Loading Effect on Particle Size Measurements by Inertial Sampling of Albuterol Metered Dose Inhalers

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Purpose. The purpose of this study is to investigate the albuterol loading effect on particle size measurements by studying the effect of the amount of albuterol delivered, the number of puffs used, and the sampling techniques used in particle size measurement.

Methods. Particle size distribution profiles for different albuterol loadings were evaluated using an 8-stage cascade impactor and a sensitive HPLC electrochemical assay method. A commercial albuterol MDI (Proventil[R]) and other specially prepared albuterol MDIs were used in the study.

Results. As the amount of albuterol was increased, either by increasing the number of puffs or the amount delivered per puff, the measured MMAD increased. This increase was more prominent in some formulations (Proventil[R]) than others. Further, albuterol particles previously deposited on the valve and/or actuator didn't play a role in the observed multi-puff/loading effect.

Conclusions. The collection of the least amount of aerosol in a cascade impactor provides a better estimate of MMAD, as it minimizes modifications of the collection surfaces.

KEY WORDS: albuterol; MDI; particle-size measurement; loading effect; HPLC-EC.

INTRODUCTION

Metered dose Inhalers (MDIs) present a convenient way for the delivery of therapeutically active drugs. Albuterol MDIs are commonly used in the treatment of obstructive pulmonary diseases. Since the expiration of the albuterol patent in the United States, there has been considerable debate on meeting FDA regulatory requirements for albuterol generic equivalents. Generic drug products must be shown to be pharmaceutically equivalent and bioequivalent to the innovator product. Several comparative *in vitro* bioequivalence tests (unit spray content, particle size distribution, spray pattern, and plume geometry) were established by the Division of Bioequivalence (1). After the publication of Cyr *et al. (2),* attention has been focused on testing dose uniformity of single doses rather than the average of many doses, as has been done in the past (3,4). Measurement of particle size distribution is important in testing micronized bulk drug substance, MDI formulations stability, lot-to-lot variability, and for predicting *in vitro-in vivo* correlation. Such prediction could be analogous to dissolution testing of solid dosage forms. The Division of Bioequivalence (1) requires the determination of particle size distribution by at least two methods, one of which must be aerodynamic using a multi-stage cascade impactor. In its latest revision, the USP is also recommending the use of a multi-stage impactor for aerodynamic particle size distribution of MDIs (5). In the past, most cascade impactor measurements were made using several puffs due to low analytical sensitivity. Unlike spray content uniformity, recent recommendations (1,5) did not specify the number of puffs to be used in cascade impactor particle size measurements.

Recently (6), we have shown that particle size distribution using a multi-stage cascade impactor may depend on either the number of puffs and/or the amount of albuterol used in the measurement. Also, Miller and Schultz (7) have observed that the mass median aerodynamic diameter (MMAD) is slightly smaller when measured with a smaller number of puffs. These results have prompted us to examine the albuterol loading effect on particle size measurements by studying the effect of the amount of albuterol delivered, the number of puffs used, and the sampling techniques used in particle size measurement.

A commercial albuterol product that delivers a constant 90 μg albuterol per puff (Schering Proventil[R] inhaler) as well as several albuterol MDIs (8) manufactured to deliver various amounts (9–144 μg of albuterol per puff) were used in the study. The results suggest that, in cascade impactor particle size measurements, one should use the smallest possible amount of the product to eliminate the observed loading effect. To achieve that, one must consider the use of the most sensitive and reliable analytical method available for the assay of the drug substance. New developments in electrochemical detectors makes HPLC-EC a relatively simple and rugged analytical technique suitable for routine drug assay (9).

MATERIALS AND METHODS

Reagents, solutions, and mobile phase were the same as in our previous study (6). Two different albuterol MDIs were used in the study, Schering Proventil[R] (control No. 2-BBS-212, expiration date 8/94), and especially manufactured albuterol MDIs (Armstrong Laboratories (8)). The manufactured MDIs were designed to deliver various amounts of albuterol per puff (Table 1).

Two different liquid chromatographic methods were used for the assay of albuterol: (1) High-Performance Liquid Chromatography with Electrochemical Detection (HPLC-EC), and (2) High-Performance Liquid Chromatography with Fluorescence Detection (HPLC-FD).

1. HPLC-EC System. This system was used for the assay of albuterol in all particle size measurements in this study except for the ten-puff measurements of the manufactured MDIs (ALB1–ALB6). This is the same system used in our previous study (6). EZChrom™ Chromatography Data System (Shimadzu, Columbia, Maryland, USA) was used for data acquisition and calculations.

2. HPLC-FD System. The HPLC system was operated at room temperature and consisted of a solvent delivery module (Waters 600E), an automatic sample injector (Waters 712 WISP), a fluorescence detector (JASCO 821-FP), and an integrator (Hewlett-Packard 3396A). The excitation and emission wavelengths for the fluorescence detector were set at 225 nm and 310 nm, respectively (10). The mobile phase

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Table I. Volume Delivered By Valve (μL), Albuterol Concentration in Suspension and Nominal Dose per Actuation (μg) of Products Manufactured for this Study (8)

Product	Valve Size[a] (μL)	Albuterol Conc.[b]	μg Albuterol per Actuation[c]
ALB1	25	0.25X	9
ALB2	25	0.50X	18
ALB3	25	1.00X	36
ALB4	50	1.00X	71
ALB5	62	1.00X	89
ALB6	100	1.00X	144

[a] Bespak Model BK 356, silver anodized aluminum ferrule 20 mm diameter; 25, 50, 62, and 100 μL metering sizes.

[b] The drug suspension concentrations were 0.25X, 0.50X, and 1.00X, with X being the same as that of Ventolin Inhaler.

[c] μg albuterol per actuation assuming linearity of delivery across valve sizes. Actual values were determined by *in vitro* testing (Table III).

was the same one used with the HPLC-EC system. The flow rate was set at 1.0 mL/min, and the injection volume was 100 μL. Calibration curves of albuterol standard were prepared from 20 to 2000 ng/mL. Typically, the correlation coefficients were ≥ 0.9999 and the coefficient of variation (CV) of the individual standards was less than 3%. When the standard concentration was below 20 ng/mL, the CV of the individual standards increased to unacceptable levels; therefore 20 ng/mL was set as the lower limit. Above 2020 ng/mL, the detector gave an overload message; therefore 2000 ng/mL was set as the upper limit.

Assay of Single Puff from Albuterol MDIs. Single puff assays of all MDIs both for Proventil[R] and manufactured products (ALB1–ALB6) were performed using our previously reported protocol (6).

Multi-Puff Assay from Albuterol MDIs. Andersen 1 ACFM Non-Viable Cascade Impactor Mark II with the modified USP induction port (5) was used. The air-flow was adjusted and maintained at 28.3 L/min. The MDI was shaken, and the first three puffs were discarded. The valve was rinsed with the mobile phase and dried with a stream of nitrogen gas. The vacuum pump was started and after 10 sec the first puff was delivered into the cascade impactor; after additional 60 sec another puff was delivered. This process was repeated, as needed, until the desired number of puffs (2–10) were delivered. After 5 sec the pump was turned off and the impactor was disassembled. The 8 stages (0 through 7) and the filter (Whatman GF/A filter) were transferred to individual 800-mL beakers and suitable volumes of the mobile phase were added to each beaker. In addition, the actuator, the valve, and the throat (USP 90° aluminum throat (5)) were all rinsed with appropriate volumes of the mobile phase. All 12 beakers and their contents were placed in an ultrasonic bath for 5 min to assure the complete extraction and dissolution of albuterol. The contents of each beaker were transferred to the corresponding volumetric flask. Each beaker was then rinsed with mobile phase and the rinses added to the corresponding flasks. The calculated amounts of the internal standard (Bamethane) were added to maintain a constant concentration of about 50 ng/mL and all fractions were diluted to the appropriate volumes with the mobile phase. Each fraction (1 through 12) and 8 standard albuterol solutions were injected into the liquid chromatograph.

To investigate the effect of the waiting time between puffs delivered into the Andersen Cascade Impactor, the same protocol was repeated with only 30 seconds waiting between puffs.

Another experiment was designed to test the possibility of albuterol previously deposited on the canister's valve and/or actuator being re-entrained in the spray as larger particles when several puffs are delivered into the cascade impactor. The amount of albuterol deposited at the top cone, filter plate, and all seven jet plates of the impactor was measured in this experiment. Ten Proventil[R] canisters from the same lot (control No. 2-BBS-212, expiration date 8/94) were selected. The 10 canisters were primed serially by shaking a canister for 5 seconds then discharging one puff to waste. The priming steps were repeated until a total of 4 puffs were discharged from the canister. The canister was then removed from the actuator, and both the canister and valve were washed with methanol and dried with a stream of nitrogen gas. The canister was inserted into an actuator that was previously washed with methanol and dried. The assembled MDI was inserted into the USP throat attached to an Anderson Cascade Impactor. The vacuum pump was started and after 10 seconds one puff was discharged. After 5 seconds the vacuum pump was turned off and the next canister was primed and sampled. This procedure was repeated until all 10 canisters were sampled. The impactor was disassembled and the 8 impaction stages and filter were transferred to nine 800-mL beakers. The "O" ring was carefully removed and the 8 jet plates and filter holder were placed in nine 1000-mL beakers. A volume of mobile phase equal to about 60% of the volumetric flask used for each part (impaction stage, jet plate or filter holder) was added to the beakers. The actuators, valves, throat, and top cone were thoroughly rinsed with mobile phase. The washing of all 10 actuators was collected into one beaker and the washing of all 10 valves was collected into a second beaker. All 22 beaker fractions were placed in the ultrasonic bath for 5 minutes. The contents of each beaker were then transferred to the corresponding volumetric flask. Each beaker was rinsed with mobile phase and the rinses added to the corresponding flasks. The appropriate amount of internal standard was added to each volumetric flask to maintain an internal standard (Bamethane) concentration of about 50 ng/mL. Volumetric flasks were then diluted to volume with the mobile phase. Due to the high sensitivity of the electrochemical detector, some samples needed further dilution prior to the analysis. All samples and 8 standard albuterol solutions were injected into the liquid chromatograph.

RESULTS AND DISCUSSION

Albuterol Assay. The electrochemical detection method (6) was used in all particle size experiments, except for the ten puff measurements of the specially prepared MDIs (ALB1–ALB6). The electrochemical detector sensitivity was essential in the quantitative measurement of albuterol in cascade impactor fractions, where albuterol concentrations

were as low as 1.2 ng/mL. Fluorescence detection provided good sensitivity for albuterol concentration above 20 ng/mL.

Albuterol Particle Size Analysis. In this study, 4–6 replicate experiments were performed for all particle size measurements. The method used to collect and extract albuterol seemed to provide complete recovery of delivered albuterol. An average of 83.5% of the declared albuterol dosage (amount delivered past the actuator) was recovered (SD = 3.26, n = 37). This is considered an advantage of using cascade impactors in particle size measurements, as it accounts for masses of delivered drug. Particle size measurements were evaluated by utilizing the probit computer program (method I) as outlined before (6). Plots of the calculated probit versus the log of the effective cut-off diameters (ECD, ECD = 1.10 through 5.8 μm) for both a single and ten puff delivery is illustrated in Fig. 1. Applying linear regression to the plots in Fig. 1 (r^2 = 0.996 and 0.994 for single and ten puffs plots, respectively)), produced the values of 1.79 and 2.16 (for the mass median aerodynamic diameter, MMAD) and 1.79 and 1.60 (for the geometric standard deviation, GSD), for the single puff and ten puff measurements, respectively.

In addition, particle size diameter graphs were generated (method II), where effective cut-off diameters (ECD = 0.43–9.0 μm) were plotted versus the cumulative percentages of albuterol greater than the stated size (CUM % GT) on log–probability papers (Fig. 2). Fig. 2 shows the dependance of particle size distribution on the number of albuterol puffs delivered. Linear regression was applied to the five middle points only (ECD = 1.10 through 5.80 μm). It has been shown recently (11,12), that applying linear regression to all points on the non-linear log-probability plots will result in unreliable MMAD and GSD values. The MMAD value was determined to be the value of ECD at a cumulative percentage greater than stated size of 50% and the GSD was the quotient of the MMAD divided by the value of ECD at a cumulative percentage greater than stated size of 84.13% (6).

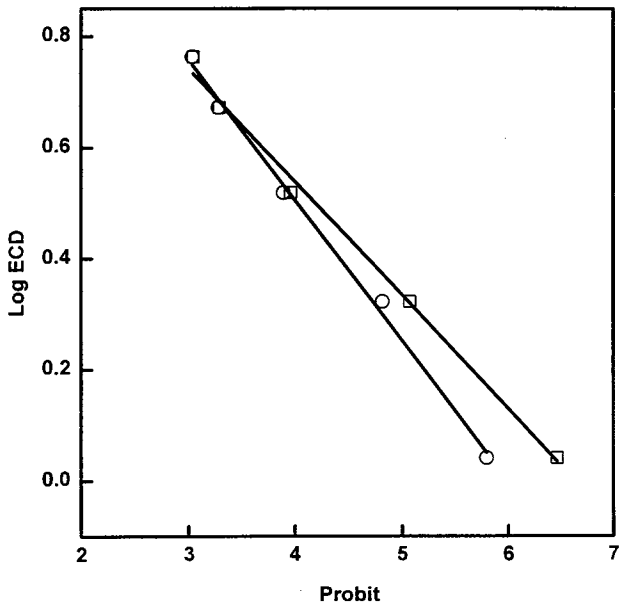

Fig. 1. Logarithm of effective cut-off diameter (1.10–5.8 μm) versus Probit (linear probability scale, (6)) for single (○) and ten (□) puffs.

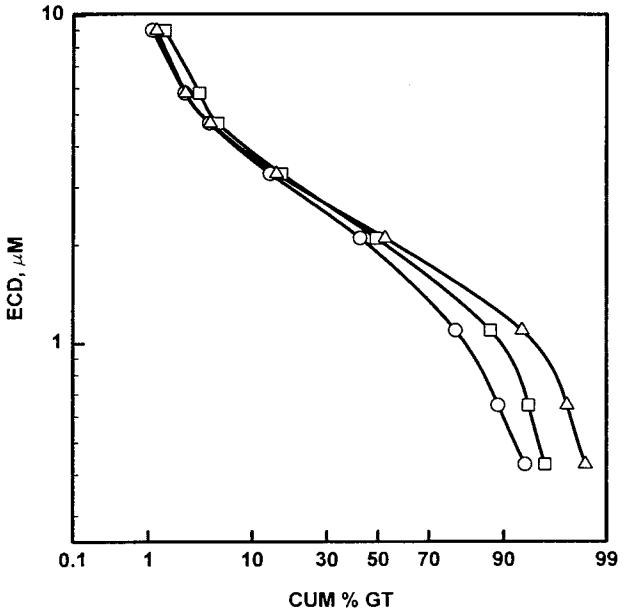

Fig. 2. Effective cut-off diameter (μm) versus cumulative percentage oversize by mass for 1 (○), 4 (□) and 10 (△) puffs.

In most cases MMAD and GSD values obtained using both methods (I, II) were identical.

Multi-Puff/Loading Effect

First, we examined the effect of the amount of albuterol delivered into the cascade impactor, using Proventil^R MDIs. Here, a successive number of albuterol puffs (1, 2, 4, 8, and 10), 60 seconds apart, were delivered into an Andersen Cascade Impactor. Particle size distribution graphs, obtained by plotting the percentage of albuterol collected at each stage versus ECD, indicated a dependance on the number of albuterol puffs delivered. The same trend was observed when albuterol puffs (1, 2, 4, and 10) were delivered into the cascade impactor with only 30 seconds waiting time between puffs. The results did not show a dependance of the measured MMADs and GSDs on the waiting time between puffs. The results summarized in Table II show that measured MMAD values increase as the number of delivered puffs increase. As expected, our ten puffs measurements are in an agreement with previously reported literature values (12–14). Previous loading of collection surfaces of the upper impactor stages in multi-puff experiments, may result in smaller particles being trapped by larger particles previously deposited on upper cascade impactor plates. Such trapping, where more particles are deposited on upper plates with larger cut-off diameter leading to artificially higher MMAD values, can provide an explanation of the observed multi-puff effect. Our observations can be used to illustrate problems associated with relying on masses to measure particle size diameters; where a larger particle may be equivalent in mass to several smaller particles (15). Fig. 3 provides a clear illustration of the dependance of MMAD on the number of delivered puffs. Also, we found that measured GSDs decreased as the number of puffs increased (Table II). Such decrease in measured GSDs may be attributed to an apparent decrease in particles polydispersity. Is the observed

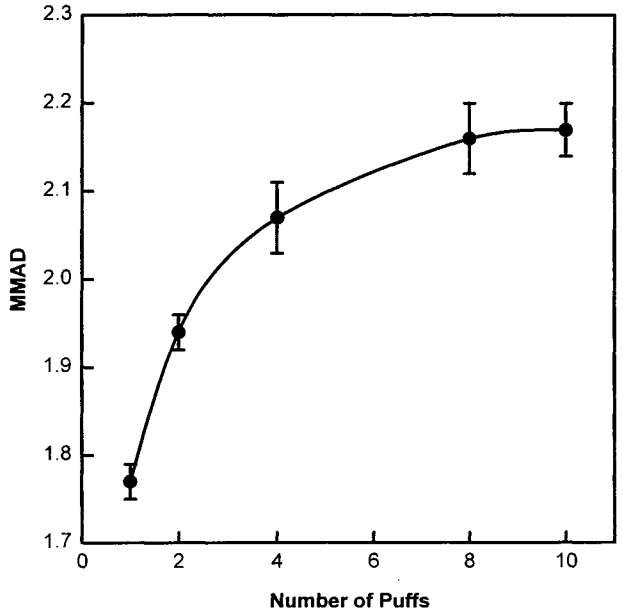

Fig. 3. Mass median aerodynamic diameter (MMAD, μm) versus the number of puffs sampled. (Proventil[R] was sampled at 60 second intervals). Error bars represents the standard deviation (n = 6).

Table II. Effect of the Number of Puffs Sampled on the Mass Median Aerodynamic Diameter (MMAD, μm) and Geometric Standard Deviation (GSD). Standard Deviations (SD) are Shown for Each Value

A. One Minute Wait between Puffs		
Number of Puffs (n[a])	MMAD ± SD	GSD ± SD
Single Puff[b] (4)	1.77 ± 0.02	1.81 ± 0.04
Two Puffs (4)	1.94 ± 0.02	1.80 ± 0.10
Four Puffs (4)	2.07 ± 0.04	1.74 ± 0.04
Eight Puffs (4)	2.16 ± 0.04	1.66 ± 0.02
Ten Puffs (6)	2.17 ± 0.03	1.64 ± 0.02
B. 30 Seconds Wait between Puffs		
Number of Puffs (n)	MMAD ± SD	GSD ± SD
Single Puff[a] (4)	1.77 ± 0.02	1.81 ± 0.04
Two Puffs (5)	1.96 ± 0.01	1.88 ± 0.11
Four Puffs (6)	2.12 ± 0.03	1.73 ± 0.04
Ten Puffs (6)	2.26 ± 0.03	1.64 ± 0.01

[a] Number of replicates.
[b] MMAD value of 1.78 ± 0.01 was reported earlier (6).

multi-puff/loading effect dependant on the number of puffs, or is it related to the amount of drug delivered?

Second, we studied the effect of varying the amount of albuterol delivered per puff. Particle size measurements were performed on the specially prepared albuterol MDIs (Table I; ALB1–ALB6). Both the MMADs and GSDs were measured (Table III). The results show an increase in the measured MMAD as a function of delivered albuterol, for both single and ten puff measurements. However, the increase in the measured MMAD from single puff to ten puffs was smaller (2.5–13.7%) in comparison to Proventil[R] (22.6%). These results indicate that the increase in measured MMAD values by increasing the amount of albuterol delivered may be dependant on aerosol formulation. Tables I and III reveal the unexpected difference between predicted versus actual albuterol delivered per puff. That difference was more pronounced in ALB1 and ALB2 products. In most cases (Table III), the normalized amounts of albuterol delivered through the valve in the ten puff experiments were larger than the amounts delivered in single puff measurements. Also, the measured MMAD values of these formulations (ALB1–ALB6) were accompanied by a larger error (%RSD = 0.50–4.65) in comparison to Proventil[R] (%RSD = 0.51–1.93).

Third, we examined the possible effect of albuterol particles previously deposited on the canister's valve and/or actuator on particle size measurements in the multi-puff experiments. In this experiment ten *different* canisters/actuator combinations were used (as described in the experimental section). The results, in comparison to other ten puff experiments (where only one canister/actuator was used), and single puff measurements are summarized in Table IV. The results obtained are similar in many aspects (MMAD, GSD) to the other ten puff measurements, indicating that albuterol particles previously deposited on the valve and/or actuator didn't play a role in the observed multi-puff/loading effect. The main differences are: an increase in the amount of albuterol deposited on the actuator(s) (233 μg versus 126–153 μg), and in the total albuterol recovered (996 μg versus 899–926 μg). The increase in total albuterol recovered (70–97 μg) may be due to the increase in albuterol deposited on actuator(s) (80–107 μg). The total albuterol recovered and albuterol deposited in actuator(s) in this experiment (where 10

Table III. Mass Median Aerodynamic Diameter (MMAD, μm), Geometric Standard Deviation (GSD) and Dose Delivered (μg/puff) by One or Ten Actuation of Six Albuterol Products Manufactured for this Study (8)

Single Puff Measurements[a]				Ten Puff Measurements[a]		
MMAD	GSD	Albuterol Delivered (μg/puff)	Product[b]	MMAD	GSD	Albuterol Delivered (μg/puff)
2.84	1.59	3.76	ALB1	2.91	1.62	5.57
3.02	1.61	8.40	ALB2	3.14	1.57	13.76
3.01	1.60	36.56	ALB3	3.26	1.58	36.85
2.94	1.59	66.86	ALB4	3.30	1.54	70.89
3.08	1.60	81.8	ALB5	3.44	1.57	95.16
3.13	1.59	146.85	ALB6	3.56	1.56	163.14

[a] Average of 6 experiments. Electrochemical detection was used in the single puff experiments, where as fluorescence detection was used in the ten puff measurements.
[b] Albuterol products are identified in Table I.

Table IV. Albuterol Particle Size Analysis and Recovery Using Andersen Cascade Impactor

Experimental Parameters	Ten Puffs: From One Canister/Actuator (n^b = 6) One Minute[a]	Ten Puffs: From One Canister/Actuator (n^b = 6) 30 Seconds[a]	Ten Puffs: From 10 Different Canisters/Actuators (n = 3)	Single Puff (n = 4)
MMAD	2.17 ± 0.03	2.26 ± 0.03	2.14 ± 0.01	1.77 ± 0.02
GSD	1.64 ± 0.02	1.64 ± 0.01	1.58 ± 0.01	1.81 ± 0.04
Albuterol Deposited in Actuator(s)	152.79 ± 11.06	125.51 ± 21.90	232.55 ± 25.81	20.59 ± 2.44
Albuterol Deposited in Throat	385.59 ± 19.27	429.86 ± 12.60	351.50 ± 10.83	41.36 ± 2.28
Total Albuterol Recovered	899.39 ± 27.91	925.89 ± 25.19	995.79 ± 25.92	104.46 ± 4.51

[a] A waiting of one minute and 30 seconds between puffs delivered, respectively.
[b] Number of replicates.

different canisters/actuators were used) is about ten times the amount obtained in single puff measurements. The amount of albuterol deposited on the top cone, filter plate, and 7 jet plates was very small (9.4 ± 0.4 μg or less than 1 μg per delivered puff).

In conclusion, this study demonstrates the importance of controlling sampling conditions in particle size measurements of MDIs using cascade impactors. The collection of the least amount of drug aerosol in a cascade impactor may provides a better estimate of MMAD, as it minimizes modifications of the collection surfaces. The use of multi-stage cascade impactors for semi-routine particle size measurements of pharmaceutical aerosols is becoming increasingly popular. Since analogies have been drawn between dissolution testing of pharmaceutical solid dosage forms and particle size measurement of aerosols and to avoid the difficulties encountered with dissolution testing in the past, attention needs to be paid to sampling protocols.

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