Effect of Drug Load and Plate Coating on the Particle Size Distribution of a Commercial Albuterol Metered Dose Inhaler (MDI) Determined Using the Andersen and Marple-Miller Cascade Impactors

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Purpose. The purpose of this study is to investigate the effect of drug load, the coating of impactor stages, and the design of cascade impactors on albuterol MDIs particle size distribution measurements. The results of the investigation will be used to explain the "*loading effect*" recently reported.

Methods. Particle size distribution parameters of a commercial albuterol MDI were measured using both Andersen (AI) and Marple-Miller (MMI) Cascade Impactors, where plates were either left uncoated or coated with silicone or glycerin. A previously validated HPLC-EC method was used for the assay of albuterol collected by the impactor and in single spray content determinations.

Results. Coating impactor collection plates had an impact on measured MMAD and GSD values for single puff measurements but very little or no effect for the multi puff measurements. Due to particle bounce, the percent of albuterol fine particles deposited in the filter and impactor finer stages (<1.10 μm in AI and <1.25 μm in MMI) in uncoated single puff experiments was much higher in comparison to either coated single puff or multi-puff (coated and uncoated) measurements.

Conclusions. Evaluation of drug load and plate coating are necessary to determine whether observed particle size distributions are representative of the generated aerosol or are the result of particle bounce and reentrainment. In order to minimize particle bounce, especially for single puff determinations, it may be useful to apply a thin layer of a sticky coating agent to the surfaces of impactor plates.

KEY WORDS: albuterol; MDI; particle size; cascade impactors; loading effect; particle bounce.

INTRODUCTION

Metered dose inhalers (MDIs) are among the most convenient respiratory drug delivery devices. MDIs have been used for the delivery of many bronchodilator and anti-inflammatory drugs to the lungs. Albuterol, a β_2-selective adrenergic bronchodilator (1), is commonly used for the treatment of asthma. In general, physicians and manufacturers recommend the use of either one or two consecutive puffs (actuations, shots or sprays) of albuterol MDIs for the treatment of asthma. The determination of unit spray content and particle size distribution have been strongly recommended by the FDA and USP as a measure of *in vitro* bioequivalence of MDIs (2,3). The content and uniformity of unit spray provides assurance of a safe and effective drug dosage delivered over the life-time of the product (200 puffs). Particle size distribution of drug particles is an important criterion in the deposition of MDIs in the lung. In the past, due partially to the lack of available analytical sensitivity, content uniformity and particle size distribution measurements (using a multi-stage cascade impactor), were performed using multiple puffs of albuterol MDIs. Recently, studies have been conducted on single puff content uniformity (4) and single puff particle size analysis (5,6).

Using impaction methods, the observed mass median aerodynamic diameter (MMAD) in single puff particle size measurements of albuterol MDIs (5,6) were found to be lower than values reported earlier where 5–40 puffs were used (7,8). The preliminary results obtained in single puff particle size distribution studies have prompted additional investigations (9,10). Attempts were made to explain the observed "*loading effect*", where higher MMAD values were observed when several albuterol puffs were delivered into an Andersen impactor. In these studies (9,10), it was suggested that when multiple puff determinations were made, drug particles deposited on the impaction plates may modify collection characteristics of the impactor plates (stages) and hence promote the premature deposition of smaller drug particles in the early cascade stages, with larger cut-off diameter, along with the larger particles resulting in *artificially higher MMAD values.*

The reliability of cascade impactor data can be influenced by factors such as wall loss, particle bounce and blow-off, deagglomeration or break-up, and the nature of collection surfaces (8,11–16). Rao and Whitby (13) concluded that particle bounce was significant when aerosol solid particles were impacting the uncoated Andersen impactor steel plates. Esmen and Lee (14) used oil coated stages in a cascade impactor to eliminate particle bounce and recommended that the use of bare metal impaction surfaces should be totally avoided. Holzner and Müller (16) were able to eliminate particle bounce by coating Andersen metal impaction plates with a thin film of Span 85. When fewer drug particles (as in a single puff measurement) enter the impactor, particles may bounce upon impaction on bare metal stage surfaces resulting in the deposition of larger drug particles at subsequent stages and causing a distortion in particle size distribution (*artificially smaller MMAD values*). In other words, two possible mechanisms have been postulated to explain the "*Loading effect*" observed with MDIs, one leading to artificially higher MMAD values when excessive drug particles are loaded on the impactor (loading/multi-puff effect) and the other leading to artificially smaller MMAD values when small amounts of particles enter the impactor and bounce off the bare metal surfaces of the early cascade stages (bounce off effect).

In this report, findings on the effect of coating impactor plates on measured particle size distribution are presented. Also, in order to examine a possible effect of the measuring device, two cascade impactors (Andersen Sampler Mark II and Marple-Miller Impactor) have been used in this investigation. Other significant aspects, such as the influence of MDI formulations

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and the effect of varying the concentration of surface active agents, are currently being investigated. Our current studies are intended to fine tune experimental protocols and methodologies used in particle size measurements of pharmaceutical aerosols using commercially available multi-stage cascade impactors.

MATERIALS AND METHODS

Chemicals, reagents, and solutions were the same as described previously (5,9). Schering Proventil® (Lot No. 4-BBS-201, expiration date 5/97) was the commercial albuterol MDI used in the study.

Chromatographic Conditions

The HPLC system was operated at room temperature and consisted of a solvent delivery module (Waters 600E), an automatic sample injector (Waters 712 WISP), and a coulometric electrochemical detector (Coulochem II, ESA). The detector was equipped with a guard cell (model 5020) operated at 900 mV and a dual-electrode analytical cell (Model 5010), where the two electrodes were set at 525 and 800 mV, respectively. Only the signal from the second detector (set at 800 mV) was monitored. EZChrom™ Chromatography Data System (Shimadzu, Columbia, Maryland, USA) was used for data acquisition and calculations. The mobile phase was prepared by mixing 400 mL of 0.25 M ammonium phosphate buffer (pH 2.8), 210 mL of methanol, and the mixture was diluted to 2.0 L with ultrapure water (5). The prepared mobile phase was filtered through a 0.2 μm nylon membrane filter. The flow rate was set at 1.5 mL/min, and the injection volume was 100 μL. Calibration curves of albuterol standard solutions were prepared from 0.5 to 1000 ng/mL and correlation coefficients were ≥0.999. Figure 1 shows examples of typical HPLC-EC chromatograms.

Particle Size Measurements and Albuterol Assay

Cascade Impactor Procedure

Andersen 1 ACFM Non-Viable Cascade Impactor Mark II (AI) and Marple-Miller Model 150 Impactor (MMI) were used in this investigation. For each MDI unit tested, the aerosol canister valve was cleaned with methanol and dried thoroughly with nitrogen gas. An actuator, that was previously cleaned with methanol, was attached to the canister, the canister was shaken for 5 seconds, and the valve was primed 5 times. The canister was weighed and its weight was recorded. A single dose was collected using the USP dose apparatus (3) (Versapor® 3 micron, 25 mm filter) at a flow rate of 30 L/min. The canister was weighed again to determine the aerosol weight delivered. The collection vessel was rinsed with the mobile phase and transferred quantitatively into a 50 mL volumetric flask. Due to the high sensitivity of the electrochemical detector employed in the assay of albuterol, 1.0 mL of the solution obtained was transferred into a 25 mL volumetric flask and diluted with the mobile phase prior to the injection into the liquid chromatograph. The spray content assay experiment was repeated four additional times to obtain a total of five single spray content determinations prior to every particle size measurement. A USP inlet (3) was attached to the impactors and the flow rate was adjusted and maintained at 28.3 L/min for the Andersen impactor and 30.0 L/min for the Marple-Miller impactor. Air flow rates were monitored using a Sierra "Top Trac" mass flow meter. The vacuum pump was started and after 10 seconds the first puff was delivered into the cascade impactor; after an additional 60 seconds another puff was delivered (when specified). This process was repeated until the desired number of puffs (up to 10 puffs) were delivered into the cascade impactor. After 5 seconds, the pump was turned off and the impactor was disassembled. In AI experiments, the nozzle plate for stage 0, 8 collection plates (0 through 7) and the filter (Whatman GF/A filter) were transferred to individual polyethylene bags (Associated Bag Co., Milwaukee, WI) and suitable volumes of the mobile phase were added to each bag using a Brinkmann Dispensette pipetter (Brinkmann Instruments, Westbury, NY). In addition, the inlet was rinsed with an appropriate volume of the mobile phase. In MMI experiments, the inlet and the filter were transferred into individual polyethylene bags and rinsed with appropriate volumes of mobile phase. The five cups were rinsed with the mobile phase. The content of each bag and cup was transferred to a corresponding volumetric flask. Each bag was then rinsed several times with portions of the mobile phase and the rinses were added to the corresponding volumetric flasks.

An additional five single spray content determinations, using the USP dose apparatus, were performed as described above after the impactor particle size measurement. Interim data analysis indicated that albuterol recovery in impactor experiments was complete and comparable to single dose determinations (Table I). From that point on, only four single spray content experiments (instead of ten) were performed, two before and two after each impactor particle size measurement. The

Table I. Albuterol Recovery (μg) in Single Spray Content and Particle Size Determinations

	Andersen Impactor			Marple-Miller Impactor		
Experiment	n[a]	Alb. Recovered, μg	Alb. Recovery[b] per Puff, μg	n[a]	Alb. Recovered, μg	Alb. Recovery per Puff, μg
Single Spray Content	145	87	87	178	87	87
One Puff Exp.	11	89	89	13	88	88
Four Puff Exp.	4	338	85	4	330	83
Ten Puffs Exp.	10	804	80	16	840	84

[a] Number of replicates.

[b] The albuterol recovery in this study (80–89 μg/puff) is lower than previously reported values (9) of 90–104 μg/puff. However, albuterol deposited in actuators (13–21 μg) was included in the recovery determination in the previous study (9).

impactor samples (AI experiments = 11; MMI experiments = 7), 10 single spray samples (five samples collected prior to the cascade experiment and five following the cascade testing), and 10 standard albuterol solutions (0.5–1000 ng/mL) were injected into the liquid chromatograph.

In the protocol described above, the following guidelines were observed in carrying out the experimental procedure: (1) Proventil canisters were shaken for 5 seconds prior to firing a shot, (2) when a canister was not used for over an hour, it was re-primed three times through a priming actuator prior to an actual single spray sample collection or a cascade experiment, (3) a single actuator, M3710 (0.022″ orifice), was used throughout the testing, and (4) neither the actuator nor the valve stem were cleaned or assayed for albuterol deposited during the sample collection process.

Impactor Plates Surface Treatment

Three surface treatments were investigated. Regardless of the surface treatment used, a cleaning procedure was used before every impactor sample collection. All plates were thoroughly cleaned with an Alonox® detergent-water mixture, rinsed thoroughly with water, rinsed with acetone, and followed by rinsing with hexane. The washing sequence was repeated twice and plates were air dried prior to use. The effect of impactor plate coating was investigated as follows:

No Surface Treatment No coating was applied and stage surfaces were left untreated (uncoated).

Silicone Surface Treatment All purpose silicone spray (Cling Surface®, ITW Fluid Products Group, St. Louis, MO) was used to coat impactor plates. Plates were laid on a flat surface and sprayed evenly. A preliminary experiment showed that this technique resulted in a coating thickness of approximately 2 μm, well in excess of the 0.3 μm thickness that has been shown to be effective with this silicone (15).

Glycerin Surface Treatment Glycerin was applied to Andersen stages and Marple-Miller cups: Approximately 1 mL of glycerin in methanol mixture (1:1) was pipetted onto the Andersen stages and Marple-Miller cups. The methanol was allowed to evaporate prior to impactor-assembly. The evaporation process took about 20 minutes prior to use in this study. The Andersen plates were mechanically textured to provide a dull finish to prevent beading of the glycerin coating. Preliminary experiments indicated that the textured plates had the same collection characteristics as plates which had not been modified.

Method Validation

Most experimental parameters utilized in this study have been validated previously (5,9). The effect of introducing new experimental parameters such as surface coating (silicone and glycerin), the plate cleaning scheme, and the use of plastic bags for sample preparation, on experimental accuracy and chromatographic separation and detection was examined. The newly introduced experimental variables had no effect on chromatographic separation and detection of albuterol (Fig. 1) and only a minimal effect on the recovery of albuterol (Table I).

RESULTS AND DISCUSSION

Albuterol Assay

The electrochemical detection method (5,9) was used in all particle size measurements. The high detection sensitivity

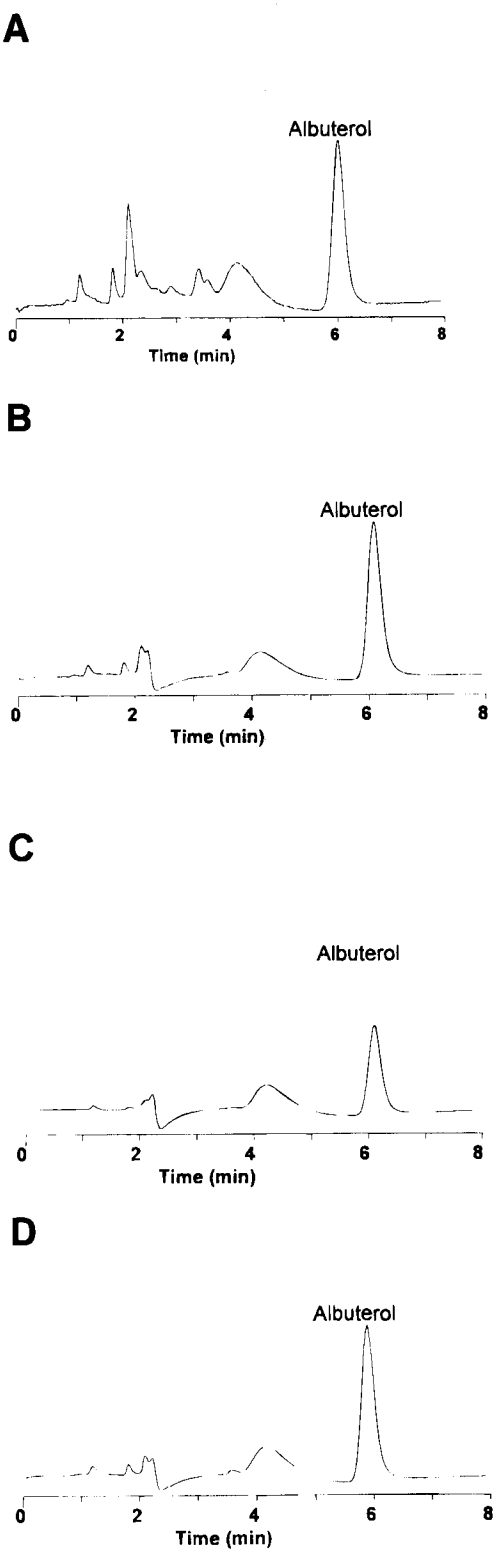

Fig. 1. Typical HPLC chromatograms obtained in the assay of albuterol using electrochemical detection: (A) albuterol standard solution, (B) impactor stage recovery of Proventil (uncoated stages), (C) impactor stage recovery of Proventil (silicone coated stages), and (D) impactor stage recovery of Proventil (glycerin coated stages). Chromatographic conditions are described in the experimental section.

was essential in the quantitative measurement of albuterol in cascade impactor fractions, where in some cases, albuterol concentrations were as low as 1 ng/mL. The chromatographic method proved to be sensitive and rugged. The presence of surface active agents and other non-albuterol ingredients in Proventil as well as silicone and glycerin in some impactor experiments had no effect on the chromatographic resolution and detection of albuterol. Typical examples of the chromatograms obtained in this study are illustrated in Fig. 1, where chromatograms of standard albuterol solution (A), a sample of Proventil recovered from an uncoated impactor stage (B), and similar Proventil samples collected from stages coated with silicone (C) and glycerin (D).

Albuterol Particle Size Analysis

In this study, 2–6 replicate experiments were performed for all particle size measurements. Even though the experimental protocol used to collect and extract albuterol in this study is simpler than the one used previously (5,9), it provided complete recovery of delivered albuterol. The nozzle plate for AI stage 0, a site not routinely assayed, collected an average of approximately 2% of the total sample recovered from the impactor. The albuterol recovery data obtained in single spray content determinations and particle size experiments are summarized in Table I. The albuterol recovered in impactor particle size determinations varied from 80 to 89 μg/puff in comparison to an average of 87 μg obtained in single spray content determinations. The albuterol recovery data obtained in this study are similar to previous results (9) where a more tedious experimental protocol was employed in albuterol extraction. The Marple-Miller impactor recovery data are comparable to the Andersen impactor data. This study and previous reports (5,9) show that particle size measurements using cascade impactors are useful not only in providing drug particle size distribution but unit spray content may be estimated from the amount of drug delivered into the impactor.

Particle size measurements were evaluated by utilizing a probit computer program (as previously described 5,9). There has been considerable debate on the reliability of MMAD and GSD values as a measure of size distribution of pharmaceutical aerosols. MMAD and GSD values for an aerosol using a multistage cascade impactor, such as Andersen or Marple-Miller impactors, may vary depending on the calculation method utilized (17–19). If direct comparison of MMAD and GSD values is desired, values should be obtained using the same impactor and utilizing the same calculation method (17).

Effect of Albuterol Loading and Plate Coating on Particle Size Distribution

In this study, Proventil has been chosen as an example of a commercial albuterol MDI. It has been shown recently (9) that the observed loading effect on measured MMAD values was larger in Proventil in comparison to some specially manufactured albuterol MDIs. Using the probit calculation method described earlier, Table II provides a summary of MMAD and GSD values obtained for single, four and ten puff impactor particle size experiments under different experimental conditions. The MMAD and GSD values obtained for single, four and ten puff measurements, where Andersen cascade impactor was used and collection plates were left uncoated, are identical (within experimental error) to values reported previously (9). This provides a validation of the experimental protocol utilized in this study and illustrates the ruggedness of the employed analytical assay. From Table II, it is evident that coating collection surfaces has a noticeable effect on measured MMAD and GSD values for single puff measurements and very little or no effect for the ten puff measurements. This observation is valid for the two impactors (AI & MMI). It is reasonable to conclude that coating impaction stages, with either silicone or glycerin, created the sticky surface needed to eliminate the possibility of albuterol particles escaping (bouncing off) upper impactor stages.

When enough drug particles are delivered into the cascade impactor, as in the case of the ten puff measurements, increasing the stickiness of the impaction stages had no effect on measured MMAD and GSD values. Plots of the particle size distribution of single puff albuterol measurements (under different impactor coating conditions) as well as a ten puffs measurements, where impactor stages were left uncoated (Fig. 2), clearly show that particle size distribution patterns of the uncoated ten puffs and coated single puff measurements are identical and MMAD values are comparable (Table II, 2.2–2.3 for AI and 2.2–2.4 for MMI). Both silicone and glycerin have similar effects on the particle size distribution of single puff measurements (Table II & Fig. 2). Also, in the single puff measurements, the GSD is decreased upon coating the impaction stages (1.7 vs 1.9 for AI and 1.7 vs 2.4 for MMI) indicating an improvement of the collection efficiency or a decrease in particle size distribution polydispersity (11). It is possible that loading enough albuterol particles, as in the ten puff particle size measurements, causes a modification of the metallic surface of impaction stages resulting in the prevention of incoming drug particles bouncing off collection plates and escaping into lower stages. It is also possible that enough sticky excipient (oleic acid in the case of Proventil) is deposited on impactor stages, in the multi-puff experiments, to eliminate or minimize drug particles bounce off in the early stages.

It has been suggested previously that aerosol particle bounce can be eliminated by coating impactor stages (13–16).

Table II. Effect of Stage Coating on the Mass Median Aerodynamic Diameter[a] (MMAD, μm) and Geometric Standard Deviation[a] (GSD)

		Andersen Impactor		Marple-Miller Impactor	
No. of Puffs	Stage Coating	MMAD, μm	GSD	MMAD, μm	GSD
One Puff	None	1.79[b]	1.92	1.75	2.41
	Silicone	2.23	1.71	2.16	1.77
	Glycerin	2.26	1.69	2.44	1.71
Four Puffs	None	2.16[b]	1.60	2.05	1.72
	Glycerin	2.28	1.60	2.24	1.66
Ten Puffs	None	2.23[b]	1.62	2.22	1.69
	Silicone	2.33	1.71	2.32	1.68
	Glycerin	2.16	1.63	2.45	1.80

[a] Particle size measurements (MMADs & GSDs) were evaluated utilizing the probit computer program outlined previously (5,9).
[b] Values are identical (within experimental error) to those reported previously (9).

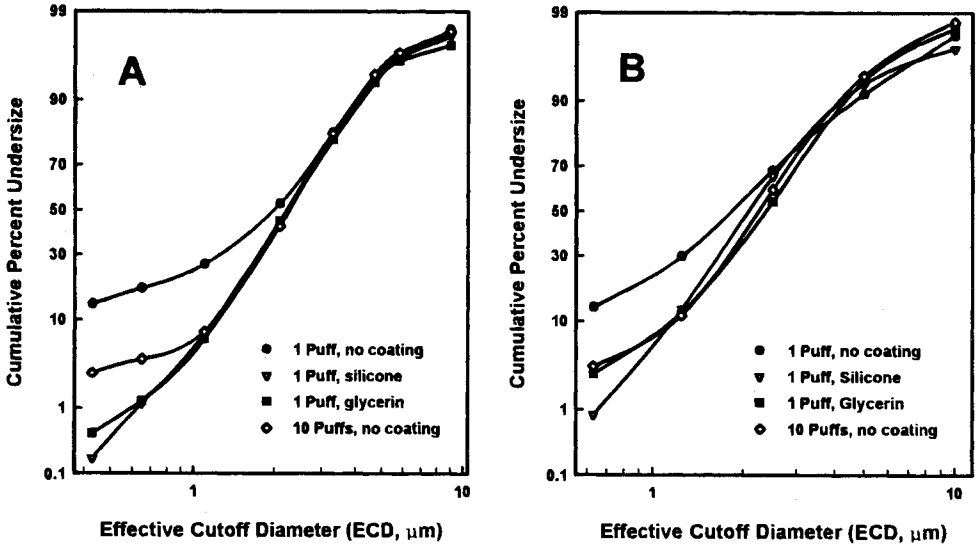

Fig. 2. Cumulative percentage undersize albuterol by mass versus the effective cut-off diameter (log scale, μm) for 1 Puff (coated and uncoated) and 10 Puff (uncoated) delivered from Proventil MDI: (A) Andersen Cascade Impactor, and (B) Marple-Miller Impactor.

When a small amount of aerosol particles is delivered into the cascade impactor, particles may bounce off collection surfaces of early impactor stages (larger cut-off diameter) and deposit at later stages (smaller cut-off diameter) resulting in artificially small MMAD values. The effect of albuterol loading and impactor plate coating on the deposition pattern of albuterol particles entering the Andersen cascade impactor (stages 0 - stage 7 and filter) and Marple-Miller impactor (cup 1 - cup 5 and filter) has been closely examined. Figure 3 depicts the deposition of albuterol delivered into Andersen impactor (A) and Marple-Miller impactor (B) under three different coating conditions (uncoated, silicone coated, and glycerin coated). To simplify the presentation, the data from the four puff and coated ten puff experiments are not included. In the uncoated single puff experiments, there is a noticeable increase in the percent of albuterol deposited in the filter and finer impactor plates (stages 6&7 in AI and cup 5 in MMI) in comparison to the coated (silicone and glycerin) single puff and uncoated ten puff experiments may be due to the bounce of drug particles as discussed before. Recently, a similar observation was made by Holzner and Müller (16), where no drug particles were deposited on the filter and only a small amount (0.1–0.3% of drug delivered) were found in the finer stages of the Andersen impactor where impactor plates were coated with a thin film of Span 85.

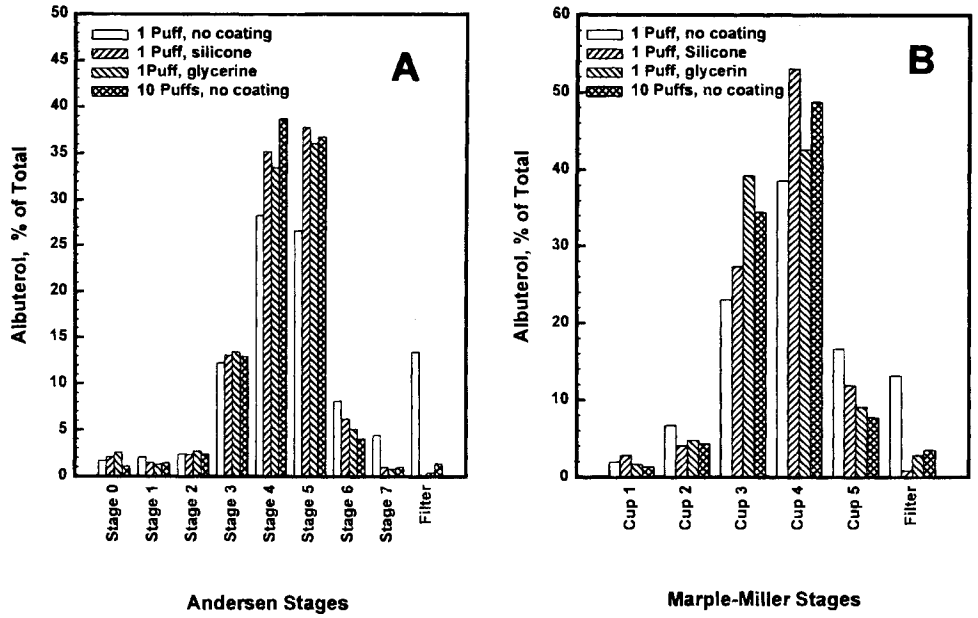

Fig. 3. The deposition pattern of albuterol from Proventil MDIs entering Andersen Cascade Impactor (A), and Marple-Miller Impactor (B).

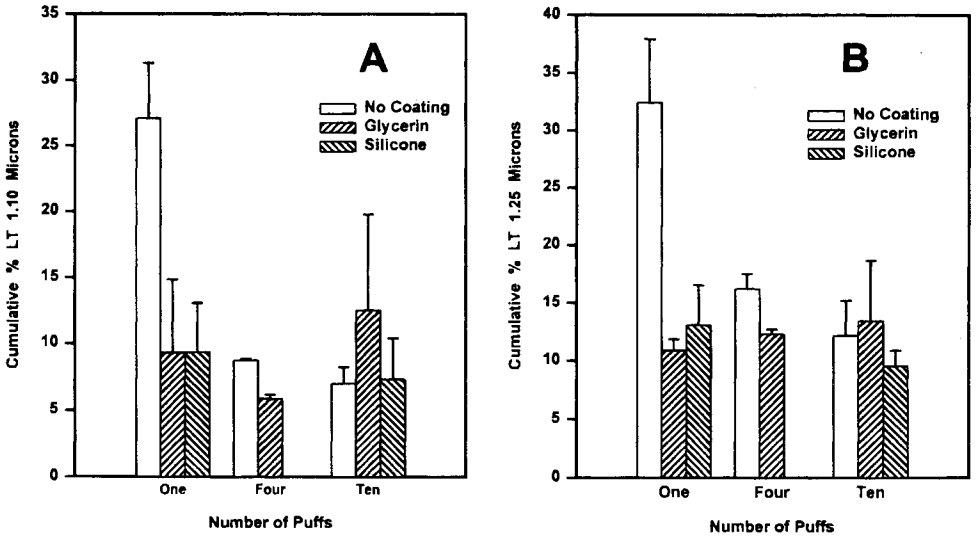

Fig. 4. The deposition of albuterol fine particles plotted as Cumulative % less than 1.10 Microns (A, Andersen Impactor) and Cumulative % less than 1.25 Microns (B, Marple-Miller Impactor) versus the number Proventil puffs used in particle size measurements. Impactors' stages were coated with silicone, glycerin, or left uncoated.

Figure 4 illustrates the deposition of albuterol fine particles plotted as cumulative % less than 1.10 μm (A, Andersen impactor) and cumulative % less than 1.25 μm (B, Marple-Miller impactor) versus the number of puffs used in particle size measurements. From Fig. 4, it is apparent that uncoated surfaces with 1 or 4 puffs from the MDI are prone to particle bounce and re-entrainment. In addition, bounce is not observed with 10 puffs on uncoated surfaces or 1 to 10 puffs on collection surfaces coated with either glycerin or silicone.

Comparison of the Marple-Miller Impactor and Andersen Cascade Impactor

Both Marple-Miller and Andersen impactors were used in this investigation to explore the possibility that the measuring device (cascade impactors) may influence the observed "*loading effect*". Tables I & II and Figures 2–4 provide a comparison of the performance of both impactors. The albuterol recovery data (Table I) and measured MMAD values (Table II) are comparable for both impactors. Particle size distribution profiles (Figure 2–4) are similar for the two impactors. In summary, the two impactors' (AI & MMI) performance was comparable in measuring particle size distribution of albuterol in this investigation and particle bounce was clearly observed with both impactors.

CONCLUSIONS

Since particle size is considered to be the most important non-biological factor affecting aerosol deposition in the lung (12), one needs to understand the techniques and calculation methods employed in particle size measurements. It is known that different measurement techniques may result in different particle size estimates (20). This study illustrates the importance of studying factors that influence particle size measurements of aerosols using multi-stage cascade impactors. Our results demonstrate that particle bounce influences particle size distribution and is the major determinant of the "*loading effect*". Even though drug particles in formulations containing surfactants or greasy excipients, such as Proventil, are considered to be sticky and therefore impaction plate coating should not be needed to prevent bouncing (21), our observations with single puff determinations dispute that view point. In order to minimize particle bounce, especially for single puff determinations, it is useful to apply a thin layer of silicone, glycerin, other coating agents to impactor plates.

Evaluation of drug loading effects is necessary to determine if observed particle size distributions are representative of the generated aerosol or arise as artifacts of particle bounce and re-entrainment.

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