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Influence of single versus multiple actuations on the particle size distribution of beclometasone dipropionate metered-dose inhalers

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Abstract

The particle size distributions of beclometasone dipropionate delivered from Becotide and Respocort inhalers after single and multiple actuations were investigated using the Andersen Mark II Cascade impactor and the drug was quantified using high performance liquid chromatography. The fine particle mass and the mass median aerodynamic diameter were calculated. An apparent increase in mass median aerodynamic diameter was observed when the number of actuations increased. In addition, the fine particle mass decreased as the number of actuations increased. When performing and analysing cascade impaction study data differences between single versus multiple actuations must be considered. Regulatory guidelines should be amended to stipulate the number of actuations to be loaded into devices used to evaluate the particle size distribution of inhaled aerosol products.

Introduction

It is generally accepted that particles with an aerodynamic diameter between 1 to 5 μm have a higher probability of penetrating deep into the lung which facilitates their therapeutic activity (Timsina et al 1994). This fraction of the emitted dose is often referred to as the fine particle mass or the respirable dose. Particles with an aerodynamic diameter greater than 5 μm are most likely to impact in the oropharyngeal cavity and induce local and systemic side effects, especially when inhaling glucocorticoids (Willey et al 1967; Toogood et al 1982). Testing and examining the particle size distribution, including the fine particle mass, of inhaler aerosols is an important quality assurance measure to ensure safety and reproducibility of the production batches. Laboratory sampling procedures are a critical factor because this may affect the interpretation of the results of an aerosol deposition study. It is appropriate that a sampling procedure which is used to evaluate inhalation aerosols should mimic the actual clinical situation in which it is used to ensure that the results simulate the clinical conditions of the inhaler usage in patients.

The United States Pharmacopoeia (USP), British Pharmacopoeia (BP), and the Therapeutic Goods Administration in Australia (TGA) set a number of requirements for testing metered-dose inhalers and dry powder inhalers. These requirements include content uniformity, labelled dose (labelled claim), particle size distribution, fine particle mass and specifications for labelling. In addition, requirements for the instrumentation used for assessing these parameters and recommendations for in-vitro impaction flow rates during the experiment have been suggested (TGA 1992; USP 1995; BP 2000). However, regulatory guidelines do not stipulate the number of actuations to be loaded into devices used to evaluate the particle size distribution. Most studies that characterize the aerosol performance delivered from different devices or comparative studies of two or more commercial devices have generally employed several actuations (5–40) of the inhaler device per determination (Holzner & Müller 1994; Barnes & Nash 1996; Smith et al 1998). Generally, this has been undertaken to enable collection of sufficient quantity of drug to allow chemical analysis due to poor sensitivity of the analytical methods employed. However, using single rather than multiple actuations when evaluating the particle size distribution of inhaled aerosols more closely parallels

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the clinical situation, where patients generally inhale one or two consecutive actuations of the anti-inflammatory or bronchodilator from the inhaler device. The usual method of assessing the drug particle size distribution of metered-dose inhalers has been to determine the fine particle mass, the mass median aerodynamic diameters, and the geometric standard deviation (Kenyon et al 1995; USP 1995).

The aim of this study was to characterize the particle size distribution after single and multiple actuations (2, 5 and 10 actuations) from two commercially available beclometasone dipropionate metered-dose inhalers.

Materials and Methods

Materials

Beclometasone (beclomethasone) dipropionate and dexamethasone 21-acetate (analytical standards) were purchased from Sigma Chemical Co. (St Louis, MO). Methanol and acetonitrile HPLC grade were purchased from Biolab Scientific (Clayton, Australia). Analytical grade acetic acid was purchased from Rhone-Poulenc Chemicals (Clayton, Australia). The beclometasone dipropionate metered-dose inhaler products were the Becotide inhaler 100 μg 200 doses, Lot no 055316 (Allen and Hanburys/Glaxo Wellcome, NSW, Australia), and the Respocort inhaler 100 μg 200 doses, Lot no 063421 (3M Pharmaceuticals, Australia).

Aerosol characterization and sampling

The Andersen Mark II Cascade impactor (Graseby Andersen, 3025, Atlanta, GA) calibrated at 28.3 L min^{-1} was used as described in detail by Feddah et al (2000, 2001). This apparatus has been well characterized in the study of particle size distribution of inhalation aerosol particles (USP 1995). This device was calibrated to collect aerosol particles in eight size categories over the following particle size range 9.0–10.0, 5.8–9.0, 4.7–5.8, 3.3–4.7, 2.1–3.3, 1.1–2.1, 0.7–1.1, 0.4–0.7 μm according to their aerodynamic diameter.

The Andersen Mark II Cascade impactor, calibrated at 28.3 L min^{-1}, was connected to a vacuum pump (Erweka, GmbH, Germany, serial no 70018) to simulate the inspiratory flow rate. The flow rate was measured by a mass flow meter (Model 822S-H-4-OV1-V1; Sierra Instruments, Monterey, CA) connected to the impactor through the induction port (USP, stainless steel 90-degree). The laboratory temperature during the study was maintained at 22 ± 2 °C and the relative humidity was 49 ± 6%.

For each beclometasone dipropionate metered-dose inhaler product, four inhalers from a single production batch were evaluated and each test consisted of at least five replicates. Each metered-dose inhaler device was shaken for 15 s before actuation then the first three actuations were discarded to waste before the beclometasone dipropionate dose was introduced into the impactor. One, two, five or ten doses were introduced into the Andersen Cascade impactor through the induction port on different occasions. There was a 1-min wait between multiple actuations, and the valve of the metered-dose device was depressed for 1 s. The vacuum pump was switched on 10 s before each actuation and allowed to continue for a further 15 s after actuation before the pump was switched off. The aerosol canister remained valve down when not in use. After each study, the cascade impactor was dismantled and each of the eight stages of the impactor, the mouth piece and the induction port were washed with 10 mL methanol to collect the deposited beclometasone dipropionate. The fraction of beclometasone dipropionate on each stage of the cascade impactor, mouthpiece, and the induction port was collected for separate quantitative analysis. A sample (100 μL corresponding to 20.7 μg) of methanolic solution of the internal standard (dexamethasone 21-acetate) was added to each sample, which was then dried at 35 °C under a stream of nitrogen. The dried residues were reconstituted in mobile phase, and 100 μL was injected directly into the HPLC. The analysis of beclometasone dipropionate was performed by HPLC using a Beckman System Gold HPLC apparatus (Beckman Instruments, Fullerton, CA), with UV detection at 242 nm and an Exsil 100/5 μm ODS, 25 × 0.46 cm (Activon Gold Pack) HPLC column. The mobile phase consisted of methanol:acetonitrile:water:acetic acid (60:10:28.3:1.7) at a flow rate of 1.7 mL min^{-1}. The standard curve in the range of 1.0–100.0 μg mL^{-1} beclometasone dipropionate in methanol was constructed using the peak area ratio with the internal standard (Feddah et al 2000).

Experimental design

Part one of this study examined the content uniformity of single actuation of beclometasone dipropionate delivered from Becotide and Respocort inhalers using two different sampling procedures. Samples were either taken at spray numbers (10, 20, 30, 40 and 50) or taken randomly after firing two actuations to the waste. This part of the study was conducted to ensure that variation in the particle size distribution for the multiple actuation vs single actuation study was not due to content uniformity problems associated with the inhalers.

Part two of the study was undertaken to compare the results of particle size distribution of single actuations obtained by random sampling with that from multiple actuations by sampling (2, 5 or 10) actuations for each canister.

Data analysis and calculations

Statistical evaluation of the results were performed using analysis of variance (JMP Statistics Software, v3.1 SAS Institute Inc., Cary, NC). The significant differences were analysed further using unpaired t-test and P-values of less than 0.05 were considered to be significant.

Fine particle mass smaller than 4.7 μm was calculated as the sum of beclometasone dipropionate mass recovered in stages 3, 4, 5, 6, 7 and the filter of the Andersen Cascade impactor. The percentage of beclometasone dipropionate

recovered in each stage of the impactor was calculated by dividing the estimated mass in each stage by the total mass delivered into the impactor (particles < 10.0 μm). A cumulative percent undersize plot was constructed by plotting the cumulative percent undersize on a probability scale against the aerodynamic diameter on a logarithmic scale. The mass median aerodynamic diameter was obtained by observation of the fiftieth percentage point. Geometric standard deviation was also obtained from this graph using the following equation 1:

$$GSD = \sqrt{(sizeX/sizeY)} \quad (1)$$

where X is the particle size at which the line crosses the 84.13% value and Y is the particle size at which the line crosses the 15.87% value.

Results and Discussion

Investigations using single actuation

The particle size distribution of beclometasone dipropionate after a single actuation delivered from Becotide and Respocort inhalers at spray numbers 10, 20, 30, 40, and 50 was compared with the single actuation particle size distribution of the same inhalers randomly sampled (Figures 1 and 2). The mean mass and the range of the mass on each stage for five replicates against effective cut-off diameter of the stage for the two products show the variation in the amount of beclometasone dipropionate observed on a given stage within the products.

The particle size distribution data obtained from the Becotide and Respocort inhalers are presented in Table 1. The first section of Table 1 presents the mean (± s.d.) amount of drug collected in each stage of the impactor. To summarize the data, particle size distribution from collective stages are presented. The sum of drug particles collected in stages 0 to 9 represents the total amount of beclometasone dipropionate delivered to the impactor. The stages containing particles < 4.7 μm are defined as the fine particle mass.

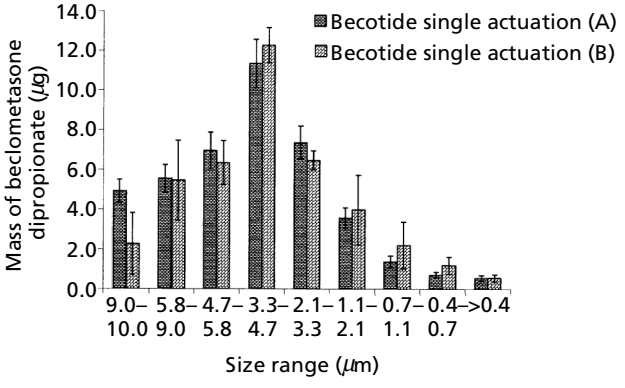

Figure 1 Particle size distribution of beclometasone dipropionate delivered from Becotide inhaler at spray numbers (10, 20, 30, 40, and 50) (A) compared with the particle size distribution of single actuation of the same inhaler randomly sampled (B). The vertical bars indicate s.d. of five determinations.

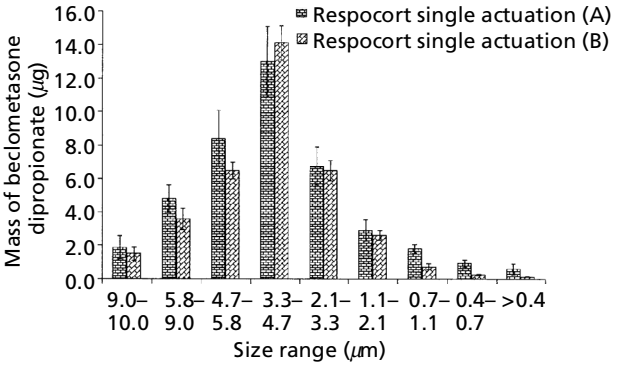

Figure 2 Particle size distribution of beclometasone dipropionate delivered from Respocort inhaler at spray numbers (10, 20, 30, 40, and 50) (A) compared with the particle size distribution of single actuation of the same inhaler randomly sampled (B). The vertical bars indicate s.d. of five determinations.

These results demonstrated that the Becotide and Respocort inhalers were comparable with respect to both the total amount of beclometasone dipropionate collected in the impactor and the fine particle mass (< 4.7 μm). The results indicated no significant differences ($P > 0.05$) in the mass median aerodynamic diameter and the geometric standard deviation observed between the two products.

Investigations using single vs multiple actuations

The particle size distribution, the fine particle mass < 4.7 μm and the size range from 0.4 to 5.8 μm for beclometasone dipropionate generated from the Becotide and Respocort inhalers are shown in Tables 2 and 3. To compare the particle size distribution of beclometasone dipropionate after single vs multiple (2, 5, or 10) actuations, each product was examined independently to identify differences between the amount of drug deposited in each specific size range, and to compare the mass median aerodynamic diameter and the geometric standard deviation for single and multiple actuations. Values of the mass median aerodynamic diameter and the geometric standard deviation are presented in Table 4 and depicted in Figures 3 and 4.

The fine particle mass (< 4.7 μm) of beclometasone dipropionate delivered from Becotide and Respocort inhalers after a single actuation was compared with that delivered from multiple actuations. It was evident that the fine particle mass decreased significantly ($P < 0.05$) as the number of actuations increased. A decrease of 21.5% and 27.4% was observed in the fine particle mass between single vs 10 actuations for Becotide and Respocort inhalers, respectively (Figures 5 and 6). When evaluating a particle size range from 0.4 to 5.8 μm the same pattern was observed, with a reduction in fine particle mass of

Table 1 Particle size distribution of beclometasone dipropionate delivered as a single actuation from Becotide 100 μg and Respocort 100 μg inhalers at an inspiratory flow rate of 28.3 L min^{-1}.

Size range (μm)	Becotide	Respocort
> 10.0	44.6 ± 9.75	51.3 ± 7.9
9.0–10.0	4.93 ± 0.59	1.85 ± 0.67
5.8–9.0	6.75 ± 1.06	4.77 ± 0.83
4.7–5.8	6.96 ± 0.93	8.41 ± 1.68
3.3–4.7	11.35 ± 1.22	12.96 ± 2.12
2.1–3.3	7.36 ± 0.83	6.75 ± 1.12
1.1–2.1	3.57 ± 0.51	2.88 ± 0.65
0.7–1.1	1.38 ± 0.29	1.78 ± 0.28
0.4–0.7	0.70 ± 0.15	0.93 ± 0.2
> 0.4	0.55 ± 0.15	0.60 ± 0.33
Collective stages		
0–9.0	43.56 ± 1.16	40.93 ± 3.22
5.8–10.0	10.48 ± 1.24	6.62 ± 1.24
Fine particle mass < 4.7 μm	24.92 ± 0.91	25.90 ± 2.44
Mass median aerodynamic diameter	3.55 ± 0.15	1.89 ± 0.1
Geometric standard deviation	3.92 ± 0.21	2.35 ± 0.1

Values given are mean ± s.d. of five replicates.

Table 2 Particle size distribution of beclometasone dipropionate after single vs multiple (2, 5 or 10) actuations of Becotide inhaler 100 μg.

Size range (μm)	Single puff (μg)	Two puffs (μg)	Five puffs (μg)	Ten puffs (μg)
> 10.0	45.75 ± 10.87	54.89 ± 5.65	42.52 ± 6.54	55.54 ± 12.25
9.0–10.0	2.26 ± 1.56	2.99 ± 0.35	4.30 ± 1.43	4.58 ± 0.52
5.8–9.0	8.33 ± 14.35	5.95 ± 0.16	6.10 ± 0.62	5.79 ± 0.47
4.7–5.8	6.36 ± 1.11	7.17 ± 0.16	6.84 ± 0.26	6.92 ± 0.49
3.3–4.7	12.28 ± 0.9	12.58 ± 0.29	11.77 ± 0.4	11.01 ± 0.57
2.1–3.3	6.48 + 0.47	6.51 ± 0.15	6.16 ± 0.32	6.09 ± 0.22
1.1–2.1	3.99 ± 1.75	3.43 ± 0.11	3.01 ± 0.4	2.41 ± 0.53
0.7–1.1	2.20 ± 1.18	1.61 ± 0.16	1.11 ± 0.14	0.65 ± 0.32
0.4–0.7	1.19 ± 0.43	0.87 ± 0.19	0.49 ± 0.04	0.36 ± 0.01
< 0.04	0.55 ± 0.18	0.49 ± 0.34	0.75 ± 0.35	0.43 ± 0.18
Total (μg)	89.39 ± 16.25	96.48 ± 1.37	83.06 ± 1.72	93.80 ± 2.12
Fine particle mass < 4.7 μm	26.69 ± 2.53	25.49 ± 1.01	23.29 ± 1.2	20.96 ± 1.05
Fine particle mass < 5.8 μm	33.05 ± 2.63	32.66 ± 1.1	30.13 ± 1.22	27.89 ± 1.52

Values are mean ± s.d. for five replicates.

15.6% and 25.3% for the Becotide and Respocort inhalers, respectively.

When the number of actuations was increased from one to ten, an increase in the mass median aerodynamic diameter of 11% and 27% was observed for beclometasone dipropionate delivered from the Becotide and Respocort devices, respectively. There were no statistically significant differences in the values of geometric standard deviation detected between single vs multiple actuations in either of the products under examination.

Previous studies using the Andersen Cascade impactor calibrated at 28.3 L min^{-1} (Miller & Shultz 1992; Nasr 1993) had obtained a lower value of the mass median aerodynamic diameter (1.8 μm) in single puff particle size measurements for salbutamol metered-dose inhalers, compared with the mass median aerodynamic diameter values (2.0–2.8 μm) reported from studies where five to 40 puffs were used (Kim et al 1985; Fults 1991).

The higher values of mass median aerodynamic diameter for beclometasone dipropionate observed in the studies employing multiple actuations suggested that the drug particles deposited on the impaction plates from the first actuations had the potential to modify the collection characteristics of the impactor plate stages. Thus some aerosolized drug particles with smaller aerodynamic diameter, which would normally penetrate to the lower stages of the impactor according to their aerodynamic diameter, were captured by these particles found in the upper stages

Table 3 Particle size distribution of beclometasone dipropionate after single vs multiple (2, 5 or 10) actuations of Respocort inhaler 100 μg.

Size range (μm)	Single puff (μg)	Two puffs (μg)	Five puffs (μg)	Ten puffs (μg)
> 10.0	35.31 ± 3.34	47.37 ± 4.16	48.22 ± 5.99	54.04 ± 4.1
9.0–10.0	1.78 ± 1.13	1.19 ± 0.27	4.54 ± 1.08	8.42 ± 0.91
5.8–9.0	4.25 ± 0.79	3.68 ± 0.58	4.32 ± 1.18	6.59 ± 0.5
4.7–5.8	7.14 ± 1.56	6.80 ± 0.83	7.69 ± 0.99	5.97 ± 0.94
3.3–4.7	13.75 ± 2.19	12.96 ± 0.53	14.98 ± 0.74	11.24 ± 0.84
2.1–3.3	7.83 ± 1.51	7.21 ± 0.9	6.33 ± 0.65	5.55 ± 0.2
1.1–2.1	3.39 ± 0.68	4.45 ± 0.73	3.39 ± 0.35	2.72 ± 1.03
0.7–1.1	3.33 ± 1.16	2.06 ± 0.33	1.31 ± 0.17	1.21 ± 0.26
0.4–0.7	1.40 ± 0.4	0.85 ± 0.09	0.97 ± 0.15	0.84 ± 0.31
0–0.4	0.87 ± 0.23	0.50 ± 0.12	0.63 ± 0.31	0.64 ± 0.14
Total (μg)	79.06 ± 9.93	87.06 ± 2.58	92.37 ± 3.45	97.22 ± 11.61
Fine particle mass < 4.7 μm	30.57 ± 4.79	28.02 ± 1.87	27.60 ± 2.04	22.20 ± 2.77
Fine particle mass < 5.8 μm	37.71 ± 6.13	34.82 ± 2.24	35.29 ± 2.32	28.17 ± 3.72

Values are mean ± s.d. for five replicates.

Table 4 Mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) of beclometasone dipropionate delivered from the Respocort and the Becotide inhalers obtained using single and multiple (2, 5 or 10) actuations.

	Single puff		Two puffs		Five puffs		Ten puffs	
	MMAD (μm)	GSD	MMAD (μm)	GSD	MMAD (μm)	GSD	MMAD (μm)	GSD
Respocort	3.75 ± 0.06	2.19 ± 0.09	3.83 ± 0.03	1.91 ± 0.23	4.15 ± 0.09	1.89 ± 0.07	4.78 ± 0.04	1.73 ± 0.15
Becotide	4.15 ± 0.11	1.91 ± 0.17	4.10 ± 0.04	1.80 ± 0.1	4.30 ± 0.24	1.79 ± 0.25	4.60 ± 0.29	1.71 ± 0.09

Values are mean ± s.d. for five replicates.

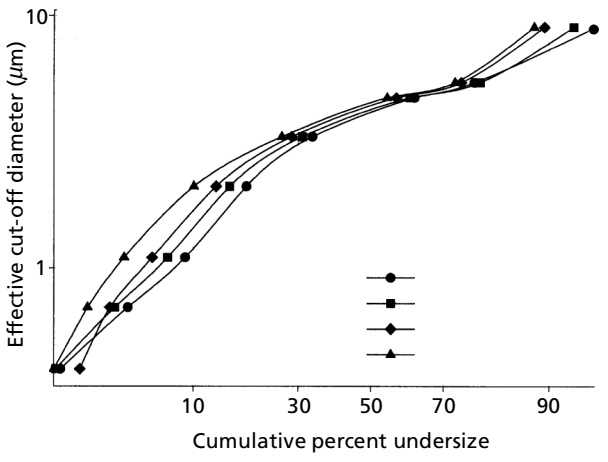

Figure 3 Cumulative mass percentage of particles against log diameter showing percent mass of particles with diameter less than a certain size for Becotide 100 μg inhaler. Mass median aerodynamic diameter was obtained from 50% undersized particles.

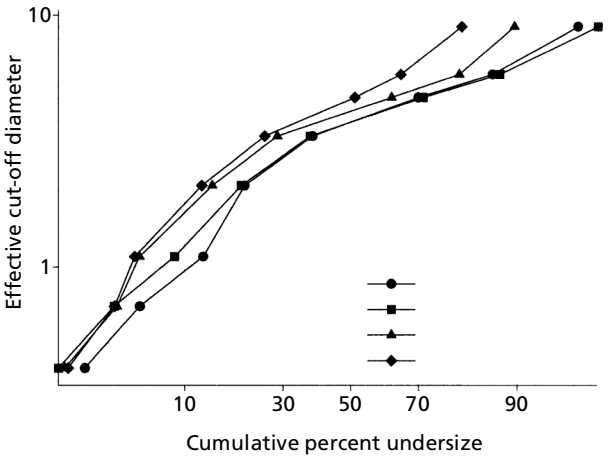

Figure 4 Cumulative mass percentage of particles against log diameter showing percent mass of particles with diameter less than a certain size for Respocort 100 μg inhaler. Mass median aerodynamic diameter was obtained from 50% undersized particles.

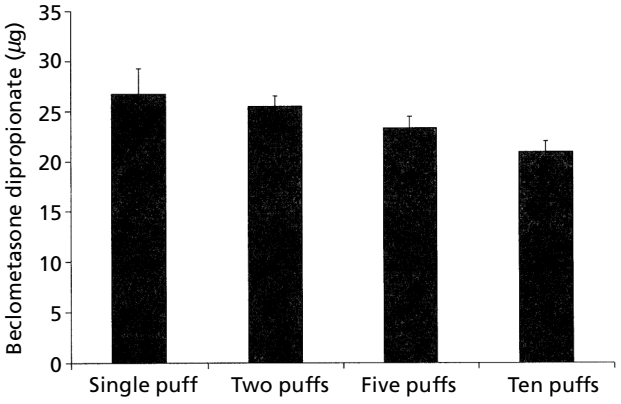

Figure 5 Fine particle mass of beclometasone dipropionate delivered from Becotide inhaler at inspiratory flow rate of 28.3 L min^{-1} from a single and from multiple (2, 5 or 10) actuations. The vertical bars indicate the s.d. of five determinations. *$P < 0.05$ compared with single puff.

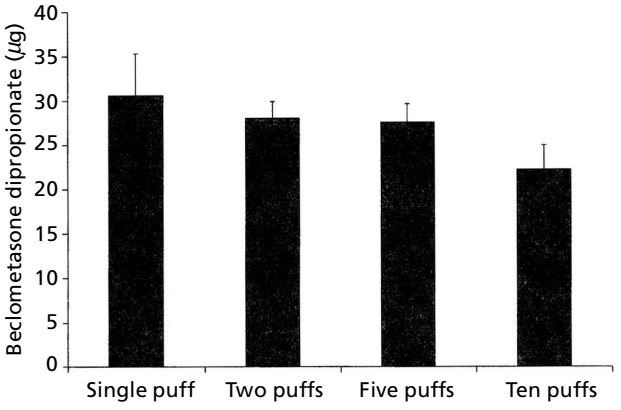

Figure 6 Fine particle mass of beclometasone dipropionate delivered from Respocort inhaler at inspiratory flow rate of 28.3 L min^{-1} from a single and from multiple (2, 5 or 10) actuations. The vertical bars indicate the s.d. of five determinations. *$P < 0.05$ compared with single puff.

of the impactor by the process of interception impaction (Hinds 1982; Hickey 1992). It was likely that the bounce phenomenon and re-entrainment in the air stream became reduced as the number of actuations increased. The increase in the retention of smaller particles on the higher stages of the impactor by these mechanisms resulted in an artificially higher mass median aerodynamic diameter compared with that observed after a single actuation of the inhaler.

Conclusions

These results were consistent with findings in the literature and suggested that there were significant discrepancies between single and multiple actuations in estimating fine particle mass and mass median aerodynamic diameter from aerosol cascade impactor studies. In the specific requirements for testing metered-dose inhaler products the number of actuations should be specified when submitting applications for new and generic metered-dose inhaler products to regulatory agencies for approval. In addition, aerosol scientists and the scientific community should not ignore the number of actuations employed in pharmaceutical investigations of aerosol inhalers but should develop standardization procedures and policies to deal with the variability and discrepancies that may result when single vs multiple actuations are characterized.

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