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Cascade impaction methods for dry powder inhalers using the high flowrate Marple-Miller Impactor

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Abstract

The magnitude and effects of stage overload and particle re-entrainment in the new, Marple-Miller cascade impactor (MMI) were evaluated at 60 liter/min by sampling and determining the aerodynamic size distributions from two, excipient-free, powder inhalers (Turbohaler™ and Spinhaler™) according to a variety of experimental protocols. Drug distributions were compared statistically, for both inhalers, following single dose experiments in the presence and absence of silicone oil impactor stage coating and between single dose and multiple dose experiments in its presence. Stage coating was found to be essential to prevent re-entrainment of drug from both inhalers. One or $\leqslant$ 25 dose sampling was shown to produce valid results provided impaction stages were coated for the 0.5 mg Bricanyl Turbohaler (44.7 $\pm$ 9.6% of emitted dose $<$ 5 μm; overload and re-entrainment was evident following sampling of 40 doses). One or 2 dose sampling was shown to produce valid results for 20 mg Spinhaler, provided drug capture was enhanced further by also coating the aerosol inlet port to MMI (10.7 $\pm$ 1.3% of emitted dose $<$ 5 μm; overload of stage 1 and re-entrainment was evident following sampling of 2 doses in the absence of a coated aerosol inlet port). The absence of significant re-entrainment could be shown most effectively by statistically comparing values for percent deposition, at different sites in the stage-coated impactor, between single and multiple dose ($\geqslant$ 2) experiments. Such an experiment should be performed for each type of inhaler and formulation to be tested, as a means of validating the exact impaction technique to be used for size distribution analysis of powder inhaler emissions.

Keywords: Aerodynamic particle size analysis; Dry powder inhaler; Cascade impactor; Re-entrainment; Particle bounce

1. Introduction

A review of aerodynamic particle sizing methods suitable for use with different dry powder inhalers (DPIs), at different flowrates, is currently being undertaken by the USP's Advisory Panel on Aerosols (Byron et al., 1994). The Panel requested

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interested parties to calibrate suitable cascade impactors and liquid impingers at a variety of different flows, typical of those which are to be used during dose emission testing of powder inhalers (30–100 liter/min). The Marple-Miller Impactor (MMI) is available in two versions with published calibrations at 30, 60 and 90 l/min (MMI models 150 and 160; Marple et al., 1995) and, thus, may well be a suitable instrument. In addition, it has design features (stage cups) which facilitate sample recovery for analysis and low inter-stage drug losses (Marple et al., 1995), which may not be true for other impactors (Marple and Willeke, 1979; Hickey, 1990; Phillips et al., 1990).

A recent theoretical description of impactor cutoff characteristics (Rader and Marple, 1985) summarizes that the cutoff diameters depend on the flow rate of air through the impactor and on the physical dimensions of the instrument. According to theory for impactors with circular jets, if (1) the spacing between nozzles and impaction surfaces is in the range of 1 to about 2 times the nozzle diameter and, (less importantly) (2) the nozzle length is less than 10 times the nozzle diameter, then the collection efficiency is a function of only 2 dimensionless variables. These are the Stokes number, Stk, and Reynolds number, Re, defined as

$$Stk = [4\rho_p QCD_p^2]/[9\Pi\mu W^3] \quad (1)$$

and

$$Re = [4Q\rho]/[\Pi W\mu] \quad (2)$$

where ρ and ρ_p are air density and particle density respectively, Q is the volumetric airflowrate through the nozzle, C is the Cunningham slip factor, D_p is particle diameter, μ is air viscosity and W is the nozzle diameter. Furthermore, when $500 \leq Re \leq 3000$, the collection efficiency becomes only a function of Stk.

Stages 2 through 5 of the MMI conform to both of the above criteria for these relationships to hold, although stage 1 does not. Thus, even though Re values for stage 1 of MMI fall in the 500–3000 range (Marple et al., 1995), the following discussion applies strictly to stages 2 through 5 and only approximately to stage 1.

According to the above definitions and the Re values tabulated in Marple et al. (1995) for MMI, the flowrate ranges corresponding to nozzle Reynolds numbers between 500 and 3000 are 12–69 and 24–138 Liter/min for the model 150 and 160, respectively. Thus, within these flowrate ranges, the capture efficiency of each stage, when expressed as a function of the Stokes number (Eq. 1), does not change. In particular, the value of Stk corresponding to the 50% capture efficiency,

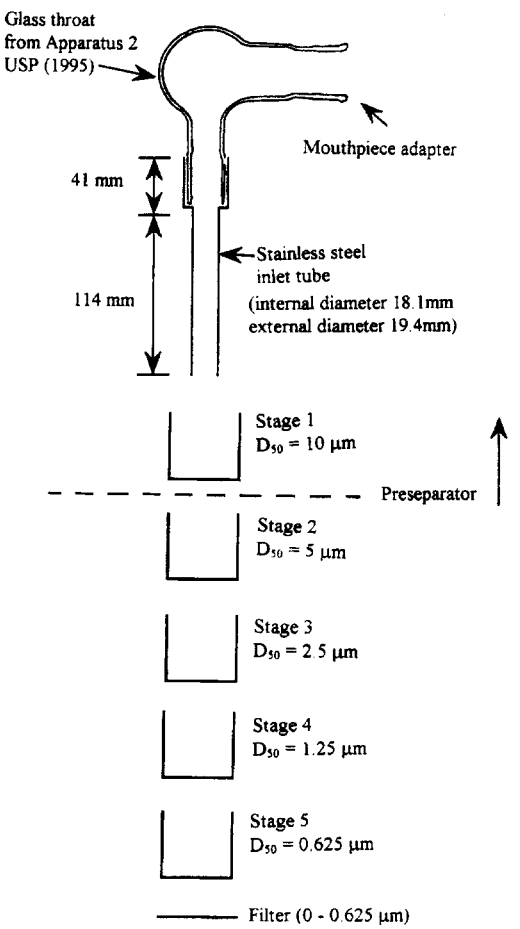

Fig. 1. Diagrammatic representation of the MMI Model 160 (Marple et al. (1995), showing dimensions of the inlet port and a schematic of the stages at which drug was collected and assayed. Values for D_{50} are the aerodynamic diameters of each stage at which the stage has a 50% capture efficiency at 60 liter/min. Drug mass was fractionated into aerodynamic size ranges (2.5–5, 5–10 μm etc.) according to common practice and USP (1995).

which is defined as the stage cutoff diameter (D_{50}, Fig. 1, is a constant value for all flowrates within the range. The cutoff diameter of any stage, at any flowrate, Q, can therefore be calculated from

$$D_{50,Q} = D_{50,Qn}\,[Qn/Q]^{1/2} \quad (3),$$

where D_{50} is the stage cutoff diameter (aerodynamic diameter which is captured with 50% efficiency, Fig. 1) and the terms Q and Qn represent the actual and the nominal flowrate, respectively. Using Eq. 3 to calculate cutoff diameters for the model 160 MMI at 90 Liter/min, based on calibration values collected at 60 Liter/min (Marple et al., 1995), values for stages 1–4 are 7.8, 4.1, 2.1 and 1.0 μm, respectively, compared to their experimental counterparts of 7.2, 4.0, 1.9 and 0.9 μm; the calculated values lying within the errors associated with experimental impactor stage calibrations.

It appears, therefore, that MMI is a suitable cascade impactor for use with powder inhalers, as defined by the USP Aerosol Panel. Even so, the panel recognized in their article (Byron et al., 1994) that stage overload and particle re-entrainment in the airstream passing over each impactor stage can be a problem when characterizing powder aerosols by impaction. They recommended the use of the 'preseparator' atop the Andersen Mk II cascade impactor (to remove particles with aerodynamic diameters > 10 μm, and thus minimize stage overload), as well as impaction-stage coating with an adhesive substance (throughout the impactor) to minimize particle bounce and re-entrainment (Byron et al., 1994; Esmen and Lee, 1980). Unfortunately, the Andersen preseparator has been shown to have broad cutoff characteristics, and its particle capture performance is quite similar to the Andersen stage 0 (Vaughan, 1989; Mitchell et al., 1988). In this study, we chose to use stage 1 (Fig. 1) of the MMI (which, at 60 liter/min, collects particles > 10 μm, Marple et al., 1995) as a well calibrated, particle impactor, in place of a purpose-built preseparator. Furthermore, we have evaluated the MMI as an instrument to determine the aerodynamic size distribution from two marketed, excipient-free, powder inhalers (Bricanyl Turbohaler™ and Intal Spinhaler™, UK formulation), which differ in the important respects of drug substance (terbutaline sulfate and cromolyn sodium) and dose (0.5 and 20 mg label claims), respectively. Also, because the probable effects of stage coating with silicone oil (as a powder adhesive substance), and the collection of multiple, as opposed to single doses per experiment, were not obvious to us, we compared the results of 7 (Turbohaler) and 5 (Spinhaler) different experimental protocols for use with MMI. Specifically, we examined the effects of stage coating and the number of doses sampled upon apparent particle size distribution data before and after transformation according to the current USP data treatment method (United States Pharmacopeia, 1995).

2. Methods

2.1. Protocol

Aerodynamic particle size distributions from two powder inhalers were determined using the model 160 Marple-Miller Impactor (MMI, MSP Corporation, Minneapolis, MN), according to a variety of experimental protocols (Table 1). The effects of coating the stages of MMI, and collecting multiple doses from each inhaler (Turbohaler, 1 through 40; Intal Spinhaler, 1 or 2, Table 1), were compared statistically in order to examine the experimental importance of particle re-entrainment and stage overload upon the measurements. In the Spinhaler experiments, the effect of reducing the drug mass reaching stage 1 (upon the resultant particle size distributions) was also studied by coating the throat and inlet tube to the impactor in protocols XI and XII. All experiments were replicated (Table 1) in order to examine their precision. Only excipient-free inhalers were studied (in which micronized drug alone was aerosolized) in order to assess the 'material -' or 'drug-dependence' of the results in this impactor (without the complication of unknown excipient loads accumulating on each of the stages). Bricanyl Turbohalers (Astra-Draco, Lund, Sweden; batch TD407, supplied as a gift by the manufacturer) were tested as an example of a low dose inhaler (label claim = 500 μg terbutaline sulfate

Table 1
Summary of experimental protocols

Product	Protocol number	Sites coated	Number of doses	Number of experiments
Bricanyl[a]	I	None	1	10
	II	All stages	1	10
	III	All stages	5	2
	IV	All stages	10	2
	V	All stages	15	2
	VI	All stages	25	2
	VII	All stages	40	2
Intal[b]	VIII	None	1	5
	IX	All stages	1	10
	X	All stages	2	3
	XI	Stages + inlet	1	5
	XII	Stages + inlet	2	5

[a]Turbohaler, label claim = 0.5 mg terbutaline sulfate.[b]Spinhaler with Spincaps (UK product), label claim = 20 mg cromolyn sodium.

per metered dose). Intal Spinhalers and Spincaps (Fisons Pharmaceuticals, Loughborough, UK; batches ATK11B and JE4501B1, respectively) were selected as a high dose inhaler (label claim = 20 mg cromolyn sodium per metered dose) and obtained commercially from the UK (note that Intal USA contains lactose as a diluent).

2.2. Equipment

The Model 160 Marple-Miller Impactor (MMI, MSP Corporation, Minneapolis, MN) was used as supplied with its designated vacuum pump and calibrated flowmeter. The impactor was assembled according to the manufacturer's instructions (as described by Marple et al., 1995), with the exception that a purpose-built aerosol inlet port was used to replace the 'entry port' (designed for use with pressurized inhalers) of that publication. Fig. 1 shows the inlet port used in the present study which was comprised of the 'glass throat' from the 'twin stage impinger' or USP Apparatus 2 (United States Pharmacopeia, 1995) inserted into the conical taper of a stainless steel tube supplied by MSP Corporation, for use with MMI. The assembly was airtight and enabled mouthpiece adapters used with the twin stage impinger also to be used with MMI.

2.3. Cascade-impaction

The air flowrate through the assembled MMI was adjusted, at the vacuum pump, with an unprimed Turbohaler (or an Intal Spinhaler containing an empty capsule) inserted into the mouthpiece adapter (to ensure an airtight seal, Fig. 1). All experiments were performed at 60 ± 3 liter/min (Hindle and Byron, 1995). Prior to each experimental protocol, the MMI was disassembled and cleaned. The inlet port and individual stages were washed [white spirit (silicone oil solvent), followed by water, followed by acetone] and allowed to dry, and a clean 47 mm diameter, glass fiber filter (Type A/E, Gelman Sciences Inc., Ann Arbor, MI) placed in the terminal filter holder below stage 5 (Fig. 1). According to the protocol being followed (Table 1), the internal surfaces of stages 1–5 and/or the inlet port (Fig. 1) were either left clean or were coated with silicone oil. This was performed by spraying, for 2 × 5 s periods, from a pressurized aerosol canister (Silicone Release Spray #316, Dow Corning Corp., Midland, MI), held at a distance of 15 cm from the surface to be coated. A 3-min interval at room temperature was allowed after each spray, to ensure complete propellant evaporation. The MMI was reassembled and the vacuum pump switched on. The inhaler was loaded according to the manufacturer's instructions and inserted into

the mouthpiece adapter (Fig. 1) for 20 s, thus sampling the entire aerosol output of the inhaler. The inhaler was removed, reloaded and the sampling procedure repeated 0–39 times (Table 1), after which the vacuum pump was switched off. Drug was collected by washing and sonicating the throat and mouthpiece adapter, the inlet tube, stages 1–5 of the MMI and the final filter, and diluting to volume with water, the wash solvent for both terbutaline sulfate and cromolyn sodium. Each drug was assayed according to Hindle and Byron, 1995. In order to validate each experiment by mass balance (as proposed in Byron et al., 1994), average drug capture per dose was compared to emitted doses determined independently at 60 liter/min (Hindle and Byron, 1995).

2.4. Data treatment and statistics

Mean amounts of drug ($\pm$ sample standard deviation) collected from the throat, inlet tube, stages 1–5 and filter were determined for each of the protocols described in Table 1 (Table 2). Aerodynamic particle size distributions were generated using the method described and tabulated in the USP for each of these average data sets, with two provisors: first, that drug captured on stage 1 of the MMI was not included as part of the size distribution (this only included drug collected on stages 2 and below) and, second, that Fig. 2c of Chapter 601 in USP was not followed because it wrongly implies that incorrect co-ordinates should be plotted as percent undersize vs aerodynamic diameter (United States Pharmacopeia, 1995). Briefly, the throat, inlet tube and stage 1 was treated as a well-designed preseparator (Byron et al., 1994). Thus, all material retained on stage 1 and above had aerodynamic diameters > 10 μm; the upper size limit of drug in this category was unknown. Size distributions were generated for drug which penetrated the MMI to stage 2 and below. Cumulative percentage mass undersize was plotted (probability scale) for each protocol versus the individual stage cut-off diameters, D_{50} (Fig. 1 at 60 liter/min; Marple et al., 1995), on a log scale ('log probability plots'). To standardize the method of deriving values for mass median aerodynamic diameter (MMAD) and geometric standard deviation (σ_g) from each data set, MMAD (aerodynamic diameter above which 50%, of the total drug mass $<$ 10 μm, resides) was determined by graphically interpolating between points either side of the 50 percentile on the log probability graph. Similarly, geometric standard deviation was determined from the ratio, $MMAD/d_{16\%}$, the denominator corresponding to the aerodynamic diameter possessed by the 16 percentile which was also determined by graphical interpolation. Values for MMAD and σ_g, emitted doses (ED) and percents of those doses less than 10 μm (% on stage 2 and below) and 5 μm (fine particle percentage, Byron et al., 1994) were compared statistically between experimental protocols (Table 2) using single factor analysis of variance (ANOVA; each variable was compared between each pair of protocols, as described in the footnote to Table 2). Probability, P, values ≤ 0.05 were regarded as an indication of statistical significance.

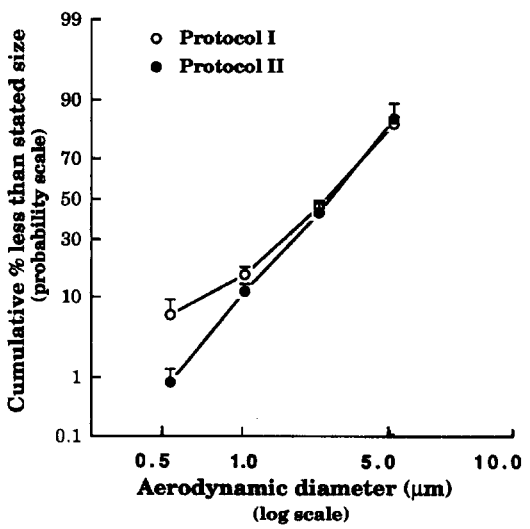

Fig. 2. Log-probability plots comparing the aerodynamic particle size distributions of single dose studies with the Bricanyl Turbohaler™, in the presence and absence of coating (Protocols II and I, respectively, Table 1).

Table 2
Mean (S.D.) particle size distributions and emitted doses from Bricanyl Turbohaler (protocols I–VII) and Spinhaler (VIII–XII) after 60 liter/min sampling in MMI. Protocols printed in bold type were used as statistical comparators.

Protocol[a]	Cumulative % less than stated size[b] (μm)					% of ED <10 μm[c]	MMAD (μm)[d]	σg[e]	ED (% of label claim)[f]	% of ED in presep[g]	% of ED <5 μm[h]
	10	5	2.5	1.25	0.625						
I	100.0	83.5 (2.1)	46.1 (3.1)	*16.1 (2.6)	*6.5 (3.0)	*63.5 (4.0)	2.67 (0.17)	2.56 (1.26)	79.5 (15.7)	*36.5 (4.0)	*53.0 (3.9)
II	*100.0*	*85.1 (4.1)*	*42.8 (5.7)*	*11.3 (2.1)*	*0.8 (0.5)*	*52.2 (9.3)*	*2.81 (0.24)*	*1.94 (0.16)*	*77.5 (6.4)*	*47.8 (9.3)*	*44.7 (9.6)*
III	100.0	83.3 (0.1)	38.7 (2.1)	9.6 (0.3)	0.9 (0.3)	55.5 (4.6)	2.89 (0.00)	1.89 (0.04)	*92.2 (3.3)	44.5 (4.6)	46.3 (3.9)
IV	100.0	81.4 (1.9)	34.9 (1.2)	8.1(1.0)	0.9 (0.2)	58.1 (4.2)	3.03 (0.07)	1.84 (0.04)	85.6 (14.8)	41.9 (4.2)	47.3 (2.3)
V	100.0	83.3 (1.4)	35.3 (0.6)	8.2 (0.8)	1.1 (0.1)	61.2 (2.7)	2.96 (0.10)	1.77 (0.02)	75.5 (9.3)	38.8 (2.7)	51.0 (1.4)
VI	100.0	82.4 (0.1)	*33.4 (0.6)	*7.9 (0.8)	1.3 (0.0)	55.1 (3.4)	3.10 (0.03)	1.87 (0.04)	85.4 (3.7)	44.9 (3.4)	45.4 (2.7)
VII	100.0	*74.4 (6.5)	*30.8 (3.2)	*7.4 (1.1)	1.0 (0.1)	53.8 (11.6)	*3.39 (0.30)	1.93 (0.09)	74.7 (1.8)	46.2 (11.6)	39.7 (5.2)
VIII	100.0	48.2 (2.8)	18.1 (0.6)	5.1 (1.1)	1.5 (0.4)	28.9 (2.6)	5.10 (0.33)	2.17 (0.16)	86.5 (7.2)	71.1 (2.6)	13.9 (0.9)
IX	*100.0*	*54.4 (7.7)*	*19.9 (2.9)*	*3.8 (1.7)*	*1.0 (1.3)*	*28.7 (5.3)*	*4.71 (0.75)*	*2.10 (0.35)*	*80.8 (11.0)*	*71.3 (5.3)*	*15.5 (3.3)*
X	100.0	57.0 (3.6)	21.5 (3.4)	3.9 (0.8)	0.8 (0.2)	**37.9 (3.5)	4.33 (0.28)	2.02 (0.08)	83.3 (5.5)	**62.1 (3.5)	**21.7(3.01)
XI	*100.0*	*49.9 (3.9)*	*18.6 (1.3)*	*5.3 (0.7)*	*1.4 (0.3)*	***21.5 (2.6)*	*4.95 (0.42)*	*2.18 (0.10)*	*76.7 (5.1)*	***78.5 (2.6)*	***10.7 (1.3)*
XII***	100.0	46.6 (2.6)	16.6 (1.4)	5.8 (0.8)	1.8 (0.3)	22.7 (1.9)	5.29 (0.28)	2.23 (0.16)	83.2 (3.4)	77.3 (1.9)	10.6 (1.4)

[a]Table 1; [b]based on drug masses collected on Stage 2 and below (Fig. 1); [c]% of emitted dose on Stage 2 and below; [d]by interpolation; [e]geometric standard deviation by interpolation; [f]emitted dose $\times$ 100/label claim; [g]% on Stage 1 and inlet port; [h]Fine particle fraction $\times$ 100.*Statistically significant difference compared to Protocol II (single dose with coated stages); $P < 0.05$ (ANOVA).**Statistically significant difference compared to Protocol IX (sIngle dose with coated stages); $P < 0.05$ (ANOVA).***No significant differences compared to Protocol XI (single dose wIth coated stages and inlet); $P < 0.05$ (ANOVA).

3. Results and discussion

3.1. Bricanyl turbohaler

The overall mean emitted dose ± relative standard deviation (RSD) measured across protocols I–VII was 79.93 (± 13.7)% of the 0.5 mg label claim. This value was consistent with the recent results from Meakin et al. (1995), determined at 60 Liter/min over 10 s using the 'twin stage impinger' (Apparatus 2, United States Pharmacopeia, 1995). However, the emitted dose was significantly different to the value of 62.5 (± 13.7) determined earlier on Bricanyl batch SH 53 by Hindle and Byron (1995) using the new, USP advocated method, for emitted dose testing and the Dosage Unit Sampling Apparatus for Dry powder Inhalers (Byron et al., 1994). This difference begged the question of whether, for Bricanyl, the emitted dose was different because of inhaler batch variations (Meakin et al., 1995) or because the new USP sampling method modified the dose collected (by using a fixed volume, valve-controlled, air-throughput of 4 liters at 60 liter/min; Hindle and Byron, 1995). However, when the 'USP' method (Hindle and Byron, 1995) was employed to determine the emitted dose uniformity of the present Bricanyl batch, TD 407, we obtained 76.44 (± 8.04)%, which differed insignificantly from the value of 79.93 (± 13.7)% determined with MMI. Thus, for Bricanyl at least, it appeared to be possible to use either MMI or the USP apparatus to determine the emitted dose; although in vitro dose emissions differed significantly between Bricanyl batches SH 53 (purchased through pharmacy outlets in the UK) and TD 407 (obtained direct from Astra Draco). Furthermore, for the latter batch in the present study, there was no significant difference between the emitted doses determined in any of the study protocols, except Protocol III, in which the emitted dose was found to increase (92.2% of label claim; Table 2). Most importantly, total drug capture in all of these cascade impactor experiments (Protocols I–VII) was consistent with the known dose emission characteristics of Bricanyl Turbohaler and the extremely low wall losses in MMI (Marple et al., 1995).

Table 3
Mean (S.D.) mass deposition of terbutaline sulfate in the MMI following sampling according to Protocols I and II (P I and P II; n = 10)

Collection site	Amount of terbutaline sulfate (μg)	
	P II (coated stages)	P I (uncoated stages)
Throat**	98.8 (15.4)	108.0 (31.0)
Inlet**	6.4 (2.3)	8.2 (1.6)
Stage 1**	80.4 (38.0)	29.4 (5.2)*
Stage 2	29.5 (7.3)	41.7 (8.3)*
Stage 3	85.9 (19.0)	95.1 (22.8)
Stage 4	63.8 (13.7)	75.1 (15.1)
Stage 5	21.1 (5.0)	24.2 (5.0)
Filter	1.7 (1.1)	15.8 (3.7)*

*Statistically significant difference compared to Protocol II (single dose with coated stages); $P < 0.05$ (ANOVA).**Throat, inlet and stage 1 comprise the preseparator (Fig. 1).

Table 2 summarizes the data collected according to protocols I–VII (Table 1). The parameters measured in the single dose Turbohaler experiments in the presence and absence of stage coating (Protocols II and I, respectively) differed significantly in a number of cases (indicated by asterisk, Table 2). The deposition pattern in the absence of silicone coating was consistent with particle bounce and re-entrainment (Esmen and Lee, 1980), even in the case of this small dose, pure drug inhaler (Turbohaler contains no diluents; total mass deposited in MMI was approximately 250 μg). The raw data modification which was introduced by the presence of coating is shown in further detail in Table 3 and Fig. 2. Significantly reduced drug retention on Stage 1, part of the preseparator in this case (Fig. 1), resulted from the use of uncoated stages, as expected. The subsequent redistribution of the drug masses in the uncoated MMI were much less predictable. Table 3 (uncoated, protocol I) shows that while stage 2 retention was increased (from stage 1), stages 3–5 were statistically unaffected; re-entrained material (from uncoated stage 2) presumably cascaded from stage 2 to 3, 3 to 4, 4 to 5 and 5 to filter, with only the latter showing a significant increase in the uncoated experiment.

These results show the importance of coating impactor stages prior to powder inhaler testing but they remain, of course, specific to Turbohaler, the uncoated MMI and the 20 s sampling time used in this study. Fig. 2 shows the results of the graphical data transformation recommended by United States Pharmacopeia (1995) for the same data sets. These plots of cumulative % undersize versus aerodynamic diameter are based only on drug penetrating the preseparator (< 10 μm in this case; Table 2). Clearly, in the absence of stage coating, plot curvature increased on a log-probability scale, as a direct result of re-entrainment and particles cascading down MMI. Of greater interest in this case was the insignificant differences detected between protocols I and II in the mass median diameter and geometric standard deviation; both terms were insensitive to dramatic shifts in drug distribution in the aerosol sampling apparatus. The fine particle fraction (% emitted dose < 5 μm), however, increased significantly in the absence of stage coating showing this to be the more sensitive derived parameter as a determinant of MMI misuse.

Table 2 (Protocol III–VII) also shows the effects of multiple dosing upon the particle size distribution results for Turbohaler using silicone coated MMI stages. Duplicate experiments were performed with 5, 10, 15, 25 and 40 doses. There were no detectable differences between the data from protocols II to VI, showing that this coating technique successfully prevented particle re-entrainment for up to (and probably beyond) 25 doses (total mass in the MMI approximately = 6 mg). After 40 doses however, particle size distributions and mass distribution in the stage-coated MMI became significantly different from that seen following single dose collection. Fig. 3 illustrates these differences between protocols I and VII following the USP method (United States Pharmacopeia, 1995). In this case (protocol VII), MMAD showed significant differences compared to protocol II while the fine particle fraction (% < 5 μm) differed insignificantly ($P > 0.05$ — ANOVA). While the mass of drug retained by the preseparator after 40 doses was found not to differ significantly compared to Protocol II ($P > 0.05$ — ANOVA; proving the efficiency of stage 1 as a preseparator), there was thus some evidence that terbutaline sulfate from Bricanyl could overload stage 2 (or perhaps the adhesive coating at the loci at which drug deposition was concentrated) and progress down the impactor after sampling 40 doses or more. These experiments serve to illustrate the validation of MMI quite clearly; it was unimportant whether 1 or up to 25 doses were collected, provided the stage coating was performed as described earlier.

3.2. Intal Spinhaler

The overall mean emitted dose ± relative standard deviation (RSD) measured across protocols VIII–XII was 81.8 (± 10.0)% of the 20 mg label claim; a value consistent with the dose emission characteristics of Spinhaler (Hindle and Byron, 1995) and advocated mass balance requirements for USP cascade impaction with DPIs (Byron et al., 1994).

Particle size distribution data for this 20 mg dose, pure drug, powder inhaler was surprisingly different to that seen with Turbohaler. There were no significant differences between single dose, per-

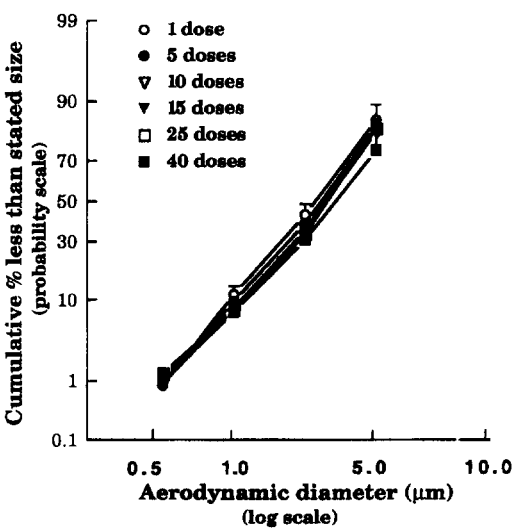

Fig. 3. Log-probability plots comparing the aerodynamic particle size distributions from studies of single and multiple dose experiments with the Bricanyl Turbohaler using coated stages (Protocols II–VII, Table 1). Significant differences were not observed until 40 doses were sampled (Table 2).

centage cromolyn sodium powder deposition in MMI, irrespective of whether or not impactor stages were coated with silicone oil (Table 2, protocols VIII and IX). The mass of powder depositing in the most highly loaded stage (stage 1 of the preseparator) was approximately 10 mg for both protocol VIII and IX. This value was very much greater than that required for terbutaline sulfate to bounce and cascade down the MMI in the absence of stage coating (Table 3 and Fig. 2). These results imply that particle re-entrainment, just like particle dispersion from powder inhalers and their formulations, is a strong function of the material being dispersed. Cromolyn sodium leaving Spinhaler may adhere much more strongly to stage 1, or cohere to itself once deposited, or both, in a much more effective manner than terbutaline sulfate from Turbohaler. Nevertheless, when a 2-dose sampling regime was employed with Spinhaler and the stage-coated MMI (protocol X), total preseparator (and stage 1) retention decreased while % of emitted dose < 5 and/or 10 μm increased significantly. Unfortunately, the absence of statistical equivalence between the 1- and 2-dose cases (protocol IX and X, Table 2) prevents us from declaring unequivocally that single dose size distributions from Spinhaler (protocol VIII and IX, Table 2) are valid results. In spite of this evidence that 2 doses of cromolyn created overload conditions in the coated MMI, the cumulative size distributions (Fig. 4) were indistinguishable (implying that extra drug from the second dose, which penetrated below stage 1, was then spread among the remaining stages of MMI in the same ratios as the first dose). Protocols XI and XII were performed to test the hypothesis that a reduced drug mass penetration of MMI would enable 1- and 2-dose measurements on Spinhaler to be determined to be statistically equivalent. The mass retention on the inlet port (protocol XI, % of ED in preseparator) was significantly greater when single doses were sampled from Spinhaler using silicone-coated throat and inlet tubes (protocol XI compared to IX, Table 2; coating the glass throat increased its drug deposition from approximately 3 to 6 mg). As a result of this increase, there was a significant reduction in the mass of cromolyn sodium penetrating to stage

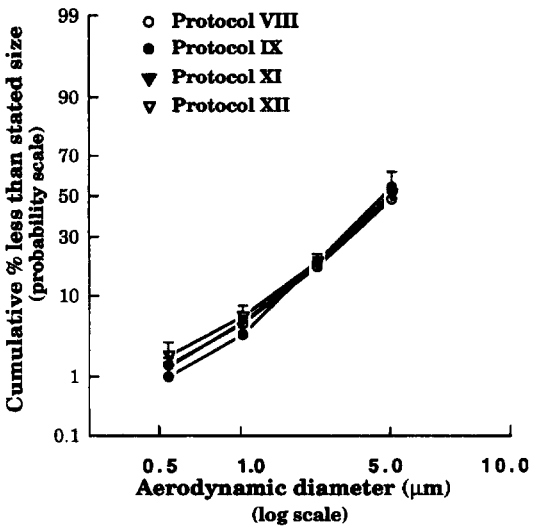

Fig. 4. Log-probability plots comparing the aerodynamic particle size distributions of single dose studies with the Intal Spinhaler, with no stage or inlet coating (Protocol VIII), with coated stages (Protocol IX), and with the throat, inlet tube and stages coated (Protocol XI). The plot also shows results from the 2-dose study in which throat, inlet tube and stages were coated, Protocol XII (Table 1).

2 and below (% of ED < 10 μm, Table 2). Most importantly, there was no significant difference between the data collected following 2 doses and 1 dose, provided both the inlet port and the stages were coated (protocol XII versus XI, Table 2). Thus, both protocol XI and XII can be declared valid with respect to re-entrainment while protocols IX (single dose with coated stages) and VIII (single dose without stage coating) may or may not be valid for use with MMI. Moreover, Fig. 4 and the values for MMAD (Table 2) show the importance of reviewing all of the drug deposition data between protocols. There were no significant differences ($P > 0.05$ — ANOVA) observed between measured MMADs or log-probability plots for any of protocols VIII–XII.

4. Conclusions

Stage overload, particle bounce and re-entrainment may all be problems when the aerosol output from powder inhalers is characterized by cascade impaction. Stage coating was essential for

aerodynamic size analysis of terbutaline sulfate in MMI at 60 liter/min (and to validate the size analyses for cromolyn sodium from Spinhaler following single dose collections). The coating procedure was simple, the impactor was easy to use and emitted-dose recovery showed that MMI had negligible wall losses in any of the investigated inhaler-sampling protocols. While the thorough validation protocols in this study were able to resolve re-entrainment problems, it was difficult to predict the experimental variable(s) which showed greatest sensitivity to re-entrainment and drug transfer between impactor stages. Technique validation should be specific to the drug and inhaler being tested; data for the 2 drugs terbutaline sulfate and cromolyn sodium implied that the adherent powder masses retainable by individual impactor stages were drug-dependent, even when stages were coated with silicone oil. Furthermore, to prove the absence of significant particle re-entrainment, percentage drug deposition within the stage-coated impactor should be compared directly between single and multiple dose protocols, rather than comparing the results of data transformations, like MMAD and fine particle dose. Those parameters may, or may not, change significantly as a result of stage overload and re-entrainment.

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