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RESEARCH PAPER

Entry Port Selection for Detecting Particle Size Differences in Metered Dose Inhaler Formulations Using Cascade Impaction

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ABSTRACT

Different sized glass entry ports were evaluated for their drug collection efficiency during aerodynamic particle sizing of metered dose inhalers (MDIs) using cascade impaction. A comparison was made between collection efficiency in the entry port, impactor plates, and filter using the 1 L, 2 L, and 20 L glass entry ports and the USP and twin impinger entry ports. Entry port losses were dependent on the size of entry port selected, with 1–2 L ports showing optimal recovery on impactor plates, compared to the USP entry port. The 1 L entry port was further compared with the USP entry port in its ability to discriminate between subtle changes in particle size distribution (PSD) in an investigational hydrofluoroalkane (HFA)-based MDI formulation. Deliberately induced differences during product manufacture were easily detected using the 1 L entry port with the Andersen cascade impactor. The USP port was unable to distinguish among products with small particle size differences. An alternative entry port such as the 1 L glass entry port used in this study may provide better means of characterizing the PSD during formulation development and stability testing of MDIs.

Key Words: Metered dose inhaler (MDI); Cascade impaction; Entry port.

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INTRODUCTION

Particle size distribution (PSD) of the emitted dose of a metered dose inhaler (MDI) is the most important factor affecting its functionality. Particle size of aerosol plumes from an MDI is not only dependent on the drug particle size, but is also influenced by the formulation components, storage condition, actuator design, orifice diameter, valve, and the mouthpiece. Aerodynamic particle sizing using a multistage cascade impactor is well accepted as the most appropriate method for characterizing inhalation aerosols.[1] The device fractionates the aerosol cloud into several size fractions, which are then quantified using a sensitive drug assay. Since only the drug is assayed, changes in drug particle size as a function of formulation or packaging components and storage stability can be assessed using cascade impaction during product development.[2]

However, for the cascade impaction technique to distinguish between small formulation or process-induced changes and particle size changes upon storage at accelerated stability conditions, a large amount of the emitted dose needs to be sampled onto the impactor stages.[3,4] Although commercially available cascade impactors are well characterized with respect to airflow and stage cutoff diameters, the use of an entry port to connect the impactor to the MDI is not well established.[5,6] Several attempts have been made to model the entry port on the human oropharynx.[7] The rationale for this has been that the human throat acts as an effective filter for material emitted from an oral MDI, because of the ballistic component of the aerosol plume. This has led to the design of an induction port, fabricated from aluminum or stainless steel and now accepted in the European and US Pharmacopoeias.[8] Although, the so-called ''USP-throat'' represents an over simplification of the human anatomy, it serves the purpose of filtering the coarse aerosol as would perhaps the human throat. However, due to its unique geometry, the USP-throat suffers from the disadvantage of filtering an abnormally large quantity of the emitted dose. In addition, it allows for reduced evaporation of droplets inside the emitted aerosol plume. This leads to inadequate sampling of the representative aerosol inside the cascade impactor. This has been routinely overcome by firing additional shots into the impactor, but this technique does not realistically sample the labeled dose, usually one or two shots for most aerosol products.[9]

A cascade impaction technique that can discriminate between small changes in the aerosol particle size distribution (PSD) during formulation development and under stability conditions would greatly benefit the product development scientist. With this goal in mind a systematic study was undertaken to select a sampling port, which allows for adequate sampling of the drug particles or droplets inside the cascade impactor. Initial studies were performed using chlorofluorocarbon (CFC)-based MDIs and several entry ports were evaluated. The second part of the study was used to compare the selected entry port with the USP-throat for sampling efficiency of CFC and hydrofluoroalkane (HFA) aerosols. The ability of either entry port was evaluated for its ability to discriminate between products deliberately manufactured to show subtle changes in the PSD.

MATERIALS AND METHODS

Study 1: Selection of Entry Port

Pressurized metered dose inhalers (MDIs) containing beclomethasone dipropionate (Vanceril®, Schering-Plough, Kenilworth, NJ) were used in this study. This CFC-based MDI delivered 42 μg/actuation. A six-stage Impaq cascade impactor (Impaq AS-6, California Measurements, Sierra Madre, CA) was used to analyze the aerosol PSD at an airflow of 12.5 liters/min. The aerodynamic cutoff for the stages were 16, 8, 4, 2, 1, and 0.5 μm for stages 1–6, respectively,[10] and a filter was used downstream of stage 6. Three different glass entry ports were used in addition to the Twin Impinger glass throat[11] and the USP-throat. The chamber volumes of these glass entry ports were 20 liters, 2 liters, and 1 liter. The twin impinger entry port had a volume of 100 mL. Schematic drawings of the entry ports with exact dimensions are depicted in Fig. 1. A modified inlet cone was used to connect the glass entry ports to the impactor.

The MDIs were shaken and actuated three times to prime and allowed to stand for 3 min. The can was then fitted with a fresh actuator, shaken, and four actuations were discharged into the cascade impactor-entry port set-up at 30 sec intervals. The entry port and cascade impactor plates were rinsed with appropriate volumes of methanol. The filter was also removed and extracted with methanol. All samples were analyzed using a validated high-performance liquid chromatography (HPLC) method for the drug substance, with a limit of quantitation (LOQ) of 0.03 μg/mL.

Study 2: Comparison of Impaq and Andersen Impactors

The selected entry port from the above study (1 L) was used with Impaq-6 and Andersen cascade impactors,

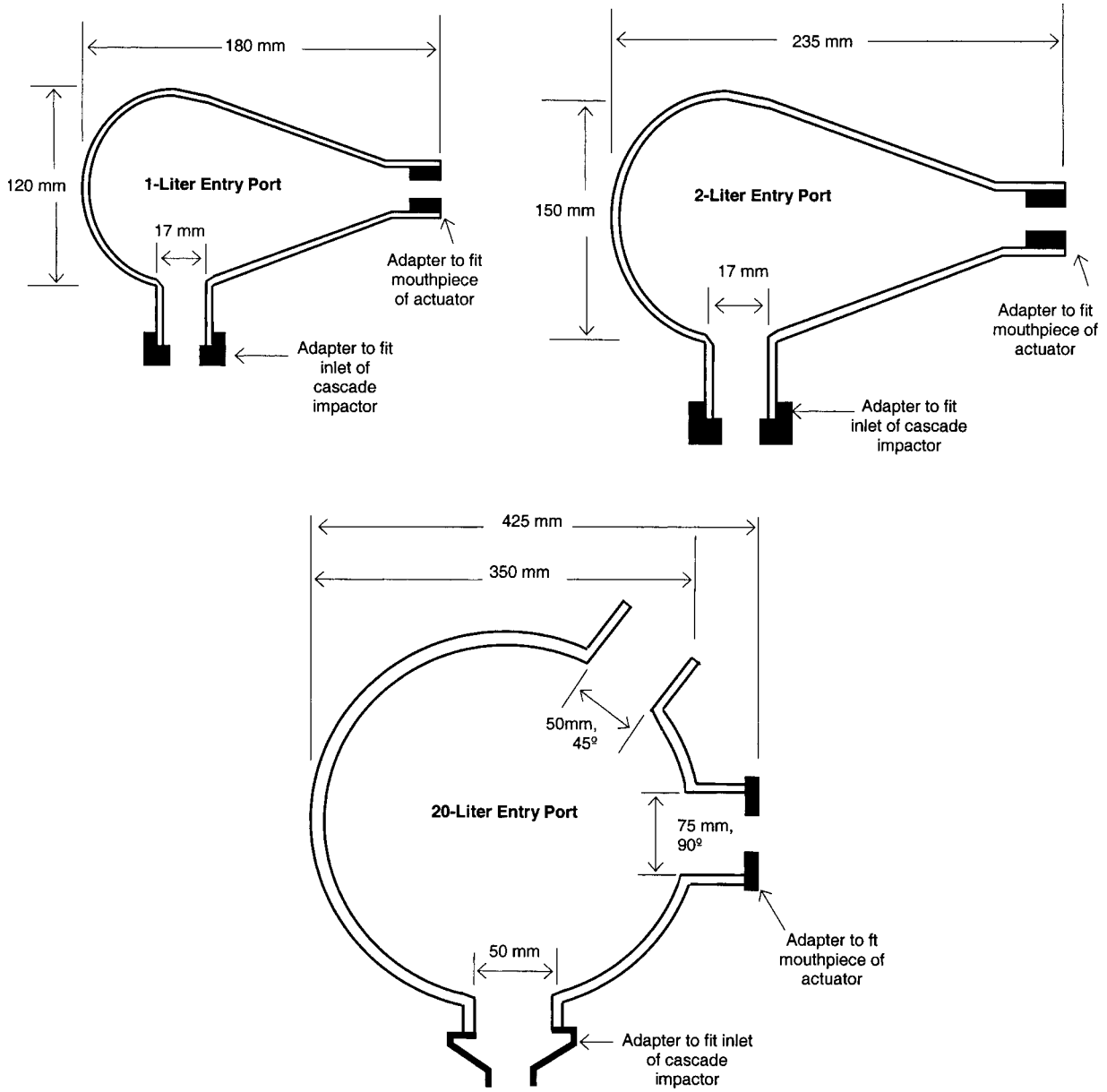

Figure 1. Schematic diagrams with actual dimensions for the 1-liter, 2-liter, and 20-liter entry ports used in the study.

to compare PSDs. This study was performed using an eight-stage Andersen cascade impactor at a flow rate of 28.3 liters/min (Mark II, Graseby-Andersen, Smyrna, GA). The Impaq-6 was operated at the flow rate of 12.5 liters/min as described above. The CFC-based beclomethasone dipropionate MDI, delivering a dose of 42 μg/actuation, was used in these studies. The sampling and analytical procedures used were identical to those described in the above section. The amount of drug collected on plates and filter was used to calculate the cumulative percentage of undersize drug distribution. The mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) were calculated from the fit of percentage cumulative undersize data to log normal distribution. For comparative purposes, the fine particle fraction was estimated by fitting the cumulative undersize data from the impactors to a log normal distribution and calculating the particle mass <5.5 μm from this linear regression. The estimation from a linear regression plot was needed because the two impactors have different cut-off sizes.

Study 3: Comparison of USP and 1-Liter Entry Ports

An additional study was undertaken with the Andersen cascade impactor and the two entry ports (1-Liter and USP entry ports). In this study an investigational HFA-based steroid formulation was deliberately manufactured to exhibit particle size differences in the finished product. In the first part of this study (Study 3A), two drug substances with varying particle size distributions were used. The drug substance particle size was measured using a Sympatec laser diffraction unit attached to a RODOS powder disperser (Sympatec, Clauszellerfield, Germany). The drug was homogenized in a surfactant/alcohol mixture and HFA-227 was then added to the drug mixture in a pressurized tank. The entire formulation was pressure filled into commercially available 14-mL aerosol cans crimped with a 63 μL valve and fitted with a common oral actuator mouthpiece. All cans were crimped and filled using laboratory aerosol equipment (Pamasol/DH Industries, Essex, UK). The MDI cans obtained from the two drug substance lots are referred to as ''Coarse DS'' and ''Fine DS.''

In the second part of the study (Study 3B), the product was manufactured using a common ''two-step'' manufacturing technique, in which the appropriate amount of drug concentrate (drug+surfactant+alcohol) was filled into empty aerosol cans. These cans were then crimped with the valve and pressure filled with the appropriate amount of propellant. The samples for impaction testing were taken from the beginning and end of batch manufacture to allow for crystal growth during manufacture. The manufactured MDIs had a dose delivery of 100 μg/actuation. The MDI cans obtained from the beginning and end of the batch manufacture are designated as ''Start of Fill'' and ''End of Fill,'' respectively.

Each can was primed four times prior to testing. Two test actuations from each inhaler were fired into the impactor. Three cans were tested for each entry port (1-Liter and the USP Entry Ports). Drug samples were analyzed using a validated HPLC method with spectrophotometric detection. The mass of drug collected on each stage or accessory was calculated as a percentage of total drug recovered in the apparatus, which included the entry port, stages, filter, and stage housings. The mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) were estimated based on the USP method. The fine particle mass was defined as percentage of drug $\leq$4.7 μm.

RESULTS AND DISCUSSION

Selection of Entry Port

Three glass entry ports (EP) were selected for evaluation along with the glass EP of the Twin Impinger and the metal USP entry port. The volumes of the selected glass entry ports were 1 L, 2 L, and 20 L, while the twin impinger EP had a volume of 100 mL. The percentage of ex-actuator drug delivered to the impactor plates and/or the entry port was profoundly affected by the size of the EP. The six-stage Impaq cascade impactor was used to perform the EP screening, due to its ease of use and the sample collection being less labor intensive compared to the Andersen cascade impactor. Drug recoveries from the different EPs and from the impactor plates and filter are summarized in Table 1. The total recoveries were comparable for all entry ports evaluated. The impactor recoveries were markedly higher for the 1-liter and

Table 1. Drug recoveries in different entry ports and the cascade impactor (Impaq) using the CFC beclomethasone dipropionate MDI.[a]

	Entry port recoveries				
	20-Liter	2-Liter	1-Liter	Twin impinger	USP
Impactor plates and filter (μg)	127.0 (2.0)	120.6 (5.1)	145.9 (3.2)	83.8 (8.7)	43.8 (5.1)
Entry port (μg)	31.8 (1.3)	12.5 (1.3)	14.9 (0.5)	74.2 (8.2)	111.0 (6.1)
Total ex-actuator (μg)	158.8 (2.2)	133.1 (5.5)	160.8 (2.8)	158.0 (4.9)	154.8 (10.0)
% Recovered on plates and filter	80.0 (0.8)	90.6 (0.9)	90.7 (0.4)	53.1 (5.0)	28.2 (2.0)
% Recovered in entry port	20.0 (0.8)	9.4 (0.9)	9.3 (0.4)	46.9 (5.0)	71.8 (2.0)

[a]Average (SD); n=3 cans; four actuations per can.

2-liter entry ports compared to the USP and twin impinger entry ports. Optimal drug recoveries of 90% on impactor plates were obtained with the 1-liter and 2-liter entry ports. Entry port recoveries were comparable and lowest for the 1-liter and 2-liter entry ports. The smaller, twin impinger, and USP entry ports retained most of the drug delivered from the actuator. Although drug recovery inside the 20-liter entry port was higher compared to the two smaller EPs, it was suboptimal when compared to the 1- and 2-liter EPs.

A comparison of the percentage of the ex-actuator dose recovered inside the entry ports is shown in Fig. 2. The losses with the 1- and 2-liter EPs were minimal at around 9% compared to the other entry ports evaluated, especially the smaller EPs. As could be expected based on several previous reports, the USP entry port showed the highest drug retention of 72%.

The percentage of drug recovered on the impactor plates and the filter as a function of the EP volume is depicted in Fig. 3. From the data it is evident that there is an optimal volume of the EP, which maximizes impactor recovery. From the results obtained in this study it appears that the optimal chamber size should be in the range of 1–2 liters. This agrees well with the earlier work of Van Oort, Gollmar, and Bohinski, who showed that from chambers ranging in size from 250–5000 mL, 1000 mL gave the optimal impactor collection efficiency, although they suggested that a 500-mL chamber was sufficient in providing effective drug deposition on impactor plates.[12] Other factors besides size such as the chamber geometry and spray orientation could potentially affect drug deposition. Earlier studies have ruled out the importance of MDI spray orientation, although orientation and geometry become significant with smaller entry ports.[12] Based on our present study we suggest that the 1-liter EP will provide better collection efficiency, although the 1- and 2-liter EPs showed identical drug collection efficiency. This is because the smaller 1-liter chamber uses much less solvent rinse and enables better drug quantitation compared to the 2-liter EP. In addition, the unique shape of the glass fabricated 1-liter EP allows for proper expansion of the aerosol plume exiting the actuator inside the chamber (Fig. 1). This allows adequate droplet evaporation and minimal wall deposition before the aerosol is drawn into the impactor.

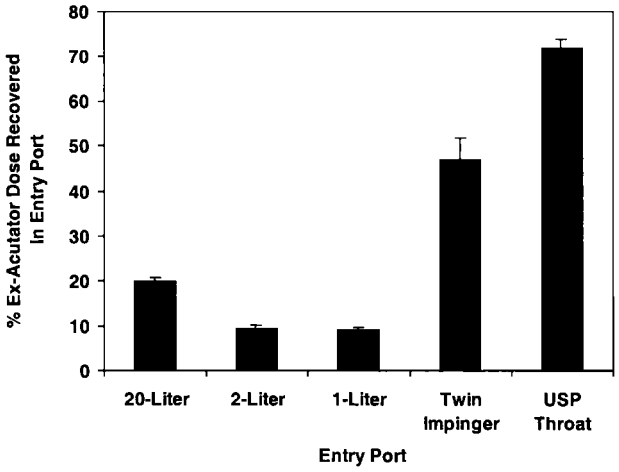

Figure 2. Percentage recovery of the ex-actuator dose in different entry ports used in the study; N=3, average and SD.

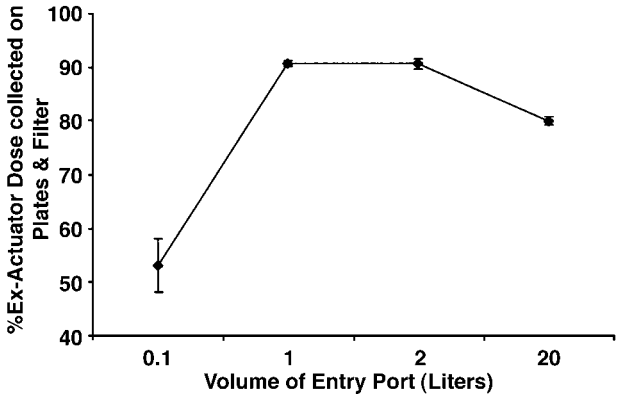

Figure 3. Plot of percentage of the ex-actuator dose recovered on the impactor plates and filter as a function of the entry port volume.

The eight-stage Andersen cascade impactor is now well accepted by all compendia as the impactor of choice for characterizing PSD of pharmaceutical aerosols. In order to adapt the 1-liter entry port to the Andersen cascade impactor, a bridging study was performed with the same CFC aerosol product used in the EP screening study. Particle size distribution

Table 2. Comparison of aerodynamic particle size data obtained from CFC beclomethasone MDIs using the Impaq and Andersen cascade impactors.[a]

	Impaq	Andersen
MMAD (μm)	2.7 (0.1)	2.9 (0.2)
GSD	1.8	1.8
%FP[b]	88.3 (0.6)	86.0 (2.0)

[a]Average (SD), n=3 cans; four actuations per can.
[b]Particle mass <5.5 μm estimated by fitting cumulative undersize data to a log-normal distribution.

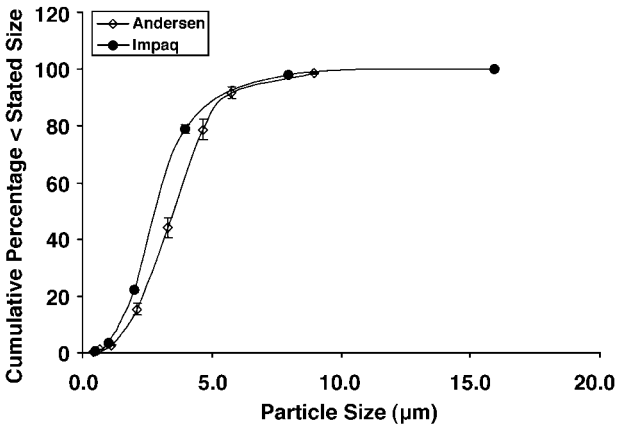

Figure 4. Comparison of the cumulative undersize distribution data for CFG-based beclomethasone dipropionate aerosol as measured using the Impaq-6 and Andersen cascade-impactors.

from three cans was evaluated for each impactor using the 1-liter entry port. Since the two impactors operate at different airflow rates and have different effective cut-off diameters for the stages, a comparison was made by fitting both cumulative undersize data to a lognormal distribution. The fine particle fraction was estimated from the fit as that particle mass less than 5.5 μm. The mass median aerodynamic diameter (MMAD), geometric standard deviation (GSD), and fine particle mass (<5.5 μm) for data from both impactors, using the 1-liter entry port, are summarized in Table 2. The data obtained using the beclomethasone MDI was closely comparable using both the impactors with the 1-liter EP. The MMAD and percentage of fine particle mass for the Impaq and Andersen impactors were 2.7 μm, 88%, and 2.9 μm, 86%, respectively. The cumulative undersize distributions from both impactors, using the 1-liter entry port are shown in Fig. 4 and are very similar.

Comparison of USP and 1-Liter Entry Ports

An investigational HFA-steroid suspension MDI was formulated under conditions that produce subtle particle size differences in the finished product. The ability of the two entry ports, 1-liter, and the USP to differentiate between the formulations was studied. In the first part of this study MDI suspension formulations were prepared using two drug lots micronized under slightly different process conditions (Study 3A). The feed rate was changed during micronization to produce a coarser and finer drug substance.[13] The median particle size of the two drug substance lots, measured using laser diffraction, was 1.2 μm and 1.3 μm for the slower and faster micronized lots, respectively. These lots were used to prepare suspensions in MDIs using the so-called ''one-step'' manufacturing process that has been shown to minimize crystal growth during manufacture. In this method the drug is homogenized in an alcohol-surfactant mixture and the HFA 227 is added to the mixture in a pressurized tank. The entire formulation was then pressure filled into empty cans, which were previously crimped with a 63-μL metered dose valve. A summary of aerodynamic particle size data obtained using both entry ports is shown in Table 3. The impactor recoveries for both batches were 89% using the 1-liter entry port and 37% with the USP entry port. Minimal EP losses of 6% were observed for both batches with the 1-liter EP, whereas the USP port showed a much higher EP loss of around 60%. Although MMADs obtained with both entry ports were similar for the coarse and fine drug substance, a significant difference was observed with the fine particle mass <4.7 μm. The 1-liter EP was better able to differentiate between the coarse and fine drug lots with respect to fine particle mass <4.7 μm ($p<0.05$). In contrast, the USP port gave a fine particle mass of 26–27% for both batches.

Table 3. Summary of aerodynamic size data of HFA suspension MDIs made using different particle size drug lots with the 1-liter and USP entry ports[a] (Study 3A).

Entry port	Sample	Total recovery (%)	Recovery in EP (%)	Recovery on impactor (%)	% FP (<4.7 μm)	MMAD (μm)	GSD
1-Liter EP	Fine DS	97	6.3	89	76[b]	2.6	1.9
	Coarse DS	98	6.4	89	68[b]	2.8	2.0
USP EP	Fine DS	89	61	37	27[c]	2.8	2.5
	Coarse DS	89	60	37	26[c]	3.0	2.4

[a]Data from 3 cans; two actuations per can.
[b]$p<0.05$.
[c]No significant difference.

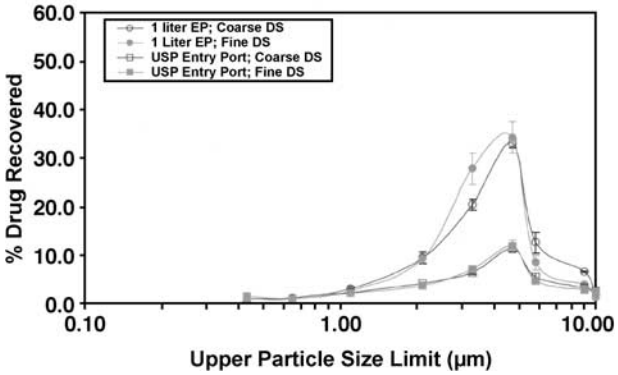

Figure 5. Andersen cascade impactor particle size distribution profile comparison between the USP and 1-liter entry ports using HFA-based MDI aerosols prepared using different particle size drug substance lots.

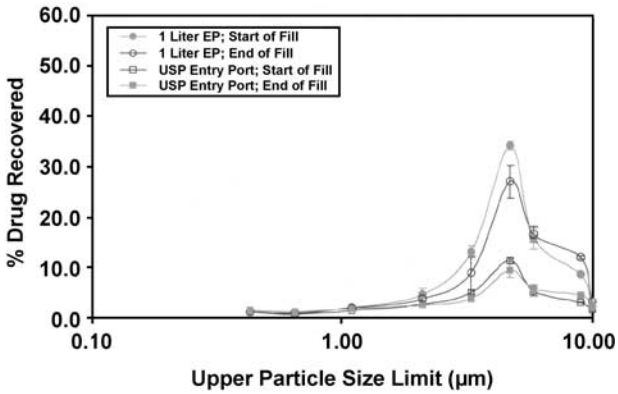

Figure 6. Andersen cascade impactor particle size distribution profile comparison between USP and 1-liter entry ports using HFA-based MDI aerosols sampled from the beginning and end of the ''two-step'' manufacturing process.

The PSD profiles plotted using the cascade impactor data for coarse and fine batches are shown in Fig. 5. The distributions are similar when intra-EP comparison is made. However, the 1-liter EP shows much better differentiation between batches made with the coarse and fine drug substance lots (Fig. 5).

In the second part of the study the same drug substance lot was used to manufacture MDIs (Study 3B). However, processing conditions were deliberately altered to induce crystal growth by ''Ostwald ripening.''[14] In this method the homogenized drug concentrate consisting of drug, alcohol, and surfactant is accurately weighed into the aerosol can. The can is then crimped with a valve and pressure filled with HFA 227. The time lag between beginning and end of the manufacturing process allows for prolonged exposure of the drug to the alcohol-surfactant mixture. This enables finer drug particles to go into solution and achieve supersaturation in the concentrate. When propellant HFA-227 is added to this mixture the drug precipitates onto larger drug particles, owing to its insolubility in the propellant. Eventually, this leads to a shift in the PSD towards higher sizes. The MDI cans were taken from the beginning and end of the manufacturing process to simulate large shifts in particle sizes and tested by cascade impaction. However, particle size changes due to processing conditions may be too small to be detected by routine testing. The ability to detect such small shifts in particle size was evaluated using the 1-liter entry port during cascade impaction. A summary of aerodynamic particle size data obtained using the 1-liter and USP entry ports is shown in Table 4. The impactor recoveries using the 1-liter EP were 84% and 77%, with cans taken at the beginning and end of batch manufacture, respectively. The USP entry port gave similar but markedly lower impactor recoveries of 34% with cans from beginning and end of the manufacturing process. Differences in fine particle mass <4.7 μm for the beginning and end samples were virtually indistinguishable when tested with the USP entry port. In contrast, the 1-liter EP

Table 4. Summary of aerodynamic size data of HFA suspension MDIs made under manufacturing conditions that promote crystal growth with the 1-liter and USP entry ports[a] (Study 3B).

Entry port	Sample	Total recovery (%)	Recovery in EP (%)	Recovery in impactor (%)	% FP (<4.7 μm)	MMAD (μm)	GSD
1-Liter	Initial	94	10	84	57[b]	3.2	2.2
	End	93	17	77	44[b]	3.6	2.3
USP	Initial	86	63	34	23[c]	3.0	2.5
	End	90	63	34	21[c]	3.3	2.8

[a]Data from 3 cans; two actuations per can.
[b]$p<0.001$.
[c]No significant difference.

showed statistically significant differences in fine particle mass between cans tested from the beginning and end of the process ($p<0.001$).

As summarized in Fig. 6, particle size changes attributed to crystal growth during product manufacture were easily discernible using the 1-liter EP compared to the USP EP.

CONCLUSIONS

The Food and Drug Administration's (FDA) Guidelines for Industry on MDIs and dry powder inhalers (DPIs), issued in October 1998, suggests that the equipment and accessories for evaluating the particle size distribution of the emitted dose should be selected so that the majority of the dose is introduced into the cascade impactor stages for fractionation.

Therefore, in the study several glass entry ports of varying size were evaluated for drug recovery during cascade impaction testing of suspension MDIs. Drug losses in the entry port were a function of the EP volume. An optimal EP size of 1–2 liters is needed to obtain good impactor recovery, thereby resulting in greater particle size distribution in a discriminatory capacity. The 1-liter entry port was then compared with the USP entry port to distinguish subtle particle size changes in the finished product. The USP entry port is perhaps more anatomically correct in filtering out a large part of the emitted dose as may occur in vivo. It may have utility in investigating in vitro–in vivo correlation and provide an estimate of drug deposition in the lung. However, use of the USP entry port during product development testing may actually mask subtle changes in the product, which is more likely to be detected by the 1-liter entry port. The detection of small particle size changes during formulation development may provide valuable information and aid in preventing such changes during product manufacture and upon long-term storage. The 1-liter entry port in conjunction with the Andersen Cascade Impactor is proposed as a development and/or quality control tool for determining the aerodynamic particle size distribution. However, it is not being recommended as a device to predict lung deposition.

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