Effects of Sampling Chamber Volume and Geometry on Aerodynamic Size Distributions of Metered-Dose Inhalation Aerosols Measured with the Andersen Cascade Impactor

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INTRODUCTION

Particle size is an important factor in determining regional deposition and efficacy for inhalation aerosols (1). In a research and quality-control setting, particle sizing serves to examine two major aspects of the formulation. The first is a quality-control check of the micronization process and the second is to ascertain whether particle growth is occurring under normal and stressed storage conditions.

The two most widely accepted methods currently used to characterize the plume emitted from a pressurized metered-dose inhaler (MDI) are optical microscopy and cascade impaction. Optical microscopy has its merits but it is a tedious, time-consuming technique and typically characterizes only a small portion of the dose delivered. In addition, the method usually relies on the use of a solvent to remove surfactant, so that individual particles are characterized rather than drug clusters or aggregates which may have existed in the original aerosol. Cascade impaction, in contrast, classifies particles in terms of their aerodynamic diameter. Because the particle size distribution measured by cascade impaction is within a turbulent airflow, and because the entire dose delivered from the MDI is characterized rather than a small portion of it, cascade impactor studies should yield better *in vivo–in vitro* correlations. Formulation factors that will affect the results obtained using cascade impaction that are not a result of the bulk drug or stressing are concentration of drug and surfactant in the formulation and the vapor pressure of the propellant mixture (2).

Two of the most popular cascade impactors used in the pharmaceutical industry are the Delron DCI-6 cascade impactor (now manufactured by Sciarra and Cutie) and the Andersen Samplers Inc. 1 ACFM nonviable ambient sampler. The Delron cascade impactor is a six-stage cascade impactor which comes equipped with a sampling chamber amenable to use with MDIs. The Anderson cascade impactor is an eight-stage cascade impactor which does not come equipped with a suitable sampling chamber. Individual pharmaceutical companies using the Andersen cascade impactor, therefore, usually design their own sampling chamber, which may be of any size or shape.

The size of the sampling chamber should significantly affect the respirable dose or respirable fraction but the effect on the measured mass median aerodynamic diameter (MMAD) is uncertain. Recently the FDA has suggested that the sampling chamber used in cascade impaction studies have a volume of no less than 500 mL and an unobstructed pathlength between the actuator orifice and the far side of the sampling chamber of no less than 13 cm (3). We investigated the effect of the sampling chamber design on the measured particle size distribution, respirable dose, and sampling chamber deposition using the Andersen cascade impactor and commercially available Ventolin. This investigation covered a range of volumes from 250 to 5000 mL, with introduction orifice angles of 90, 135, and 180° from the cascade impactor inlet. For comparison, the Twin Impinger throat (50 mL) as a sampling chamber was also investigated.

MATERIALS AND METHODS

An Andersen Samplers Inc. Mark II 1 ACFM nonviable ambient sampler was used and operated according to manufacturer's recommendations at a flow rate of 28.3 L/min. The effective cutoff diameters (ECD) for this apparatus are 9.0, 5.8, 4.7, 3.3, 2.1, 1.1, 0.7, and 0.4 μm for stages 0 to 7, respectively. Glass impaction plates were used and the preseparator was removed. The preseparator was replaced with a modified inlet cone constructed of aluminum and designed to accept a male 24/29 ground-glass joint (Fig. 1). The sampling chambers were constructed of glass, with the input orifice having a female 29/32 joint, so that a standard Twin Impinger rubber mouthpiece could be used to seal the opening around the actuator during testing. The volumes of the sampling chambers used were 250, 500, 1000, 2000, and 5000 mL, with orientations of 90, 135, and 180° from the cascade impactor inlet (see Fig. 2). The Twin Impinger throat (50 mL, 90°) was taken from a standard Copley Twin Impinger apparatus (4).

Three commercially available Ventolin inhalers were tested with each sampling chamber volume and orientation investigated. Ten priming shots were discharged to waste, with 30 sec of shaking before each shot. The inhaler was removed from the actuator and the actuator and valve were cleaned with methanol. Two seating shots were performed prior to the assay. The inhaler was shaken for 30 sec, the pump turned on, and then the mouth of the actuator inserted into the mouthpiece and one shot delivered. The inhaler was removed and shaken for 5 sec and another shot was delivered to the apparatus. This was repeated until 10 actuations were discharged into the apparatus. After the last actuation, the airflow was allowed to continue for a further 60 sec.

The mass of drug deposited in the sampling chamber was determined by rinsing the chamber as well as the rubber mouthpiece with methanol, collecting the washings, and quantitating by HPLC. Deposition on each stage of the cascade impactor was determined by collecting the dose depos-

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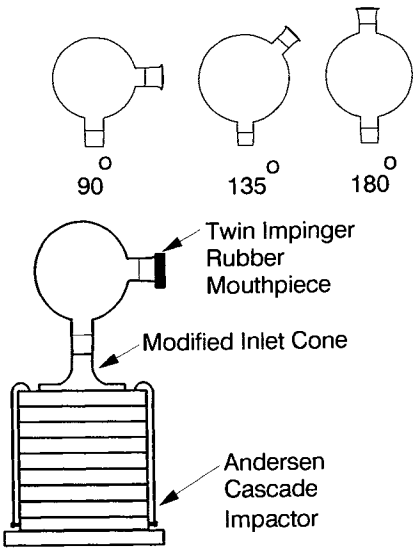

Fig. 1. Schematic of the modified Andersen cascade impactor and the various orientations of sampling chambers used.

ited on the impaction plate as well as on the surface and walls of the stage itself and quantitating by HPLC. (The %RSD of the total drug recovered in the 48 experiments was about 3%.) The column used in the HPLC system was a 5-μm Spherisorb ODS (10 cm × 3-mm i.d) operating at a flow rate of 0.85 mL/min and a column temperature of 50°C. The UV detector was set at 276 nm and the injection volume used was 100 μL. The mobile phase consisted of 800 mL of methanol + 300 mL of 0.1% aqueous ammonium acetate solution filtered through a 0.45-μm filter.

Calculations

The assay data were converted to cumulative mass percentage less than stated size vs particle size using the known particle size cutoffs for each stage of the cascade impactor. Using RS/1 software (BBN Software Products Corp.), the cumulative mass percentage data were converted to a probability axis via a function which performs an inverse normal probability transformation. A log-probability plot of the particle size vs the cumulative mass percentage data could then be generated by computer, from which the MMAD could be determined. The MMAD corresponds to 0 on the inverse normal probability scale, 1 and −1 on this scale correspond to 84.13 and 15.87%, respectively. If the particle size distribution was truly log-normal, the data set would yield a straight line. Since the data did not yield a straight line, the most weight in determining the best fit was given to the data points between 20 and 80 cumulative mass%, or the points corresponding to stages 2 through 5. A weighted fit was approximated by simply fitting a line to these center four data points using the least-squares method and interpolating the MMAD at $x = 0$ (Fig. 2).

The respirable fraction of the dose delivered was defined as the mass fraction of particles having an aerodynamic diameter of less than 5.8 μm. It was determined by summing the amount of drug deposited on stages 2 and below in the Andersen cascade impactor divided by the amount of drug assayed leaving the actuator in each experiment.

RESULTS AND DISCUSSION

Sampling Chamber Deposition and Respirable Dose

As illustrated in Fig. 3, the fraction of the dose deposited in the sampling chamber decreases as the volume of the chamber increases. The results show that there is a sharp decrease in the sampling chamber deposition when the chamber volume is increased from 50 to 1000 mL and that further increases in chamber volume result in only small additional decreases in the sampling chamber deposition. This trend can be related to the velocity profile of the aerosol spray. The velocity of the aerosol as it exits the actuator orifice of the MDI is of the order of 30–50 m/sec (5). A rapid deceleration then takes place as the aerosol comes in contact with air. Therefore, as the unobstructed pathlength from the actuator orifice to the far side of the sampling chamber increases with an increase in chamber volume, the probability of impaction of the particles on the sampling chamber wall is reduced. As one might expect, the 90 and 135° orientations yielded significantly higher deposition than the 180° orienta-

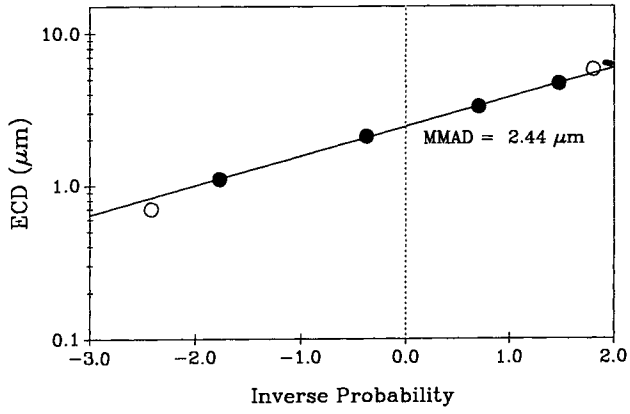

Fig. 2. Plot of the logarithm of the effective cutoff diameter of the impactor stage (log ECD) versus the inverse normalized probability of the cumulative mass percentage data. The filled circles represent the points used in the regressional analysis.

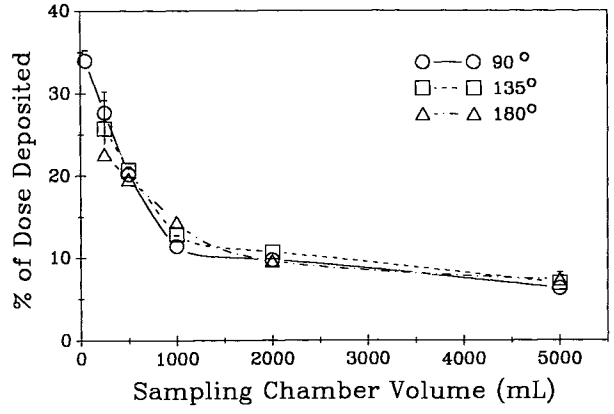

Fig. 3. Plot of the fraction of the dose deposited in the sampling chamber and the volume and orientation of the sampling chamber. Error bars are standard deviations ($n = 3$) and are not shown if they are less than the symbol size.

tion for the smallest chamber size tested (250 mL), but somewhat surprisingly, for volumes of chambers greater than 250 mL there appears to be no significant difference in the mass deposition resulting from a change in the direction of sampling chamber. This indicates that a sampling chamber between 250 and 500 mL provides sufficient volume/distance to allow the particles to decelerate to the velocity of the airstream and avoid impaction with the sampling chamber wall.

Because of the small amounts of drug deposited in stages 0 and 1 of the cascade impactor in these studies, the total dose delivered was approximately equal to the sum of the respirable dose and the dose deposited in the sampling chamber. Therefore, one would expect the effect of volume on the respirable dose to be opposite that seen for sampling chamber deposition, and this trend is illustrated in Fig. 4.

The respirable fraction obtained with the Twin Impinger throat was 63%. These results are slightly higher than those reported by Hallworth and Westmooreland (56%) (6) using the Twin Impinger apparatus and much higher than those reported by Phillips *et al.* (38%) (7). The different respirable fractions obtained by different authors using the Twin Impinger apparatus may be attributable to the effects that the operator and the operating conditions have on assay results (8).

A larger sampling chamber may be desirable in terms of assay ruggedness because the respirable dose obtained using a Twin Impinger sampling chamber is sensitive to ambient temperature (8) and it may be more sensitive than sampling chambers of larger volume/distance. If this temperature effect is also observed in a clinical setting, the twin impinger sampling chamber would be a better choice for *in vitro* testing than the 500-mL sampling chamber proposed by the FDA.

Mass Median Aerodynamic Diameter

Figure 5 shows the effect that the sampling chamber volume has on the measured MMAD. Little difference exists among the MMADs obtained using the 50-, 500-, and 1000-mL throats, all of which yielded an average MMAD of approximately 2.4 μm. These results agree with those obtained by Kim *et al.* (9), who found values of MMAD ranging from 2.4 to 2.6 μm. Increasing the volume of the chamber from 1000 to 2000 mL results in a small but sudden increase in the MMAD to approximately 2.6 μm. This 8% increase in the MMAD is not accompanied by any corresponding discontinuities in the respirable dose or sampling chamber deposition data and is unaccounted for. Again, the orientation of the aerosol inlet has no effect on the measured results for volumes greater than 250 mL. In the case of the 250-mL sampling chamber, the higher MMAD observed for the 180° sampling chamber relative to the other orientations reflects the higher deposition observed in the upper stages of the impactor. It is interesting to note that since the MMAD and particle size distribution are unaffected by a volume change from the Twin Impinger throat (50 mL) to 500 mL, one may see a similar lack of effect when an MDI is tested with and without a spacer attachment.

An analysis of the MMADs obtained for the 1000- and 2000-mL 90° throats shows them to be significantly different ($P = 0.0001$) by a Student's t test. A plot of the differences between normalized 2000-mL 90° throat data and normalized 1000-mL 90° throat data (Fig. 6) can be used to analyze stage-by-stage the differences in mass deposition contributing to the measured increase in MMADs. From this plot we

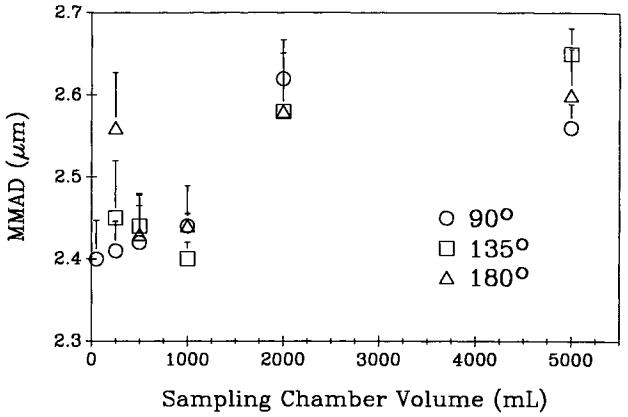

Fig. 5 Plot of the measured mass median aerodynamic diameter (MMAD) and the volume and orientation of the sampling chamber. Error bars are standard deviations ($n = 3$) and are not shown if they are less than the symbol size.

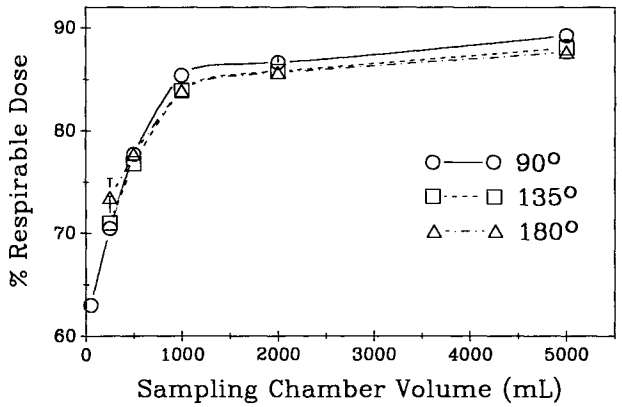

Fig. 4. Plot of the percentage respirable dose and the volume and orientation of the sampling chamber. Error bars are standard deviations ($n = 3$) and are not shown if they are less than the symbol size.

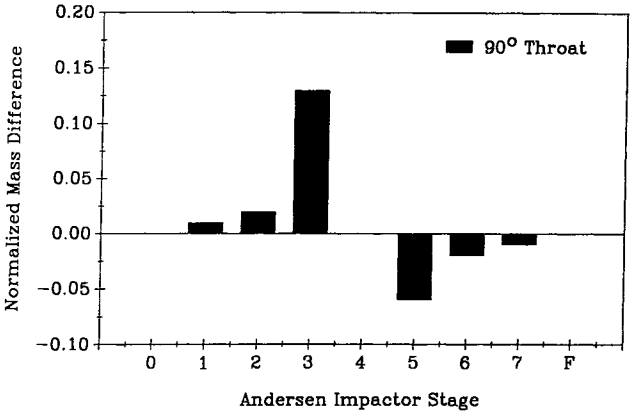

Fig. 6. Plot of the differences between the normalized 2000-mL sampling chamber and the normalized 1000-mL sampling chamber with a 90° orientation.

can see that when the 2000-mL throat is used, more drug is deposited on stage 3 of the impactor (ECD = 3.3 μm) and slightly less is deposited on stage 5 (ECD = 1.1 μm), which accounts for the small increase in MMAD. Apparently a sampling chamber of 2000 mL or larger allows adequate volume and/or distance for expansion and deceleration of the aerosol cloud, so that the propellants may fully evaporate to form dry drug clusters or aggregates before either impacting on the chamber wall or entering the cascade impactor. It is also possible that the increase in residence time of the aerosol cloud in the larger volume sampling chambers may result in reaggregation of drug particles due to electrostatic attraction, resulting in an increase in the MMAD

CONCLUSIONS

As the volume of the sampling chamber is increased, the percentage respirable dose increases and the sampling chamber deposition decreases. The effect is more pronounced for sampling chambers with volumes of 500 mL or less and less pronounced as the volume is increased to 1000 mL or more.

The particle size distribution and measured MMAD are not affected by the volume of the sampling chamber over the volume range studied, except for a small increase (8%) occurring between 1000 and 2000 mL. The Twin Impinger throat yields the same MMAD as the 500-mL throat, which has been suggested by the FDA.

The angle of introduction of the aerosol into the sampling chamber appears to have little or no effect on the measured MMAD, percentage respirable dose, or mass deposition in the sampling chamber for sampling chambers greater than 250 mL. For the 250-mL sampling chamber, the lowest sampling chamber deposition and highest respirable dose were observed for the 180° throat, as expected.

From these investigations, it appears that the resultant MMAD obtained from cascade impaction is independent of the volume and orientation of the sampling chamber used, although shape, which may be a factor, was not investigated. The respirable dose obtained is greatly affected by the volume of the sampling chamber. It may be desirable to have a sampling chamber of approximately 1–2 L so that most of the dose delivered from the MDI is characterized by cascade impaction. On the other hand, a smaller volume sampling chamber (volumes and distances consistent with the anatomy of the upper airways) may be more sensible because it may have better discriminatory power in assessing the clinically significant factors affecting formulation design, yielding better *in vivo–in vitro* correlations. The purpose and use of cascade impaction should be defined before a decision on sampling chamber is made. Will the sole purpose of its use be to detect changes in the input drug or formulation or will it be used as a method eventually to be correlated with *in vivo?* In many respects this is similar to the evolution of dissolution testing. The general view in the present climate is to approximate as well as possible what the patient receives, and therefore a smaller sampling chamber should be used. This belief is demonstrated in the recent guidelines issued by the British Pharmacopeia and United States Pharmacopeia (10). These investigations also show that the MMAD obtained using a small-volume throat is the same as that with a large-volume throat, therefore a small-volume throat should be used because both particle size and a more realistic respirable dose are assessed.

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