the NGI first, the comparatively slow mass transfer from the inhaler to the NGI is particularly evident at low V^*, where both TEM (119±49 µg) and $T_{CI\ system}$ (84.0±35.0 µg) were well below their equivalent values when V^* was 1.98 at the compendial 4-L sample volume (TEM=277.0±26.1 µg; $T_{CI\ system}$=211.6±28.5 µg). Interestingly, at the intermediate condition where V^*, at 0.49, was just less than 50% of the internal dead space, both TEM and $T_{CI\ system}$ (252.5±20.9 and 190.4±19.8 µg, respectively) were insignificantly different from their corresponding values at the compendial sample volume [paired t test for each metric, $p \geq 0.27$]. Likewise, values of the ratio $T_{CI\ system}$/TEM increased marginally from 70.5±0.9% at the lowest V^* (0.25) to 75.4±1.9% at the intermediate value of 0.49 and to 76.2±3.2% when V^* was highest (1.98) [one-way ANOVA, p=0.04]. Both findings indicate that mass transfer of the aerosol bolus from the Cyclohaler® DPI was completed as far as the pre-separator of this CI system even when V^* was <1.0.

In contrast to the Cyclohaler® data, bolus transfer from the higher resistance HandiHaler® DPI was complete when V^* was at its minimum value of 0.25 with the NGI-based measurements, since TEM at this condition (2.15±0.21 mg) was insignificantly different to the corresponding values at both intermediate (2.20±0.08 mg) and compendial (2.40±0.09 mg) sample volume for V^* [one-way ANOVA, p=0.14]. The comparable values of $T_{CI\ system}$ at low and intermediate values of V^* (1.44±0.12 and 1.43±0.06 mg, respectively) increased to 1.65±0.05 mg at the compendial sample volume, but the change was only marginally significant [$p \leq 0.025$].

Returning to the Cyclohaler® DPI data, but this time considering the ACI, the use of the two different flow control systems provided comparable outcomes when V^* was 3.46 (4 L volume per the compendial method) in terms of both TEM and $T_{CI\ system}$ (284.6±16.9 and 224.1±15.2 µg,

respectively, for the TPK controller) and 275.7±11.3 and 211.9±11.3 μg, respectively, for the timer-solenoid valve option [$p \geq 0.33$]). A similar outcome was evident with the corresponding HandiHaler® DPI data, where values of TEM (2.40±0.06 mg (TPK); 2.36±0.03 mg (timer-solenoid valve)) and $T_{CI\ system}$ (1.55±0.05 mg (TPK); 1.55±0.04 mg (timer-solenoid)) were each also comparable [$p \geq 0.41$]. However, it is perhaps more pertinent in the context of this article to examine how these metrics compared when V^* was reduced to its lowest value for the ACI system (0.43). When the TPK controller was used with the Cyclohaler® DPI, TEM and $T_{CI\ system}$ were 210.2±69.3 and 162.2±61.5 μg, respectively, and with the timer-solenoid flow control option, these values were 212.2±98.1 and 158.0±78.4 μg, respectively. When the TPK controller was used for the measurements with the HandiHaler® DPI, TEM and $T_{CI\ system}$ values were 2.31±0.06 and 1.58±0.08 mg. These values were quite close to those observed for the timer-solenoid valve option, 2.31±0.10 and 1.52±0.08 mg. Thus, at the lowest values of V* for the ACI system, the TPK controller results and the timer-solenoid results were the same for both drug products.

The measurements of time to reach 90% of the nominal flow rate of 60 L/min (T_{90}) for the two flow controllers with and without ACI in the supplementary experiment using a critical orifice instead of a DPI are summarized in Table VIII. The TPK flow controller was well within the pharmacopeial specification of <100 ms for valve opening/closing time with or without the presence of the additional dead space in the ACI system that lengthened the average rise time (T_{90}) from 10 to 33 ms. These rise times are typical of current flow control equipment and correspond with air acceleration rates between 100 and 30 L/s/s. On the other hand, the rise times of the timer-solenoid controller were more than an order of magnitude longer, at 203 and 315 ms alone and with the ACI system present, and likely reflect the fact that in this older design, solenoid valve operating characteristics had not been selected for optimum opening/closing speed. The air acceleration rates associated with these longer rise times are 5 and 3 L/s^2, respectively.

The APSD measurements are the ultimate diagnostic of successful size analysis and are therefore illustrated in cumulative mass of API-weighted form in Figs. 6, 7, and 8 for the measurements with the three CI system configurations undertaken with the Cyclohaler® DPI. Corresponding data determined using the HandiHaler® DPI are provided in Figs. 9, 10, and 11.

The measurements with the NGI have the advantage that the upper-bound size limit of 12.7 μm aerodynamic diameter at 60 L/min is defined by the pre-separator for which a calibration is available (12). It should be noted that the mass

Table VIII. Comparative Evaluation of Rise Times to 90% of Nominal Flow Rate of 60 L/min (T_{90}; mean±SD) Using a Critical Orifice to Provide a Pressure Drop of 4 kPa for the Timer-Solenoid and TPK Flow Controller Systems

Test arrangement	T_{90} (ms)
TPK alone	10.0±0.0
TPK with ACI	33.0±2.9
Timer-solenoid valve alone	203±2.9
Timer-solenoid valve with ACI	315±5.0

ACI Andersen eight-stage cascade impactor

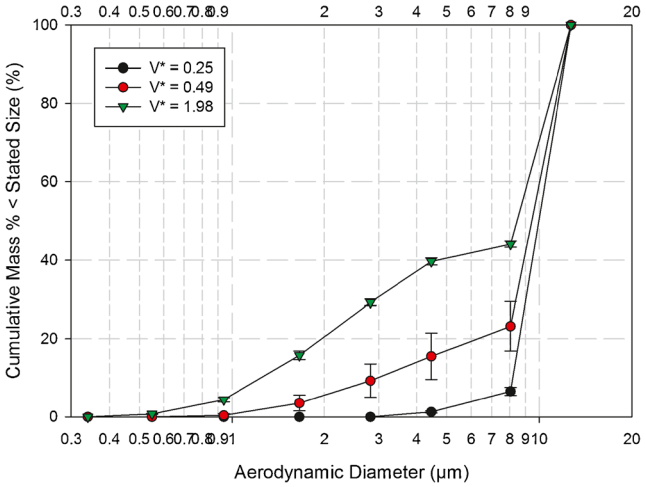

Fig. 6. APSDs for product Y from Cyclohaler® DPI with NGI at 60 L/min

passing the pre-separator and entering the first stage of the NGI is included in these NGI-generated APSDs; hence, their shapes are different from the normal representation of impactor data in which the upper-bound size would not include the pre-separator and therefore be limited to 8.1 μm aerodynamic diameter. Furthermore, since they are scaled to the total mass of API penetrating beyond the induction port, by definition, the cumulative mass corresponding to the upper-bound size is 100% at all values of V^*. The earlier study had confirmed that the compendial sample volume of 4 L was adequate for the aerosol bolus to have travelled through the entire system and be fully size fractionated (4), so APSDs obtained with V^* of 1.98 can be considered as representing the reference or fully size-analyzed state. Given this background information, it is therefore easy to see for measurements with either DPI (Fig. 6 and 9) that the compendial sample volume (V^*=1.98) was required to achieve adequate size fractionation throughout this CI system. Even if bolus mass transfer to this CI system had been quicker from the HandiHaler® DPI, as implied by the comparisons with TEM and $T_{CI\ system}$ previously discussed, the full 4-L sample volume was still needed to complete the size fractionation process with this CI.

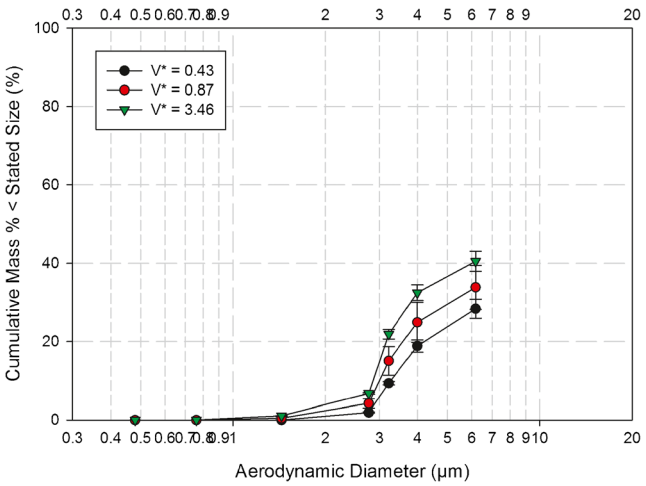

Fig. 7. APSDs for product Y from Cyclohaler® DPI with ACI/TPK controller at 60 L/min

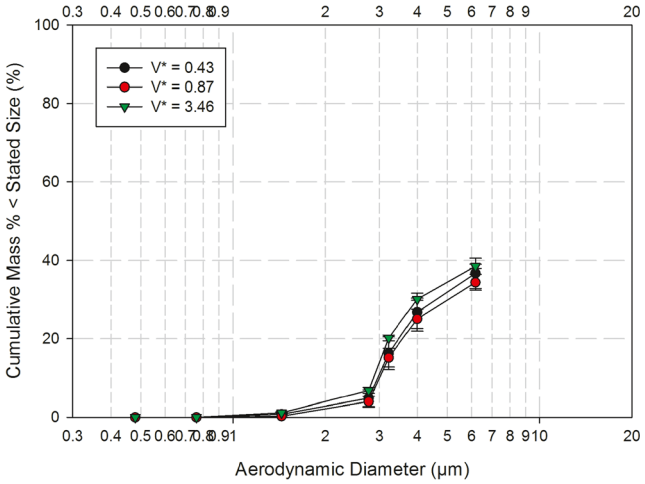

Fig. 8. APSDs for product Y from Cyclohaler® DPI with ACI/timer-solenoid valve flow control at 60 L/min

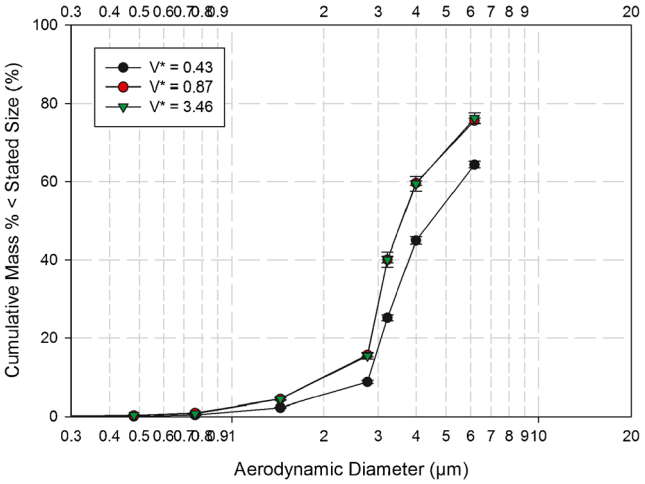

Fig. 10. APSDs for product Z from HandiHaler® DPI with ACI/TPK controller at 60 L/min

In the case of the ACI-based APSDs, the upper-bound size was limited to 6.2 μm aerodynamic diameter, as neither the first stage (stage 0) nor the pre-separator has a suitable calibration at 60 L/min. However, since the cumulative size-fractionated mass was scaled to the total mass of API that reached as far as the pre-separator, these non-sizing components are included in the denominator of the calculations, with the result that the maximum cumulative mass that can be represented in this way was well below 50%. Despite this ordinate scaling limitation, the APSDs for both DPIs demonstrated a remarkably good agreement regardless of *V** throughout the range evaluated. The choice of flow controller had only a marginal influence on these ACI-measured APSDs from either DPI, despite the evidence previously presented, of more rapid bolus transfer to the CI system in the case of measurements with the Cyclohaler® DPI.

DISCUSSION

These measurements were not intended to provide either *in vitro* comparative performance data following the compendial methodology or to simulate clinical use of either DPI. Rather, the flow rate was fixed at 60 L/min from both inhalers in order to provide a consistent comparison between the different CI systems on the basis of an equivalent volume of air containing the aerosol bolus at different elapsed time intervals from initiation of sampling after opening the valve to the vacuum pump. The alternative approach of fixing the pressure drop at 4 kPa would have made it necessary to vary sample times widely for the two inhalers to achieve comparable sample volumes. More importantly, sampling times for the low-resistance Cyclohaler® DPI evaluated at the upper limit of 100 L/min set by the pharmacopeias would have needed to be as short as 0.3 s to achieve the smallest sample volume (0.5 L) required to make this investigation meaningful. This time interval is close to the limit of capability for the response time for the electromechanical components comprising the flow control systems and would therefore likely have resulted in an unacceptable decrease in measurement precision.

Given this constraint in methodology, the data obtained from this extension of the original investigation to encompass DPIs representing the extremes of flow resistance

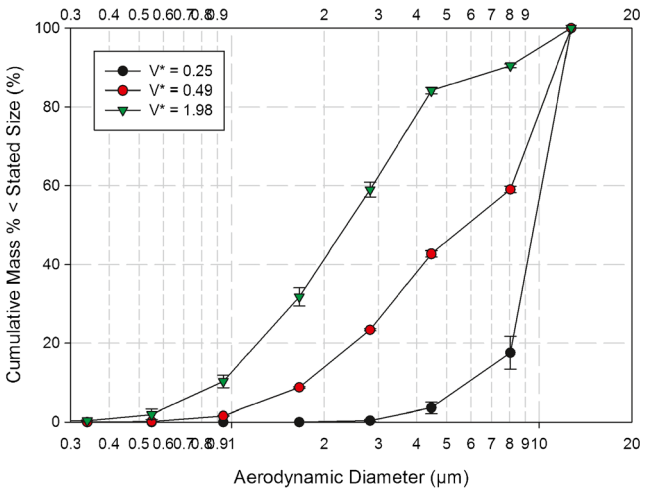

Fig. 9. APSDs for product Z from HandiHaler® DPI with NGI at 60 L/min

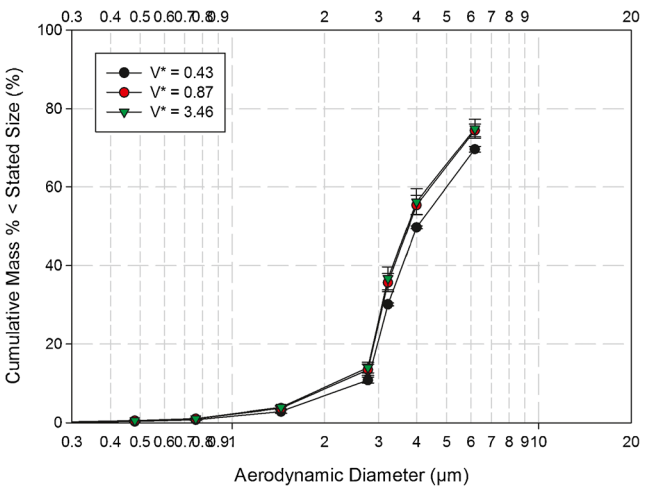

Fig. 11. APSDs for product Z from HandiHaler® DPI with ACI/timer-solenoid valve flow control at 60 L/min

nevertheless confirmed that the aerosol bolus transfer and complete subsequent size fractionation in the NGI required almost two complete sample volumes to be taken (V^*=1.98). By contrast, this state was reached at a significantly earlier stage with the ACI ($V^* \geq 0.87$) used with either TPK or timer-solenoid valve flow control options.

The differing behavior between the three CI configurations observed in particle size fractionation behavior is best illustrated if the cumulative mass-weighted APSDs for each inhaler (Figs. 12, 13, and 14) are expressed in terms of ratios between test and reference conditions for each DPI, where TEST represents measurements made at each of the two V^* values smaller than at the single reference (compendial) sample volume. In the ideal situation, the test/reference ratio would be expected to remain at unity irrespective of particle size. Each plot therefore contains two curves for each DPI, one at intermediate and the other at the lowest value of V^*. Under these circumstances, the low to intermediate ratios associated with the NGI data (Fig. 14) dramatically demonstrate the resulting incomplete size fractionation of the aerosol bolus from either inhaler when the compendial sample volume of 4 L was reduced. Furthermore, the steepness of the positive slope associated with each curve is indicative of the extent of incomplete penetration of the aerosol to the most distal impactor stages including the micro-orifice collector that functions similar to the back-up filter in the ACI for the collection of the finest particles penetrating all size fractionating stages. Interestingly, the differences between the curves obtained with either of the inhalers were small, whether V^* was either 0.25 or 0.49. On this basis, even though the aerosol bolus appears to have arrived slightly earlier at the pre-separator in the sampling process from the high-resistance HandiHaler® DPI compared with Cyclohaler®-generated aerosols (based on the data presented in Fig. 14), the time needed for correct size fractionation in the CI itself was only marginally affected.

Conversely, the corresponding plots comparing test and reference conditions for the ACI-sampled aerosols from the HandiHaler® DPI show that size fractionation was close to completion, except for perhaps the finest particles when V^* was as small as 0.87 (Fig. 14). Even when V^* was at its smallest value of 0.43 with this CI, the extent of size fractionation with

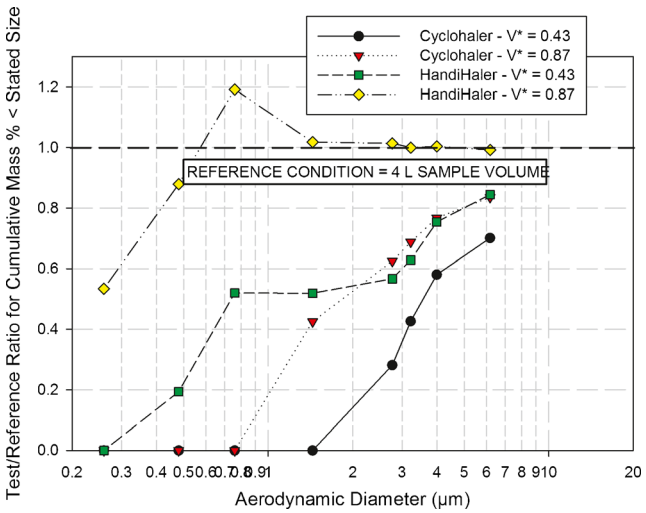

Fig. 13. ACI-TPK controller-based APSD ratios for Cyclohaler®- and HandiHaler®-generated aerosols at medium and lowest values of normalized volume (V^*)

the aerosol emitted from the HandiHaler® DPI was comparable to that with the NGI for the intermediate value of V^*(0.49) associated with the measurements with the latter system. On the other hand, the steeper slopes of the curves associated with measurements made sampling Cyclohaler® DPI-generated aerosols are indicative that these had barely begun to be size fractionated by the ACI (irrespective of flow control arrangement), even when V^* was set at 0.87. It therefore appears that for this CI system, the more rapid transfer of the aerosol bolus from the higher flow resistance HandiHaler® DPI to the pre-separator may have also influenced the speed of the subsequent size fractionation process.

These findings are important because they indicate that for the ACI at least, the flow resistance of the inhaler has a part to play in determining the extent of the anomalous outcomes at V^*<1.0. The differences in aerosol measurement

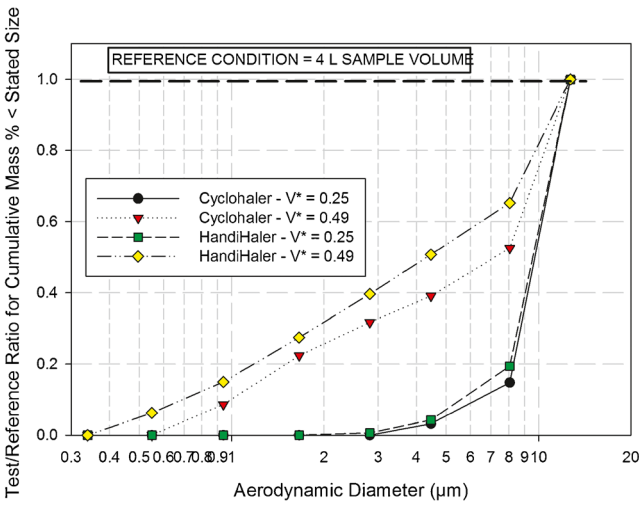

Fig. 12. NGI-based APSD ratios for Cyclohaler®- and HandiHaler®-generated aerosols at medium and lowest values of normalized volume (V^*)

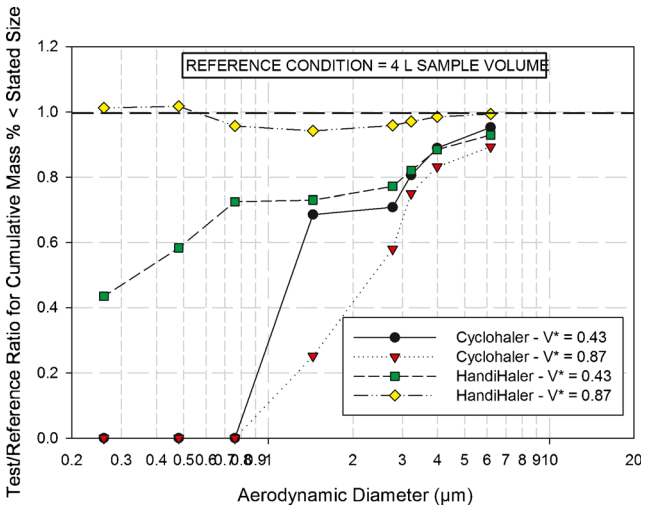

Fig. 14. ACI-timer-solenoid valve flow control-based APSD ratios for Cyclohaler®- and HandiHaler®-generated aerosols at medium and lowest values of normalized volume (V^*)

behavior that were observed between the ACI and NGI systems in the preceding investigation had been attributed primarily to the CI system, rather than to the inhaler (4). It now seems that by comparing ACI system performance with DPIs representing a wider range of flow resistance, the kinetics of aerosol bolus transport from the DPI and its subsequent size fractionation in this CI may both be influenced by flow resistance, confirming the hypothesis underlying the purpose of this extension study. Intuitively, such an outcome might be expected, given the substantial divergence in pressure-flow characteristics that are related to the differing geometries of the internal aerosol passageways when the two inhalers are compared side-by-side (Fig. 15) In support of this finding, it is germane that Shur *et al.*, in an extensive study examining both experimentally and through CFD modeling the aerosol bolus transfer through both the same DPIs (13), observed that there are noticeable differences in aerosol transfer between them even when the same formulation and capsule (Spiriva®, Boehringer Ingelheim) were evaluated in both devices to eliminate formulation/capsule differences. Their experimental investigation was restricted to the NGI with its pre-separator; however, they observed that the magnitudes of the differences in the API mass deposition profiles within the CI between these two DPI devices appeared to be flow rate dependent, becoming most pronounced for the upper stages (stages 1–4) of the NGI when the flow rate was increased from 39 to 55 L/min, the higher value being quite close to the 60 L/min fixed flow rate of the present study. They concluded that the high flow resistance of HandiHaler® DPI contributed to more efficient bolus transfer at the higher flow rate, compared with the Cyclohaler® DPI. The present investigation was not designed to provide a "heads-on" comparison of the mechanics of powder to aerosol formation between these DPIs so that each DPI was evaluated with capsules containing formulation intended for that device and that the mass loadings in the capsules used were different. Nevertheless, despite this limitation, the work of Shur *et al.* (13) taken with the present findings indicates that DPI flow resistance as well as CI system dead space can both contribute to the kinetics of aerosol formation at each capsule exit aperture, followed by its transfer to the measurement apparatus and subsequent size fractionation therein. In summary, it is acknowledged that the kinetics of powder transfer from capsule within each DPI to the entry of the CI system under investigation may also play a part in determining the overall speed of transfer and subsequent size fractionation. Further investigation is therefore merited with drug products in which the flow rate-dependent aerosolization characteristics have been published. Furthermore, the findings from the present study will now need to be verified as a key component in any CFD modeling of the aerosol transport processes to the ACI and NGI systems that should follow this experimental investigation.

It is acknowledged that DPI testing with the ACI at 60 l/min can also be undertaken with modified stage configurations −0 and −1, removing stages 6 and 7 to enable the same number of stages overall to be retained (14). This arrangement provides for a more useful range of cut-point sizes in the critical size range from 0.5 to 5.0 μM aerodynamic diameter. However, the presented study was confined to the original ACI configuration that is described in the Pharmacopeial Compendia prior to 2014. It is recommended that similar measurements be undertaken at a future time using the alternative ACI configuration, given it increasing importance now that the option to use this approach has been confirmed as being introduced in 2014 with the next revision of Chapter 601 of the US Pharmacopeia. (15).

This investigation, like its predecessor (4), has demonstrated that while the NGI behaves as might be expected from a consideration of the way flow is propagated progressively from the control valve back through the CI system to the inhaler when the valve opens at the start of sampling, the ACI when used with either flow control arrangement (TPK controller or timer-solenoid valve) may give comparable APSDs at smaller sample volumes. Complete penetration and size fractionation of the aerosol bolus by this impactor should not occur when V^* is less than unity, unless there is some way that particle transport through the apparatus is enhanced. In the previous article, the anomalous behavior of the ACI was attributed to maldistribution of the radial flow profile through the uppermost stages where the collection plates are annular rather than solid across their sections (4). Since this article was written, new experimental evidence for

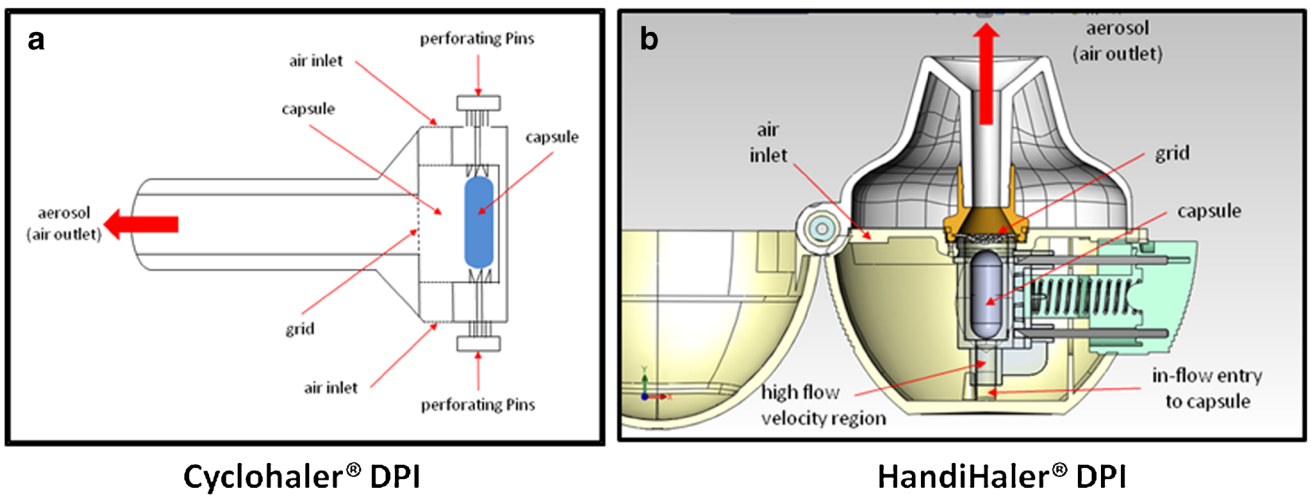

Fig. 15. **a**, **b** Air flow pathways through the two DPIs investigated

such behavior has come from the uneven particle deposits reported from an ACI calibrated with 5.0 μm aerodynamic diameter monodisperse oleic acid particles close to the nominal cut-point size of the stage 1. The concentric annular deposits on the collection plate located under the outermost and innermost of the four concentric nozzle rings of this stage are markedly more intense than those located under the inner two nozzle rings (Fig. 16). Furthermore, there is visual evidence for greater entrainment of the incoming particles that took place with the flow passing to the following stage for particles whose trajectories coincided with either the outermost or innermost nozzle rings (16). Nevertheless, even though this deposition pattern indicates that aerosol progression through the ACI may be more complex than in the NGI, it is important to be aware that the latter still functions correctly in accord with its stated calibration (12) as an inertial particle size fractionating apparatus when evaluating DPI performance using the sample volume of 4 L which is recommended by the pharmacopeias (1,2).

Flow rise time values ($T_{90\%}$) determined for both TPK and timer-solenoid valve flow controller systems (Table VIII) demonstrated the improved capability of the more modern TPK design in which solenoid valve opening/closing times were optimized and in accordance with the compendial guideline. Nevertheless, the resulting APSDs determined from either DPI were not greatly influenced by the choice of flow controller. It can therefore be concluded that flow controller capability in terms of $T_{90\%}$ is not likely to be a confounding parameter with DPI flow resistance in connection with the transport and subsequent size fractionation of DPI-generated aerosols, if current equipment such as the TPK controller or its successor that has a similarly short valve opening/closing time of 25 ms (TPK2000, Copley Scientific Ltd.) is used.

A significant limitation to the present study was the inability to reduce V^* for the measurements made with the ACI systems to a value closer to 0.25 that was achievable with the NGI system by virtue of its larger internal dead space. However, sample times would have had to be reduced to 0.29 s, a value that was impractical with either of the flow control systems available. In any case, such data would likely have only extended the observed trends seen reducing V^* from 0.87 to 0.43.

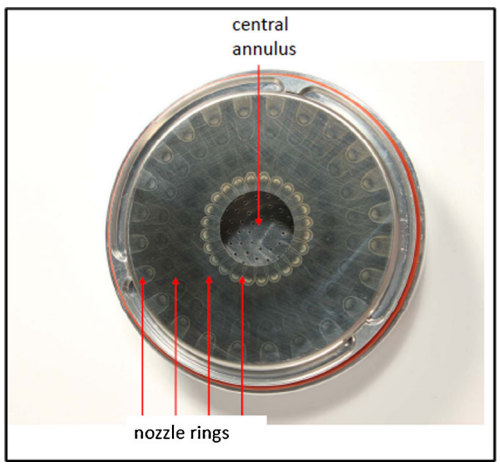

Fig. 16. Uneven radial deposits of 5 μm aerodynamic diameter monodisperse calibration particles on stage 1 of an ACI operated at 28.3 L/min; the deposits under the outermost and innermost nozzle rings of this stage are more intense than those located under the inner two nozzle rings, with evidence of greater entrainment with the flow to stage 2 for the deposits under the outermost and innermost nozzle rings

It should be noted that these considerations regarding the minimum sample volume do not apply to the assessment of dose content uniformity following the compendial procedures, in which a 2-L sample is all that will be required in a planned change to the USP method (17), because the internal dead volume of the dosage unit sampling apparatus for DPI testing, including connection tubing to its flow controller, is of the order of 140 cm^3, and the inhaler is coupled directly to it without the need for induction port or pre-separator components.

Finally, these measurements have identified that the ACI behaves differently to expectations based on the concept of plug flow through the system as the vacuum initiated at start of measurement by the opening of the valve downstream of this CI propagates from the valve to the inlet. Nevertheless, its continued use as a pharmacopeial CI for the accurate measurement of DPI-generated aerosol APSDs should not be put in jeopardy, so long as the compendial volume of 4 L is sampled. Likewise, the operation of the NGI should result in accurate determinations of aerosol APSD from this OIP class when a 4-L sample is taken. It also follows from the outcomes of the present experiments that attempt to reduce this volume below 4 L, perhaps to simulate more closely the volume of an average adult inhalation, should be resisted.

CONCLUSIONS

Both this investigation into DPI testing at sample volumes commensurate with the internal dead volume of the measurement apparatus, and its predecessors have provided support for the current sample volume of 4 L to be retained in the compendial procedure for the determination of APSD with this class of orally inhaled product. In the event that additional clinical realism is sought simplistically by reducing this volume so as to obtain a more realistic simulation of patient use with broncho-constrictive disease, the result will be an unacceptable level of size-related bias in the case of the NGI. Equivalent measurements by ACI may or may not be affected as much, depending upon the flow resistance of the inhaler. The choice of flow control, either by TPK unit or timer-solenoid valve, appears to have little effect upon the determined APSD profiles regardless of DPI flow resistance, lending support for the retention of either option as part of the compendial procedure.

Nevertheless, it is, however, known that many patients are not able to achieve the high inhalation flows required to create a pressure drop of 4 kPa (18,19), as simulated in the compendial procedure, and the temptation therefore may exist to modify the procedure by reducing sample volume. The compendial procedure allows for realistic *in vitro* comparisons as a quality test of the performance of DPIs having a wide range of flow resistance by constraining both pressure drop and sample volume (20). If the intent is to explore more clinically appropriate laboratory testing methods, for example in considerations of bioequivalence, alternative procedures are available that, for example, make use of an aerosol mixing device, as part of the aerosol sampling system. The DPI on test

with this modified sampling configuration is thereby decoupled from the CI system so that the former can be evaluated with a more clinically appropriate inhalation flow rate–time profile for the intended user population, and the latter can be operated for as long as is necessary to obtain an unbiased APSD measurement (21,22).

ACKNOWLEDGMENTS

The authors wish to acknowledge the support of TEVA-Pharmachemie, Netherlands, and Boehringer-Ingelheim, Germany, for the supply of the DPI products and to other members of the Cascade Impactor Sub-Team of the EPAG for their advice and support during the experimental work and in the internal reviewing of this article.

REFERENCES

1. European Directorate for the Quality of Medicines and Healthcare (EDQM): European Pharmacopeia 6(8). Chapter 2.9.18. Preparations for inhalations: aerodynamic assessment of fine particles. Strasbourg: Council of Europe; 2010.
2. US Pharmacopeial Convention. United States Pharmacopeia; USP 33-NF 28; Chapter 601—Physical tests and determinations: aerosols. United States Pharmacopeia, Rockville, MD, USA; 2010.
3. Copley M, Smurthwaite M, Roberts DL, Mitchell JP. Revised internal volumes to those provided by Mitchell JP and Nagel MW in cascade impactors for the size characterization of aerosols from medical inhalers: their uses and limitations. J Aerosol Med. 2005;18(3):364–6.
4. Mohammed H, Roberts DL, Copley M, Hammond M, Nichols SC, Mitchell JP. Effect of sampling volume on dry powder inhaler (DPI)-emitted aerosol aerodynamic particle size distributions (APSDs) measured by the Next-Generation Pharmaceutical Impactor (NGI) and the Andersen Eight-Stage Cascade Impactor (ACI). AAPS PharmSciTech. 2012;13(3):875–82.
5. Copley M. Improving inhaled product testing: methods for obtaining better *in vitro-in vivo* relationships. Pharm Tech (Europe). 2013;37(2):1–6. Available at: http://www.pharmtech.com/pharmtech/Peer-Reviewed+Research/Improving-Inhaled-Product-Testing-Methods-for-Obta/ArticleStandard/Article/detail/804866 visited June 20, 2013.
6. De Boer AH, Bolhuis GK, Gjaltema D, Hagedoorn P. Inhalation characteristics and their effects on in vitro drug delivery from dry powder inhalers: part 3: the effect of flow increase rate on the in vitro drug release from the Pulmicort 200 Turbuhaler. Int. J. Pharm. 1997; 153.
7. Beron KL, Grabek CE, Jung JA, Shelton CM. Flow rate ramp profile effects on the emitted dose from dry powder inhalers. In: *Drug delivery to the lungs, 19*. Edinburgh, The Aerosol Society; 2008, 61–4.
8. Van Oort M, Downey B. Cascade impaction of MDIs and DPIs: induction port, inlet cone, and pre-separator lid designs recommended for inclusion in the general test chapter "aerosols" <601> Pharm Forum. 1996;22(2):2204–10.
9. Marple VA, Roberts DL, Romay FJ, Miller NC, Truman KG, Van Oort M, *et al*. Next generation pharmaceutical impactor. Part 1: design. J Aerosol Med. 2003;16(3):283–99.
10. Olsson B, Asking L. Critical aspects of the function of inspiratory flow driven inhalers. J Aerosol Med. 1994;7S1:S43–7.
11. Donovan MJ, Kim SH, Raman V, Smyth HD. Dry powder inhaler device influence on carrier particle performance. J Pharm Sci. 2012;101(3):1097–107.
12. Marple VA, Olson BA, Santhanakrishnan K, Mitchell JP, Murray S, Hudson-Curtis B. Next Generation Pharmaceutical Impactor. Part II: calibration. J Aerosol Med. 2003;16:301–24.
13. Shur J, Lee S, Adams W, Lionberger R, Tibbatts J, Price R. Effect of device design on the in vitro performance and comparability for capsule-based dry powder inhalers. AAPS J. 2012;14(4):667–76.
14. Nichols SC, Brown DR, Smurthwaite M. New concept for the variable flow rate Andersen cascade impactor and calibration data. J Aerosol Med. 1998;11:133–8.
15. United States Pharmacopeial Convention. <601 > Inhalation and nasal drug products: aerosols, sprays, and powders—performance quality tests. Pharm Forum 2014;40(2): in press.
16. Roberts DL. Calibrating cascade impactors with particles—approaches and pitfalls. Inhalation. 2013;7(2):18–23.
17. United States Pharmacopeial Convention. In process revision: <601 > Inhalation and nasal drug products: aerosols, sprays, and powders—performance quality tests. Pharm. Forum. 2013;39(1) available on-line at: http://www.usppf.com/pf/pub/index.html visited July 9, 2013.
18. Pedersen S, Hansen OR, Fuglsang G. Influence of inspiratory flow rate upon the effect of a Turbuhaler. Arch Dis Child. 1990;65:308–19.
19. Broeders MEAC, Molema J, Hop WCJ, Folgering HTM. Inhalation profiles in asthmatics and COPD patients: reproducibility and effects of instruction. J Aerosol Med. 2003;16:131–41.
20. Mitchell JP, Newman S, Chan H-K. *In vitro* and *in vivo* aspects of cascade impactor tests and inhaler performance: a review. AAPS PharmSciTech. 2007;8(4):237–48.
21. Nadarassan DK, Assi KH, Chrystyn H. Aerodynamic characteristics of a dry powder inhaler at low inhalation flows using a mixing inlet with an Andersen Cascade Impactor. Eur J Pharm Sci. 2010;39:348–54.
22. Olsson B, Borgström L, Lundbäck H, Svensson M. Validation of a general *in vitro* approach for prediction of total lung deposition in healthy adults. J. Aerosol Med. Pulmon. Deliv. 2013;26, in press.