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Aerodynamic deposition of combination dry powder inhaler formulations *in vitro*: A comparison of three impactors

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ABSTRACT

Inertial impaction is generally regarded as the 'gold standard' for the *in vitro* assessment of aerodynamic deposition of inhaled formulations. Despite the availability of several impactors, few studies have compared measurements of aerodynamic deposition using multiple impactors and none employed a combination formulation. The aerodynamic deposition of the combination dry powder inhaler (DPI) Seretide® Accuhaler®, which contains salmeterol xinafoate (SX) and fluticasone propionate (FP), was assessed using the Andersen cascade impactor (ACI), multi-stage liquid impinger (MSLI) and next generation impactor (NGI) and the results were compared. Two Seretide products were tested at flow rates of 30 and Q L min^{-1}, the latter corresponding to a pressure drop of 4 kPa across the device. Significant differences in the particle size distributions were observed when the same formulation was tested using various impactors. The ACI was found to be less suitable for DPI testing at flow rates considerably higher than 28.3 L min^{-1} due to the significant overlap in the cut-off curves of the pre-separator and stage 0. This was not the case with the MSLI but the data derived were limited by the relatively small number of stages. Deposition data determined by the three impactors were significantly different. The NGI produced good resolution and minimal inter-stage overlap and was regarded as the impactor of choice for DPI testing.
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1. Introduction

Particle size is widely accepted as an important factor in determining the aerodynamic deposition of particles within the respiratory system (Bates et al., 1966; Dolovich, 1991; Ganderton, 1997; Heyder et al., 1986; Newman and Clarke, 1983; Rudolf et al., 1990). The aerodynamic diameter of a given particle is defined as the diameter of a unit density sphere having the same settling velocity as the particle (Gonda, 1992). The aerodynamic particle size of medicinal aerosols is most commonly determined using inertial impaction (Mitchell and Nagel, 2003). Several aerodynamic particle size measuring devices are available but the most commonly used in pharmaceutical aerosols are the liquid impingers, including the multi-stage liquid impinger (MSLI), and the inertial impactors, including the Andersen cascade impactor (ACI) and, more recently, the next generation impactor (NGI).

There are only a limited number of well-designed studies comparing different, commonly used cascade impactors—none employing a combination formulation. Some researchers have compared different impactors using several inhaled products. Weda et al. (2002) and Lim et al. (2006) compared the ACI with the MSLI and reported equivalent deposition results while El-Araud et al. (1998) and Dunbar et al. (2005) suggested significant differences between the two impactors. Few reported studies have compared the ACI or the MSLI with the recently introduced NGI. Kamiya et al. (2004) employed a pressurised metered dose inhaler (pMDI) formulation in such a comparison and reported equivalence between the two impactors while Mitchell et al. (2003), who also tested pMDI formulations, reported significant differences. The different conclusions reached by the aforementioned studies may have resulted from differences in the methods employed in the testing of inhalers and the analysis of results. Differences in impactor cut-off limits at a given flow rate were often not considered in calculating fine particle fraction (FPF) values.

Abbreviations: ACI, Andersen cascade impactor; ANOVA, analysis of variance; CI, cascade impactor; COPD, chronic obstructive pulmonary disease; DPI, dry powder inhaler; DUSA, dose uniformity sampling apparatus; FP, fluticasone propionate; FPD, fine particle dose; FPF, fine particle fraction; $FPF_{<3\,\mu m}$, fraction of particles smaller than 3 μm; $FPF_{<5\,\mu m}$, fraction of particles smaller than 5 μm; GLM, general linear model; GSD, geometric standard deviation; GSK, GlaxoSmithKline; HPLC, high performance liquid chromatography; MMAD, mass median aerodynamic diameter; MOC, micro-orifice collector of the NGI; MSLI, multi-stage liquid impinger; NGI, next generation impactor; Ph. Eur., European Pharmacopoeia; PS, pre-separator; RD, recovered dose; RSD, relative standard deviation; S100, Seretide® 100 Accuhaler®; S500, Seretide® 500 Accuhaler®; SB, salmeterol free base; SD, standard deviation; SEM, scanning electron microscopy; SX, salmeterol xinafoate; w/w, weight/weight concentration; XA, xinafoic acid.

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When determining the fine particle fraction of inhalers, it is prescribed that pMDI formulations should be tested at a flow rate of 30 L min^{-1}, while dry powder inhalers (DPIs) should be tested at the flow rate producing a pressure drop of 4 kPa across the inhaler device (Ph. Eur., 2004; USP, 2006). It is thought that a pressure drop of 4 kPa is broadly representative of that produced by patients when using DPIs (Mitchell and Nagel, 2004). In comparison to pMDIs, testing DPI formulations at the required – typically higher – flow rates may reveal differences in the particle size distribution of drugs when determined using different impactors. Furthermore, unlike pMDIs, the fine particulate content generated by DPI devices is often flow rate dependent. Research into the effect of changing the air flow acceleration rate through the Rotahaler® DPI device revealed a significant increase in emitted dose and FPF values of the active ingredient (salbutamol) were achieved when a higher air acceleration rate through the DPI device was employed (Chavan and Dalby, 2002). The rate at which air accelerates at the beginning of each test is expected to be different between impactors due to inherent design differences (Roberts and Chiruta, 2007). The air acceleration rate might play an important role in the aerosolisation of particles from DPI products leading to possible differences between impactors.

Each type of impactor has different nominal stage cut-off diameters leading to difficulties in making direct inter-impactor comparisons. It has been long suggested that particles having a mass median diameter (MMD) of less than 5 μm are likely to deposit in the lower airways (Bates et al., 1966). Consequently, the fine particle dose (FPD) is defined as the mass of the active substance consisting of particles having an aerodynamic diameter of less than 5 μm (Ph. Eur., 2004). The FPD can also be expressed as a proportion of the recovered dose (FPF). However, none of the impactors used have a cut-off of 5 μm at flow rates which are typically used such as 60 L min^{-1} or the flow rates producing a pressure drop of 4 kPa across dry powder inhaler devices. The nearest cut-off limits to 5 μm for the MSLI and NGI at a flow rate of 60 L min^{-1} are 6.8 μm (stage 2) and 4.46 μm (stage 2), respectively. The ACI cut-off limits near 5 μm at 60 L min^{-1} are 6.18 or 3.98 μm (stages 0 and 1, respectively), while at the recommended flow rate of 28.3 L min^{-1}, stage 1 of the ACI is nearest to 5 μm with a nominal cut-off diameter of 5.8 μm. Interpolation techniques should, therefore, be used to calculate the FPD and % FPF values (Chan et al., 1997; Chew and Chan, 2001).

Some published reports comparing aerodynamic deposition profiles determined using different impactors resort to combining the fine particle mass recovered from all the stages downstream from the cut-off limit chosen and define the resulting fraction as their FPF (El-Araud et al., 1998). This approach results in weaknesses when comparing and contrasting the performance of different impactors in relation to the establishment of FPF equivalence. Some workers have even attempted to draw comparisons between impactors in spite of the flow rates being vastly different (Weda et al., 2002, 2004).

Considering the points discussed above, the need to investigate further the equivalence – or lack of it – of deposition data obtained using different impactors remains apparent. However, any study carried out must ensure that all of the critical parameters, such as the test flow rate, stage coating, the inclusion or omission of a pre-separator, FPF calculations, inhaler device and formulation, are all standardised. It would be particularly interesting to compare the deposition profiles of complex formulations – such as combination products – as determined using the most commonly employed multi-stage impactors. Combination formulations provide the opportunity to study drug-specific factors that may lead to different deposition profiles when determined by different impactors.

The aim of this study, therefore, was to measure the deposition profiles of salmeterol xinafoate (SX) and fluticasone propionate (FP) aerosolised from the commercially available combination DPI formulation Seretide® Accuhaler®. The specific products selected were Seretide® 100 (S100) and Seretide® 500 (S500) which contain the lowest and highest FP to SX ratios, respectively. It was proposed to employ all of the multi-stage impactors listed in the European Pharmacopoeia (2004), namely the ACI (apparatus D), MSLI (apparatus C) and NGI (apparatus E). The deposition results obtained using different impactors would then be analysed and compared.

2. Materials and methods

2.1. Quantitative sample analysis by high pressure liquid chromatography (HPLC)

The HPLC instrument used was a SpectraPHYSICS™ system (Thermoseparation Products Inc., CA, USA). The column used was a Hypersil Gold 250 mm in length with 4.6 mm internal diameter which was packed with 5 μm C18 stationary phase (Thermo Fisher Scientific, Inc., Waltham, MA, USA). The mobile phase was a mixture of methanol and 0.8% (w/v) ammonium acetate buffer at a pH of 5.5 (±0.01) in a ratio of 75:25, respectively. The buffer was made by dissolving ammonium acetate (HiPerSolv, BDH International, Poole, UK) in reverse osmosis water and adjusting the pH as required with glacial acetic acid. The mobile phase was freshly made before each analysis, filtered through 0.45 μm nylon filter and degassed by ultra-sonication for a minimum of 2 h. The flow rate was 1.00 mL min^{-1} and the temperature was maintained at 40 °C using a column heater. A UV detector set at a wavelength of 228 nm was employed. The injection volume was 20 μL, determined by means of a loop, and each sample was analysed in triplicate using a run time of 10 min. Detector signal processing was performed using the Chromeleon™ system (Dionex, Surrey, UK). All solvents used were of HPLC grade. SX standard was obtained from Vamsi Labs Ltd. (Maharashtra, India) while FP was obtained from Coral Drugs Ltd. (New Delhi, India). SX is detected as two distinct peaks; one belonging to xinafoic acid (XA) and the other to the salmeterol free base (SB). The HPLC method was validated throughout the SX and FP concentration range of 0.1–100 μg mL^{-1} and was found to be linear, accurate, precise and reproducible for both analytes. The limits of detection and quantification values for the XA, SB and FP peaks were 0.013, 0.015 and 0.011 μg mL^{-1}, respectively, while the LOQ values for the same three peaks were 0.039, 0.045 and 0.034 μg mL^{-1}, respectively.

2.2. Measurement of pressure drop across the Seretide® Accuhaler® devices

The flow rate required to produce a pressure drop of 4 kPa across the Accuhaler device was determined using the apparatus developed by Masoud (2004) as shown in (Fig. 1). Each device was placed in a sealed chamber that was connected to a calibrated flowmeter on one end and a dose uniformity sampling apparatus (DUSA) on the other end (Ph. Eur., 2004). The DUSA was connected to a powerful vacuum pump (Model no. 1423, Gast Manufacturing Inc., MI, USA). One end of a manometer (Digitron 2081P, Sifam Instruments Ltd., UK) was connected to the appropriate port on the DUSA while the other end was attached to the flow chamber in which the device had been placed. The flow rate was controlled by means of the control valve connected to the vacuum pump until the required pressure drop of 4 kPa was indicated on the manometer. Prior to the study, the flowmeter was calibrated for the volumetric flow leaving the meter using a volumetric gas meter. The flow rate, in L min^{-1}, measured by the flowmeter was identified as flow rate (Q). This routine was repeated in triplicate for each individual Accuhaler device to ensure that the correct 'bespoke' value was obtained for each device.

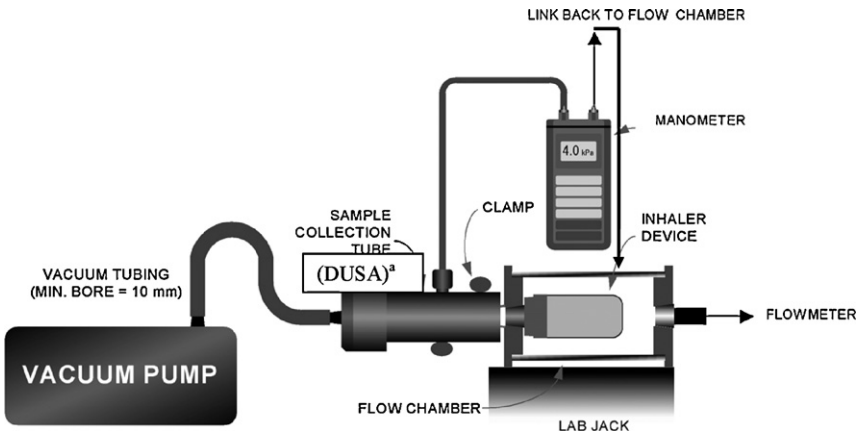

Fig. 1. Schematic diagram showing the apparatus employed to measure the flow rate required to achieve a pressure drop of 4 kPa across the Accuhaler device. A similar arrangement was used to adjust the through-the-device flow rate as required. [a]Dose uniformity sampling apparatus.

2.3. Aerodynamic assessment of particle size distribution of SX and FP from the Seretide® Accuhaler®

2.3.1. Attainment of the required flow rate for each impactor setup

Each analysis was carried out at two different flow rates, Q and 30 L min^{-1}. The process described in Section 2.2 was repeated to achieve an air flow rate of $Q(\pm1)$ and 30 (±1) L min^{-1} for each device by placing the impactor between the DUSA and the vacuum pump. The flow control valve was adjusted until the required flow rate was achieved. The device holding chamber was then removed and the Accuhaler device was directly connected to the impactor using a tightly fitting adaptor. This adaptor was specially designed for this study to provide a tight seal on the Accuhaler and minimise sample deposition on the adaptor.

2.3.2. Aerosolisation of Seretide® Accuhaler® formulations into the ACI, MSLI and NGI

Ten actuations from a Seretide® Accuhaler® were sampled into the ACI, MSLI or NGI. A total volume of 4 L of air was drawn through the inhaler device for each actuation by means of controlling the actuation time at a given flow rate. This was performed at both test flow rates (Q and 30 L min^{-1}). Two different strengths of Seretide® Accuhaler® were used. S100 (BN: B128880) contains 72.5 μg of SX (equivalent to 50 μg of salmeterol base) and 100 μg of FP, while S500 (BN: B128150) contains 72.5 μg of SX and 500 μg of FP per dose. All Accuhaler devices contain 60 pre-metered doses per device. All inhalers used were commercially purchased from AAH Pharmaceuticals Ltd. (Coventry, UK).

The ACI, MSLI and NGI were all simultaneously set up and doses were sequentially fired from the test inhaler according to pre-defined dosing sequence ensuring that any inter-device dose inconsistencies are prevented from producing bias between impactor results. The entire procedure described above was repeated 4 times using different Accuhaler devices ($n = 4$). A different dose firing sequence into the ACI, MSLI and NGI was followed for each Accuhaler device to ensure that any drug deposition differences resulting from intra-device dose inconsistencies affecting certain dose number(s) were minimised.

Any powder component deposited in the analytical equipment was recovered using a 75:25 (v/v) methanol:water mixture (termed the 'collecting solution') and was then quantified by HPLC.

2.3.3. Validation of particle recovery methods

Approximately 1.00 mg of both SX and FP were weighed and carefully sprinkled on each section of each impactor and then recovered using the standard coating and particle recovery methods employed in this study. The resultant concentrations in the recovery solution were then determined by HPLC. Validation was repeated 5 times for each section of the equipment. Validation results showed high recovery (>95%) and low variability (RSD < 5%) in all instances.

2.3.4. Calculation of impactor stage cut-off diameter at various flow rates

For all the inertial impactors used in this study, the nominal cut-off diameter of each stage is flow rate dependent. At a given flow rate, the effective cut-off diameters of the various stages were calculated from reference calibration cut-off diameters. Eq. (1) was used for the ACI and MSLI. In the case of the NGI, the cut-off values for impactor stages were calculated in accordance with Eq. (2), while the cut-off of the pre-separator stage was calculated using Eq. (3) (Table 1). The reference flow rate for the ACI is 28.3 L min^{-1}, while for the MSLI and NGI, the reference flow rate is 60 L min^{-1}.

$$D_{50,x} = D_{50,ref}\left(\frac{Q_{ref}}{Q_x}\right)^{1/2} \quad (1)$$

$$D_{50,x} = A\left(\frac{Q_{ref}}{Q_x}\right)^{B} \quad (2)$$

$$D_{50,x} = 12.8 - 0.07(Q_x - 60) \quad (3)$$

where $D_{50,x}$ is the stage cut-off at the test flow rate (Q_x). $D_{50,ref}$ is the reference cut-off at the reference flow rate (Q_{ref}). A and B are constants (Marple et al., 2003a).

Table 1
Calculated stage cut-off limits of the ACI, MSLI and NGI at flow rates of 30 and 70 L min^{-1}. The actual flow rate (Q) of each device was used in the calculations but the stage cut-off limits at the average value of 70 L min^{-1} are given here for reference.

Stage	Calculated impactor stage cut-off limit (μm)					
	Flow rate = 30 L min^{-1}			Flow rate = 70 L min^{-1}		
	ACI	MSLI	NGI	ACI	MSLI	NGI
Pre-separator	9.71	–	14.90	6.36	–	12.10
0	8.74	–	–	5.72	–	–
1	5.63	18.38	11.72	3.69	12.04	7.42
2	4.56	9.62	6.40	2.99	6.30	4.12
3	3.21	4.38	3.99	2.10	2.87	2.61
4	2.04	2.40	2.30	1.34	1.57	1.54
5	1.07	–	1.36	0.70	–	0.87
6	0.68	–	0.83	0.45	–	0.50
7	0.39	–	0.54	0.25	–	0.31

2.3.5. Aerodynamic assessment of particle size distribution using the Andersen cascade impactor

The ACI was originally calibrated to operate at a flow rate of 28.3 L min^{-1}. However, the cut-off diameter of each stage has been shown to follow Stokes' law (Srichana, 1998). The effective cut-off diameters can, therefore, be calculated at flow rates other than 28.3 L min^{-1} (Table 1).

The impaction plates of the ACI were coated by immersing them in the coating solution for 5 s thus ensuring that all surfaces were covered. The coating solution was prepared by dissolving 11 g of polypropylene glycol (975–1075 g mol^{-1}, Riedel-de Haen AG, Seelze, Germany) in 100 mL of isohexane. The ACI impaction plates were removed from the coating solution and allowed to air dry in a fume hood. A pre-separator containing 10 mL of the HPLC mobile phase solution was used in all experiments. A Whatman GF/A glass microfibre filter was placed in the filter stage of the ACI to collect any fines.

The ACI was then assembled from the filter stage through to stage 0 onto which the pre-separator was placed and the induction port attached. The ACI was subsequently connected to a vacuum pump via vacuum tubing with an internal diameter of 1 cm. The air flow was then adjusted to the required rate as described in Section 2.3.1, and the relevant Accuhaler device was attached to the impactor using a specially designed adaptor providing a good seal. Doses were then fired allowing 4 L of air to be drawn following each actuation. Once the required number of actuations had been fired, the ACI was disassembled and each impactor section was individually rinsed thoroughly, recovering any powder deposits, using a 75:25 (v/v) methanol:water mixture (termed the 'collecting solution') and samples were then taken for HPLC analysis.

2.3.6. Aerodynamic assessment of particle size distribution using the multi-stage liquid impinger

The method described in the European Pharmacopoeia for the MSLI was followed (Ph. Eur., 2004). A 70 mm Whatman GF/A glass microfibre filter was placed on the filter support and secured with the O-ring. The impactor frame containing stages 1–4 was then placed on the filter stage and secured using the snap-locks.

Collecting solution (20.0 mL) was placed in each of the four stages and a stopper was inserted to seal the stage. The MSLI was then carefully rotated to wet the internal surfaces of each stage ensuring that no liquid transfer took place between the various stages. The induction port was then securely attached and a vacuum pump was connected to the inlet port using vacuum tubing (internal diameter = 1 cm). Once the flow rate had been adjusted as detailed in Section 2.3.1, the appropriate Accuhaler device was fitted to the induction port via a specially designed adaptor forming a tight seal. The vacuum pump was then started allowing the first dose to be actuated. The pump was turned off once the required period of time to draw 4 L of air through the Accuhaler device had elapsed.

After the required number of actuations had been drawn into the MSLI, the induction port was removed and the powder deposits were recovered by thoroughly washing the internal surface of the induction port with the collecting solution. The contents of each MSLI stage were emptied carefully into separate volumetric flasks and each stage was thoroughly rinsed thrice using the collecting solution. The washing solutions were transferred into the corresponding volumetric flasks and made up to volume. The contents of each flask were mixed well, and a sample was removed for analysis.

2.3.7. Aerodynamic assessment of particle size distribution using the next generation impactor

The procedure detailed in the European Pharmacopoeia was followed. The collection cups were coated by adding approximately 10 mL of the coating solution to each cup as described in Section 2.3.5. The liquid was swirled to ensure all cup surfaces were covered and then tipped out. The cups were then left to air dry in a fume hood. An accurately measured amount of 15 mL of the collection solution was added to the pre-separator and a vacuum pump was connected to the inlet port of the NGI via vacuum tubing. The NGI was then assembled ensuring a tight seal was obtained between the pre-separator and the NGI body and between the induction port and the pre-separator. Following adjustment of the flow rate as detailed in Section 2.3.1, the relevant Accuhaler device was attached to the induction port ensuring a tight seal had been formed. The vacuum pump was then started allowing the first dose to be actuated and turned off once the required period of time to draw 4 L of air through the Accuhaler device had elapsed.

After the required actuations had been sampled into the NGI, the induction port and pre-separator were removed and deposits were recovered into separate volumetric flasks using the collecting solution. A volume of 10 mL of the collection solution was used to recover deposits on each NGI collection cup with the aid of ultra-sonication. A sample was then removed from the washings corresponding to each NGI section and analysed by HPLC.

2.3.8. Calculation of the aerodynamic deposition parameters of SX and FP determined using the ACI, MSLI and NGI

HPLC calibration curves were used to determine the concentration of drug in the samples recovered from each impactor section. From these concentration values, the total mass deposited in each section of the three impactors was calculated. The total mass recovered from each impactor for each run was then calculated as the sum of all deposits recovered.

In calculating the mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) values, the mass deposited in the induction port of all impactors and the mass deposited in the pre-separators of the ACI and NGI and stage 1 of the MSLI were not included due to the unavailability of a precise upper size limit for particles deposited in these sections. In addition, the drug can still deposit in the pre-separator (or stage 1 of the MSLI) despite having a smaller particle size than the cut-off limit of these sections due to the adhesion of fine drug particles to coarse lactose particles. These drug particles with apparently larger sizes, therefore, are not representative of the true fine particle size.

Percentage-drug-undersize figures were then constructed for each impactor according to the method described in the British Pharmacopoeia (BP, 2008). Two FPF values were calculated, $FPF_{<3\,\mu m}$ and $FPF_{<5\,\mu m}$, and were expressed as the percentage of the recovered dose having an aerodynamic diameter of less than 3 and 5 μm, respectively.

3. Results

3.1. Flow rate (Q) for Seretide® Accuhaler® devices

The average flow rate producing a pressure drop of 4 kPa varied between different Accuhalers from each Seretide product (S100 vs. S500) with the mean flow rate (Q) values (±SD) for the S100 and S500 being 67 (±3.2) and 70 (±3.6) L min^{-1}, respectively. However, there was no significant difference in Q values between S100 and S500 Accuhaler devices (ANOVA, $p > 0.05$, $n = 8$).

3.2. SX and FP recovery from impactors during deposition studies

Recovery of SX and FP from the ACI, MSLI and NGI for both Seretide formulations at both flow rates was >90%. Standard deviation values between the replicate runs ($n = 4$ for each) were small. There was no significant difference in recovery between the three impactors (ANOVA, $p > 0.05$).

Table 2
Mean deposition results ($n = 4$) of SX and FP aerosolised from the S100 product and determined using the ACI, MSLI and NGI at flow rates of 30 and Q L min^{-1}. ANOVA values compare the mean values obtained from the three impactors.

Drug	Parameter	ACI (mean ± SD)	MSLI (mean ± SD)	NGI (mean ± SD)	ANOVA
Formulation = S100; flow rate = 30 L min^{-1}					
SX	MMAD (μm)	5.21 ± 0.15	6.12 ± 0.40	6.81 ± 0.03	<0.001
	GSD	1.70 ± 0.03	2.44 ± 0.07	1.89 ± 0.02	<0.001
	$FPF_{<3\,\mu m}$ (%)	3.39 ± 0.33	6.18 ± 0.27	2.70 ± 0.09	<0.001
	$FPF_{<5\,\mu m}$ (%)	10.66 ± 0.64	11.92 ± 0.47	8.50 ± 0.19	<0.001
FP	MMAD (μm)	4.81 ± 0.13	5.62 ± 0.41	6.39 ± 0.06	<0.001
	GSD	1.71 ± 0.02	2.55 ± 0.09	2.02 ± 0.08	<0.001
	$FPF_{<3\,\mu m}$ (%)	5.30 ± 0.47	7.95 ± 0.41	4.15 ± 0.37	<0.001
	$FPF_{<5\,\mu m}$ (%)	14.68 ± 1.01	14.22 ± 0.71	10.70 ± 0.27	<0.001
Formulation = S100; flow rate = Q L min^{-1}					
SX	MMAD (μm)	3.13 ± 0.12	4.50 ± 0.12	3.79 ± 0.03	<0.001
	GSD	1.44 ± 0.12	2.56 ± 0.01	2.54 ± 0.12	<0.001
	$FPF_{<3\,\mu m}$ (%)	9.79 ± 0.98	11.16 ± 0.11	15.58 ± 0.48	<0.001
	$FPF_{<5\,\mu m}$ (%)	20.01 ± 1.27	18.23 ± 0.40	23.98 ± 0.97	<0.001
FP	MMAD (μm)	2.97 ± 0.01	4.08 ± 0.15	4.16 ± 0.07	<0.001
	GSD	1.59 ± 0.09	2.61 ± 0.09	2.09 ± 0.02	<0.001
	$FPF_{<3\,\mu m}$ (%)	12.38 ± 1.02	13.81 ± 0.19	12.37 ± 0.53	0.022
	$FPF_{<5\,\mu m}$ (%)	21.17 ± 1.18	21.54 ± 0.36	22.53 ± 0.88	0.129

3.3. Particle size distribution of Seretide measured using the ACI, MSLI and NGI

Deposition results for the S100 product obtained using different impactors at flow rates of 30 and Q L min^{-1} are shown in Table 2, while results for the S500 product at both flow rates are shown in Table 3. ANOVAs of MMAD, GSD and FPF values measured using different impactors showed statistically significant differences that were observed in several of the measured deposition parameters for both drugs, at both flow rates using both Seretide formulations. For example, there were significant differences between the MMAD values determined using different impactors, generally being smallest when measured with the ACI (Tables 2 and 3). At 30 L min^{-1} the NGI always produced the highest MMAD values. However, at Q L min^{-1}, MMAD values followed the trend ACI < NGI < MSLI. GSD values were smallest and largest with the ACI and MSLI, respectively, except for SX from the S500 at Q L min^{-1} (Table 3).

The largest $FPF_{<3\,\mu m}$ values determined at 30 L min^{-1} were obtained using the MSLI whereas the smallest $FPF_{<3\,\mu m}$ values at this flow rate were obtained with the NGI. At flow rate Q, in contrast, the ACI produced the smallest $FPF_{<3\,\mu m}$ values. FP generally produced higher $FPF_{<5\,\mu m}$ values than SX. Considering the different impactors, the NGI produced the smallest $FPF_{<5\,\mu m}$ values for both SX and FP at the test flow rate of 30 L min^{-1} and the largest $FPF_{<5\,\mu m}$ for both drugs at flow rate Q L min^{-1}.

3.3.1. Proportion of emitted dose entering impactors

In accordance with pharmacopoeial methods (Ph. Eur., 2004), fractions entering the cascade impactor (% CI fractions) were used in the calculation of FPF (Table 4). This fraction includes all particles emitted from the inhaler excluding those deposited in the induction port and pre-separator in the ACI and NGI. In the MSLI, stage 1 acts as a pre-separator and was, therefore, not included in the calculation of % CI fractions.

At flow rate Q, % CI fraction of SX was different in all three impactors. There were also significant differences in the % CI fraction for FP between the three impactors. FP generally passed the pre-separator stage more effectively than SX. When comparing the two Seretide products, SX aerosolised

Table 3
Mean deposition results ($n = 4$) of SX and FP aerosolised from the S500 product and determined using the ACI, MSLI and NGI at a flow rate of 30 and Q L min^{-1}. ANOVA values compare the mean values obtained from the three impactors.

Drug	Parameter	ACI (mean ± SD)	MSLI (mean ± SD)	NGI (mean ± SD)	ANOVA
Formulation = S500; flow rate = 30 L min^{-1}					
SX	MMAD (μm)	4.15 ± 0.09	4.05 ± 0.12	4.96 ± 0.16	<0.001
	GSD	1.65 ± 0.03	2.17 ± 0.05	1.95 ± 0.14	<0.001
	$FPF_{<3\,\mu m}$ (%)	6.45 ± 0.44	8.98 ± 0.58	5.92 ± 0.52	<0.001
	$FPF_{<5\,\mu m}$ (%)	16.00 ± 0.02	15.58 ± 0.51	13.34 ± 0.03	<0.001
FP	MMAD (μm)	4.20 ± 0.05	3.87 ± 0.17	4.78 ± 0.13	<0.001
	GSD	1.64 ± 0.00	2.27 ± 0.08	1.88 ± 0.00	<0.001
	$FPF_{<3\,\mu m}$ (%)	7.06 ± 0.09	13.13 ± 0.76	7.05 ± 0.38	<0.001
	$FPF_{<5\,\mu m}$ (%)	18.25 ± 0.63	21.61 ± 0.42	16.14 ± 0.52	<0.001
Formulation = S500; flow rate = Q L min^{-1}					
SX	MMAD (μm)	3.05 ± 0.12	3.78 ± 0.13	3.23 ± 0.05	<0.001
	GSD	1.56 ± 0.07	1.94 ± 0.08	2.12 ± 0.01	<0.001
	$FPF_{<3\,\mu m}$ (%)	9.69 ± 1.45	9.92 ± 0.26	16.09 ± 0.89	<0.001
	$FPF_{<5\,\mu m}$ (%)	17.35 ± 1.13	18.19 ± 1.63	25.12 ± 1.27	<0.001
FP	MMAD (μm)	3.07 ± 0.06	3.43 ± 0.02	3.30 ± 0.04	<0.001
	GSD	1.55 ± 0.02	2.11 ± 0.14	1.95 ± 0.02	<0.001
	$FPF_{<3\,\mu m}$ (%)	12.46 ± 1.19	14.33 ± 1.01	16.01 ± 0.71	0.002
	$FPF_{<5\,\mu m}$ (%)	22.53 ± 1.30	23.22 ± 0.67	26.44 ± 1.14	0.001

Table 4
Mean percentage (±SD) of emitted dose passing the pre-separator stage of the ACI and NGI and stage 1 of the MSLI (% CI fraction). Mean and significance of differences between impactors are shown ($n = 4$). Values in 'bold' indicate significance at the 0.05 level using Tukey's HSD test.

Product	Flow rate (L min^{-1})	(I) impactor	% CI fraction (±SD)		(J) impactor	Mean and significance of difference			
			SX	FP		SX		FP	
						($I-J$)	p-Value	($I-J$)	p-Value
S100	30	ACI	26.6 ± 0.2	31.6 ± 0.8	MSLI	−2.35	**<0.001**	0.15	0.939
					NGI	−0.40	0.558	2.17	**0.002**
		MSLI	29.0 ± 0.7	31.4 ± 0.4	ACI	2.35	**<0.001**	−0.15	0.939
					NGI	1.95	**0.002**	2.02	**0.003**
		NGI	27.0 ± 0.6	29.4 ± 0.6	ACI	0.40	0.558	−2.17	**0.002**
					MSLI	−1.95	**0.002**	−2.02	**0.003**
	Q	ACI	26.4 ± 1.2	28.0 ± 2.0	MSLI	−7.12	**<0.001**	−8.90	**<0.001**
					NGI	−12.45	**<0.001**	−9.58	**<0.001**
		MSLI	33.5 ± 1.4	36.9 ± 1.3	ACI	7.12	**<0.001**	8.90	**<0.001**
					NGI	−5.33	**0.001**	−0.68	0.795
		NGI	38.9 ± 1.2	37.6 ± 0.9	ACI	12.45	**<0.001**	9.58	**<0.001**
					MSLI	5.33	**0.001**	0.68	0.795
S500	30	ACI	26.8 ± 0.4	31.1 ± 1.5	MSLI	1.16	0.109	−3.67	**0.001**
					NGI	0.45	0.659	0.53	0.699
		MSLI	25.7 ± 0.5	34.8 ± 0.6	ACI	−1.16	0.109	3.67	**0.001**
					NGI	−0.70	0.383	4.20	**<0.001**
		NGI	26.4 ± 1.1	30.6 ± 0.0	ACI	−0.45	0.659	−0.53	0.699
					MSLI	0.70	0.383	−4.20	**<0.001**
	Q	ACI	22.0 ± 1.5	28.3 ± 1.5	MSLI	−5.50	**0.011**	−5.19	**0.002**
					NGI	−12.97	**<0.001**	−7.79	**<0.001**
		MSLI	27.4 ± 2.8	33.5 ± 1.6	ACI	5.50	**0.011**	5.19	**0.002**
					NGI	−7.47	**0.002**	−2.60	0.084
		NGI	34.9 ± 1.5	36.1 ± 1.3	ACI	12.97	**<0.001**	7.79	**<0.001**
					MSLI	7.47	**0.002**	2.60	0.084

from S100 achieved higher % CI fractions at flow rate *Q* when compared with the fraction obtained from the S500 despite both products containing a similar SX concentration in each dose.

Comparing the different impactors, the ACI and NGI produced the smallest and largest % CI fractions, respectively, at flow rate *Q* for both SX and FP. Interestingly, with the NGI, % CI fractions were always greater at flow rate *Q* compared to 30 L min^{-1} whereas the opposite trend was observed with the ACI. Similar to the NGI, % CI fractions in the MSLI were generally greater at flow rate *Q*.

Regardless of impactor, flow rate, formulation or drug used, the overall % CI fraction mean (±SD) was 30.6% (±4.46), with the range being 20.62–40.20% ($n = 96$).

3.3.2. Statistical analysis

Data obtained from both products at each flow rate were pooled and fitted to a general linear model (GLM) to evaluate general significant differences between values calculated for the same deposition parameter when determined using different impactors. The model also provides estimates for the magnitude of such inter-impactor

Table 5
Estimates of difference based on a general linear model (GLM) fitted for data obtained at a flow rate of 30 L min^{-1}. Significance is based on Tukey's HSD test and p-values obtained are shown. Mean difference values in 'bold' indicate significance at the 0.05 level ($n = 16$).

Dependent variable	(I) impactor	(J) impactor	Mean difference ($I-J$)	p-Value	95% Confidence interval	
MMAD (μm)	ACI	MSLI	**−0.324**	<0.001	−0.494	−0.154
		NGI	**−1.148**	<0.001	−1.318	−0.977
	MSLI	ACI	**0.324**	<0.001	0.154	0.494
		NGI	**−0.824**	<0.001	−0.994	−0.654
	NGI	ACI	**1.148**	<0.001	0.977	1.318
		MSLI	**0.824**	<0.001	0.654	0.994
GSD	ACI	MSLI	**−0.686**	<0.001	−0.741	−0.630
		NGI	**−0.261**	<0.001	−0.317	−0.205
	MSLI	ACI	**0.686**	<0.001	0.630	0.741
		NGI	**0.424**	<0.001	0.369	0.480
	NGI	ACI	**0.261**	<0.001	0.205	0.317
		MSLI	**−0.424**	<0.001	−0.480	−0.369
$FPF_{<3\,\mu m}$ (%)	ACI	MSLI	**−3.173**	<0.001	−3.550	−2.796
		NGI	**0.930**	<0.001	0.553	1.307
	MSLI	ACI	**3.173**	<0.001	2.796	3.550
		NGI	**4.103**	<0.001	3.726	4.480
	NGI	ACI	**−0.930**	<0.001	−1.307	−0.553
		MSLI	**−4.103**	<0.001	−4.480	−3.726
$FPF_{<5\,\mu m}$ (%)	ACI	MSLI	0.044	0.971	−0.419	0.507
		NGI	**3.702**	<0.001	3.239	4.165
	MSLI	ACI	−0.044	0.971	−0.507	0.419
		NGI	**3.658**	<0.001	3.195	4.121
	NGI	ACI	**−3.702**	<0.001	−4.165	−3.239
		MSLI	**−3.658**	<0.001	−4.121	−3.195

Table 6
Estimates of difference based on a general linear model (GLM) fitted for data obtained at a flow rate of Q L min^{-1}. Significance is based on Tukey's HSD test and p-values obtained are shown. Mean difference values in 'bold' indicate significance at the 0.05 level ($n = 16$).

Dependent variable	(I) impactor	(J) impactor	Mean difference ($I-J$)	p-Value	95% Confidence interval	
MMAD (μm)	ACI	MSLI	**−0.673**	<0.001	−0.766	−0.579
		NGI	**−0.344**	<0.001	−0.437	−0.251
	MSLI	ACI	**0.673**	<0.001	0.579	0.766
		NGI	**0.329**	<0.001	0.236	0.422
	NGI	ACI	**0.344**	<0.001	0.251	0.437
		MSLI	**−0.329**	<0.001	−0.422	−0.236
GSD	ACI	MSLI	**−0.738**	<0.001	−0.806	−0.669
		NGI	**−0.604**	<0.001	−0.673	−0.536
	MSLI	ACI	**0.738**	<0.001	0.669	0.806
		NGI	**0.133**	<0.001	0.065	0.201
	NGI	ACI	**0.604**	<0.001	0.536	0.673
		MSLI	**−0.133**	<0.001	−0.201	−0.065
$FPF_{<3\,\mu m}$ (%)	ACI	MSLI	**−1.296**	0.003	−2.193	−0.400
		NGI	**−4.006**	<0.001	−4.903	−3.110
	MSLI	ACI	**1.296**	0.003	0.400	2.193
		NGI	**−2.710**	<0.001	−3.606	−1.814
	NGI	ACI	**4.006**	<0.001	3.110	4.903
		MSLI	**2.710**	<0.001	1.814	3.606
$FPF_{<5\,\mu m}$ (%)	ACI	MSLI	**1.260**	0.008	0.289	2.231
		NGI	**−2.966**	<0.001	−3.937	−1.994
	MSLI	ACI	**−1.260**	0.008	−2.231	−0.289
		NGI	**−4.226**	<0.001	−5.197	−3.254
	NGI	ACI	**2.966**	<0.001	1.994	3.937
		MSLI	**4.226**	<0.001	3.254	5.197

differences. The differences in the MMAD, GSD and $FPF_{<3\,\mu m}$ obtained between impactors at both flow rates were all significant as were the differences in $FPF_{<5\,\mu m}$ at Q L min^{-1}. Detailed GLM-based estimates of differences between values obtained using different impactors at different flow rates and the significance of such estimates are shown in Table 5 (30 L min^{-1}) and Table 6 (Q L min^{-1}). The significant GLM model was used to estimate the various deposition parameters as determined by the ACI, MSLI and NGI. Fig. 2 shows GLM-based plots summarising trends for SX and FP obtained using the three impactors at both flow rates. The ACI is predicted to produce the smallest MMAD [Fig. 2(a) and (b)] and GSD [Fig. 2(c) and (d)] values while other trends were flow rate dependent.

3.3.3. *Conformation to the log-normal distribution*

Data generated by the NGI produced straight lines with high r^2 values (>0.99) when plotted on log-probability scales providing high confidence in MMAD and GSD calculations. MSLI data also produced high r^2 values (>0.99) although the regression derived straight lines were based on 3 points, corresponding to fractions deposited on the 3 lower stages. For example, plots of data obtained using the three impactors for SX aerosolised from the S100 product at a flow rate of 30 L min^{-1} are shown in Fig. 3(a).

ACI data, however, produced typical r^2 values ≈0.90 if deposition on all stages was considered. Visual inspection of the fitted line demonstrates a significant deviation from linearity [Fig. 3(a)]. Mass fractions depositing in the smaller stages of the ACI (5-filter) only represent a small percentage of the total % CI fraction yet these significantly affected the fitting of the regression line. The graphs were particularly linear for stages 1–4 typically corresponding to ∼70% of the total drug deposited on the ACI stages. This linear region of the curve always incorporated the MMAD and produced r^2 values which were generally >0.99. Calculations were therefore based on the representative, linear middle part of the graph [Fig. 3(a)].

Interestingly, changing the x-axis to a linear-probability scale appears to linearise the ACI data to a greater extent than a log-probability scale. The same set of data is shown on a different x-axis scale in Fig. 3(b). The MMAD, GSD and FPF values are very similar between the two plots. Unlike the ACI, a linear-probability plot causes data generated with the MSLI and NGI to deviate significantly from normality.

4. Discussion

It has been widely shown that at higher flow rates, higher doses are emitted from many DPI devices (Broeders et al., 2001; Coates et al., 2005; De Boer et al., 1996; Meakin et al., 1995; Pauwels et al., 1997; Tarsin et al., 2006; Yang et al., 2001). DPI devices depend on the patient's inspiratory effort in generating the required turbulence during inhalation with higher flow rates generating greater turbulence. Therefore, there is often a greater chance of effective aerosolisation of the powder at higher flow rates. In some cases, the dependency of the emitted dose from a DPI device on the inspiratory flow rate has been shown to produce clinically significant differences across a range of different inhaler devices (Auty et al., 1987; Pedersen et al., 1990; Tarsin et al., 2006). Consequently, it is vital that a similar test flow rate is used when comparing different impactors.

An ideal DPI product – among other features – should deliver the same emitted dose every time regardless of the inspiratory flow rate used. It should therefore be noted that the S100 product delivered a similar mean dose for both SX and FP despite the large difference between the two flow rates used. A more anticipated result, however, was observed with the S500 product where the higher flow rate (∼70 L min^{-1}) produced larger delivered doses for both SX and FP. Interestingly, standard deviation values for the emitted dose of both SX and FP from the S100 and S500 products were smaller at the higher flow rates of Q L min^{-1}. The higher flow rate is expected to produce greater turbulence and superior entrainment of particles by the air-stream possibly resulting in smaller SD values.

GSK – the manufacturers of Seretide – claim that under standardised conditions and at a flow rate of 60 L min^{-1}, the S100 and S500 Accuhalers deliver an average FP dose of 93 and 465 μg, respectively. The claimed average delivered dose of SX (expressed as the dose equivalent to salmeterol base) for both products is 45 μg (GSK, 2007). Despite the difference in flow rates used, results generated in this study are very similar to the manufacturer's claimed

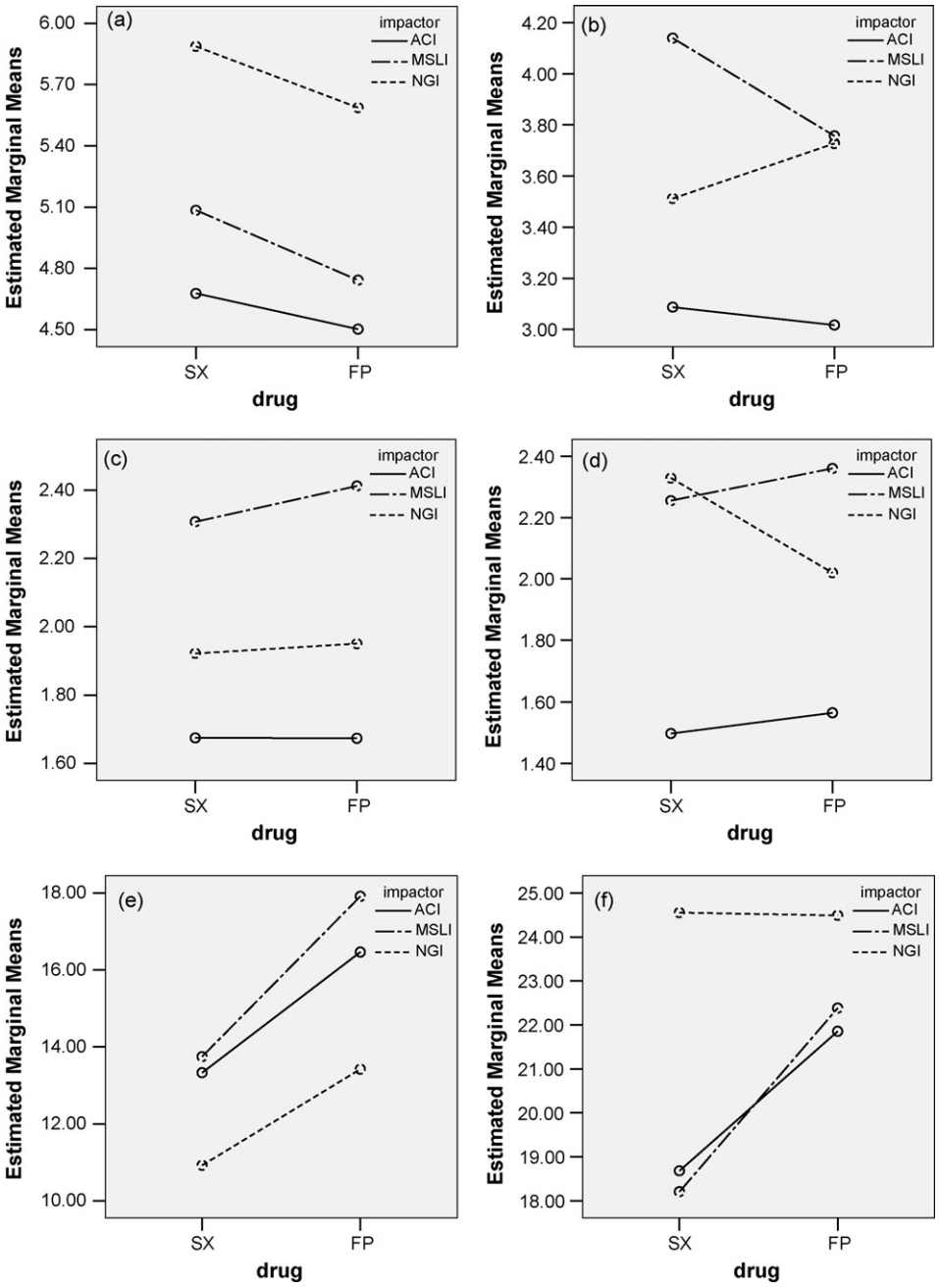

Fig. 2. Estimated marginal means based on a general linear model fitting all data points ($n = 8$). Graphs show mean MMAD (a and b), GSD (c and d) and $FPF_{<5\,\mu m}$ (e and f) at flow rates of 30 L min^{-1} (a, c and e) and Q L min^{-1} (b, d and f).

delivered doses. There is a considerable difference in the flow rate dependency between various DPI devices. While some DPI devices (e.g. Turbuhaler) have been shown to produce large differences in the emitted dose and FPF as a function of change in the flow rate (Tarsin et al., 2004, 2006), the Accuhaler device is thought to produce less variation in emitted dose and FPF values upon increasing the flow rate between 30 and 90 L min^{-1} (Prime et al., 1999). In the present study, relatively small, but significant, differences were observed in emitted dose and FPF of both drugs when the flow rate was increased from 30 to Q (~70) L min^{-1} but the differences were product and impactor dependent. Both Seretide® Accuhaler® products passed the pharmacopoeial content uniformity tests for dry powder inhalers (Ph. Eur., 2004). Recovery of SX and FP from impactors following aerosolisation was greater than 90% in all cases. Drug recovery from impactors can be used as a validation test for the deposition run. If the recovery is between 75 and 125% compared to the mean amount recovered in the content uniformity tests then the deposition data would pass the test (Ph. Eur., 2004). It has been suggested that the recovery from the ACI may be lower than that obtained from the MSLI or NGI due to greater ACI inter-stage wall losses (Kamiya et al., 2004; Marple et al., 2003a,b; Mitchell et al., 2003). Recoveries from the ACI did appear to be marginally smaller although there was no significant difference in the recovery of SX ($p > 0.05$). The recovery of FP from the NGI was greater in comparison with the ACI ($p = 0.005$). FP, particularly in the S500 formulation (where the SX:FP ratio is ≈1:7), is present in a much greater proportion than SX. Such a difference in quantity may allow for a more precise quantification due to higher concentrations of analyte in the assayed solutions which, in turn, can lead to smaller standard deviation values. There was

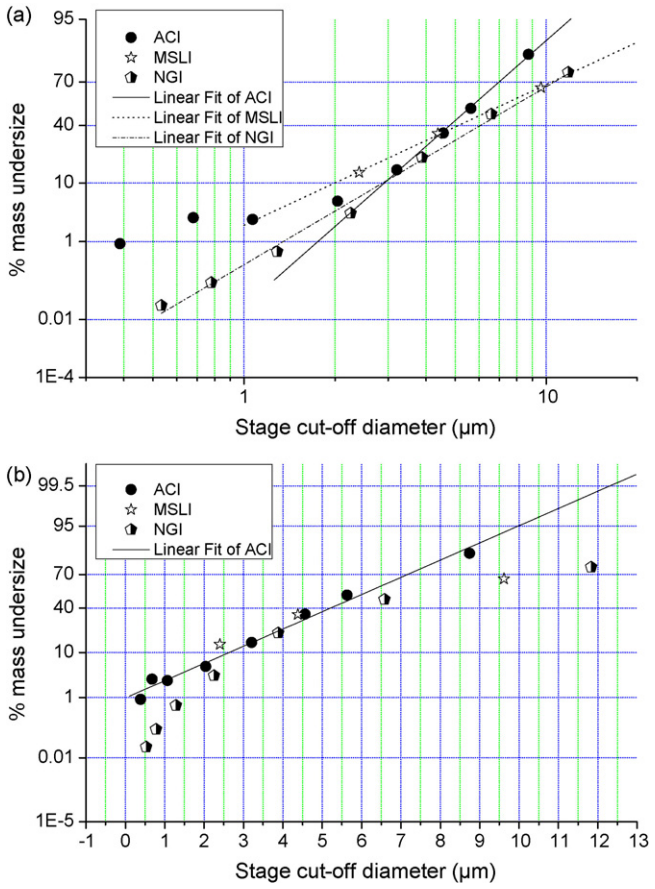

Fig. 3. Stage cut-off diameter (μm) and SX % mass undersize deposited on impactor stages plotted on (a) log-probability and (b) linear-probability scales. The data correspond to the deposition of SX from the S100 at 30 L min^{-1}. Best-fit lines were fitted through the representative middle part of each data set.

no difference between the recovery of both drugs from the NGI and MSLI.

Table 4 shows significant differences between % CI fractions for the different impactors. Thiel (1998) suggested that fractions deposited in the induction port and the pre-separator should not be included in calculating MMAD and GSD values for pMDI preparations. Inclusion of such fractions can potentiate bi-modality and subsequent deviation from log-normality. This view was also expressed for the purpose of standardising inhaler testing (Berg et al., 2002). In contrast to pMDIs, a pre-separator should always be used in DPI testing particularly with formulations containing coarse carrier particles. Consequently, drug particles depositing in the induction port and pre-separator are not necessarily large enough to do so. It is more likely that strong adhesion to coarse carrier particles and/or the formation of stable large agglomerates are responsible for such deposition. It is more appropriate, therefore, to exclude particles depositing in the induction port and the pre-separator and MMAD as well as GSD calculations in DPI testing should then be based on fractions entering the impactor.

While calibrations of the MSLI and NGI have been well established across a wide range of flow rates (Asking and Olsson, 1997; Marple et al., 2003a), the ACI is supplied calibrated only at a flow rate of 28.3 L min^{-1}. Although this calibration was performed on the original 6-stage impactor in the early 1950s, it is still supplied by the manufacturers with the Mark II ACI (Thermo Electron Corporation, 2003). The cut-off values for each stage reported from subsequent independent calibrations appear to be in broad agreement with the original one (Mitchell et al., 1988; Srichana, 1998; Vaughan, 1989). However, the ACI has been shown to suffer from significant manufacturing variability (Shelton et al., 2002) and irregularity leading to changes in the stage cut-off limits (Stein and Olson, 1997). The ACI has also been criticized for high inter-stage ('wall') losses (Kamiya et al., 2004; Mitchell et al., 1988), low stage Reynolds numbers (ACI: 110–782, NGI: 149–2938), small nozzle-to-plate distance (Mitchell and Nagel, 2004), lack of reliable stage mensuration data (Roberts and Romay, 2005), and significant overlap between the collection efficiency curves of several stages (Marple et al., 1998). These factors have been suggested to lead to increased error and variability (Miller et al., 1998; Nasr et al., 1997; Stein, 1999).

Unlike pMDI preparations, DPIs usually require flow rates higher than 28.3 L min^{-1}. However, despite the wide use and apparent acceptance of the ACI, its aerodynamic stage cut-off diameters are not well established at flow rates other than 28.3 L min^{-1} (Ph. Eur., 2004). A modification to the ACI has been proposed (Nichols et al., 1998) to allow an increase in the flow rate to 60 L min^{-1} by removing stage 7 and adding a new stage (−1). A further modification was also suggested (Nichols, 2000) by removing stages 6 and 7 and adding stages (−2) and (−1) enabling the impactor to be operated at 90 L min^{-1}. Nevertheless, unless the stage cut-off limits are calculated to accommodate operation at different flow rates, these modifications do not solve the original problem as the ACI remains a fixed flow rate impactor that should only be used if the DPI device happens to have a pressure drop of 4 kPa at precisely 60 or 90 L min^{-1}. Furthermore, there appears to be a significant overlap between the collection efficiency curves of stages −1 and 0 possibly resulting in stage −1 trapping particles that should be deposited on stage 0 (Mitchell and Nagel, 2003). More importantly, a pre-separator cannot be used with this modification since the cut-off limit of the pre-separator at 60 L min^{-1}, for example, is significantly smaller than that of stage −1 (6.86 μm vs. 8.6 μm, respectively) rendering the modification unsuitable for the vast majority of DPI testing.

However, as with other cascade impactors, Eq. (1) can be used to calculate the aerodynamic stage cut-off diameters for the ACI at flow rates other than 28.3 L min^{-1}, since its operation is based on Stokes' equations defining gas flow and Newton's laws of motion which can describe the passage of particles through different stage geometries. Srichana (1998) performed a calibration on the standard ACI at 60 L min^{-1} and demonstrated that experimentally determined cut-off limits are similar to those calculated using Eq. (1). This approach allows the ACI to be used for DPI testing and enables direct comparison between multiple impactors at different flow rates.

While there is a large cut-off gap at all calibration flow rates in the range of 30–100 L min^{-1} both between the pre-separator and stage 1 in the NGI (e.g. 14.9 μm vs. 11.7 μm at 30 L min^{-1}, respectively) and stages 1 and 2 in the MSLI (e.g. 18.4 μm vs. 9.6 μm at 30 L min^{-1}, respectively), the gap is very small between the pre-separator and stage 0 of the ACI even at the calibration flow rate of 28.3 L min^{-1} (10 μm vs. 9 μm, respectively). In fact, different workers who published ACI Mark II calibration data have reported the pre-separator cut-off at 28.3 L min^{-1} to be even smaller than 10 μm. Mitchell et al. (1988) reported the pre-separator cut-off to be 9.0 and 8.8 μm using latex and methylene blue, respectively while Vaughan (1989) found the cut-off to be 9.8 μm using dioctylphthalate. Srichana (1998) calibrated the ACI pre-separator at 28.3 and 60 L min^{-1} using silica and reported the values as 9.49 and 6.88 μm, respectively. The pre-separator cut-off, therefore, appears to be of the order of 9.5 μm. The sharpness of the cut of the ACI pre-separator is relatively poor with a GSD of ≈1.5 at a flow rate of 28.3 L min^{-1}, mainly due to the influence of gravity on the deposition of particles larger than 5 μm (Mitchell and Nagel, 2003). A computational and experimental investigation into the size-based separation performance of the ACI pre-separator revealed the existence of low velocity locations as well as interference in the flow

between adjacent nozzles which have been suggested as a possible cause for poor size selectivity (Sethuraman and Hickey, 2001). This design weakness leads to a significant overlap in the collection efficiency curves and can result in particles that might be expected to deposit on stage 0 being collected in the pre-separator. It has even been suggested that the pre-separator could 'starve' both stages 0 and 1 due to this similarity in cut-off diameter (Mitchell and Nagel, 2003). However, the efficiency of the pre-separator is likely to improve at higher flow rates due to an increased contribution of inertial impaction. The overlap between the pre-separator and stage 0 is likely to be reduced since the gravitational sedimentation component of deposition is likely to play a greater role in the deposition of particles in the pre-separator due to the presence of larger particles.

In the present study, % CI fractions were found to be impactor, flow rate, formulation, and drug dependent (Table 4). The nominal pre-separator stage cut-offs for the ACI, MSLI and NGI at 30 L min^{-1} were 10, 18.4 and 14.9 μm, respectively, whereas at Q L min^{-1} the cut-offs were 6.4, 12.0, and 12.1 μm, respectively.

At 30 L min^{-1}, % CI fraction for the ACI, MSLI and NGI were generally similar. In contrast, when the flow rate was increased to Q (~70 L min^{-1}), there were impactor dependent trends with the % CI fractions of the NGI increasing sharply, those of the MSLI increasing moderately and those of the ACI decreasing modestly. When the flow rate was increased, two opposing processes can be expected to affect the % CI fraction of an impactor. First, excluding the effects on the aerosolisation process, the increase in flow rate leads to higher momentum gained by particles and consequent smaller cut-off limits possibly resulting in a larger proportion depositing in the induction port and pre-separator and a smaller % CI fraction. Second, the increased flow rate leads to higher turbulence and a more efficient aerosolisation process allowing for increased drug particle detachment from coarse carrier particles as well as greater deaggregation of any large drug agglomerates. The increased detachment and deagglomeration produce smaller particles that can penetrate deeper into the impactor resulting in reduced deposition in the induction port and pre-separator and leading to an increase in % CI fraction. The overall effect is that a combination of the two opposing processes often produces an increase in the % CI fraction. The decrease in the cut-off limit of the pre-separator stage of the MSLI (stage 1) as a function of increasing flow rate is markedly greater than the decrease in the NGI pre-separator cut-off limit with increasing flow rate which may explain the greater increase in the % CI fraction of the NGI.

The pre-separator of the NGI was designed to act as a two-step barrier with an upper liquid-containing central cup removing larger particles and 6 nozzles leading to an impaction stage removing smaller coarse particles (Marple et al., 2003b). Gravitational sedimentation plays a significant role in the deposition of particles >5 μm thus the deposition of coarse carrier particles, typically in the size range 63–90 μm, is likely to be considerably affected by this mechanism. This was accounted for in the calculation of the cut-off limit of the NGI pre-separator through the use of Eq. (3) (instead of Eq. (2)). Interestingly, the % CI fraction of the ACI followed the opposite trend to the other impactors by consistently decreasing with increasing flow rate. This might be due to the cut-off diameter of the ACI pre-separator being considerably smaller than those corresponding to the MSLI and NGI pre-separator stages (6.4 vs. 12.0 and 12.1, at Q L min^{-1}, respectively).

There were significant differences between the impactors in determining the particle size distribution of the tested formulations. The NGI produced the largest MMAD values at 30 L min^{-1}, while the MSLI produced the highest MMAD values at Q L min^{-1}. MMAD values determined by the ACI were consistently smaller except for the S500 at 30 L min^{-1} (Table 3). This is possibly due to stage 0 being 'starved' by the pre-separator as discussed above. Selectively reducing the amount of the largest particles in the CI fraction is expected to shift the median value downwards. Interestingly, Subba Rao et al. (1997) compared the deposition of a peptide pMDI formulation using an ACI and a 150 series Marple–Miller Impactor (MMI) at 28.3 and 30 L min^{-1}. They also observed smaller MMAD values with the ACI (2.9 μm vs. 4.0 μm) and the authors attributed the differences to higher inter-stage losses of larger particles in the ACI.

Similarly, the spread of the data is reduced by decreasing the contribution of values at either end of the distribution resulting in smaller GSD values such as the selective reduction in particles depositing in stage 0 due to the overlap of collection efficiency curves of the pre-separator and stage 0 in the ACI. Olsson et al. (1998) analysed an extensive database of ACI and MSLI measured budesonide Turbuhaler samples and found the GSD values to be smaller with the ACI compared to the MSLI (1.4 vs. 2.0, respectively). Subba Rao et al. (1997) also reported smaller ACI GSD values compared to those obtained with the MMI.

The FPF however is not necessarily affected by the overlap between the efficiency curve of the ACI pre-separator and stage 0. The overlap may lead to an under-estimate of the MMAD and a decrease in the % CI fraction which has opposing effects on the FPF values. Indeed FPF values generated with the ACI were between those produced with the MSLI and NGI.

The MSLI has been shown to produce consistent results in the flow rate range of 30–100 L min^{-1} (Asking and Olsson, 1997). However, the small number of stages over a wide range of cut-off sizes limits the amount of information that can be extracted. In the testing of carrier-based DPI systems, the first stage acts as a pre-separator leaving three points on the log-probability graph. Only two of these points are <5 μm at 60 L min^{-1}. Even in the case of pMDIs, a cut-off limit of 13.0 μm is far less important than achieving greater resolution in the therapeutically relevant 0.5–5 μm size range. In this study, the MSLI produced consistent results that were broadly similar to those obtained with the ACI and NGI.

The next generation impactor was purposely designed to test pharmaceutical aerosols taking the shortcomings of other impactors into account (Marple et al., 2003b). The collection efficiency curves show minimal overlap at flow rates in the range of 30–100 L min^{-1} (Dunbar and Mitchell, 2005; Marple et al., 2003a). However, it has been suggested that particle bounce in the NGI is greater than that in the ACI. The higher Reynolds numbers at the stages inadvertently result in increased chances of particle bounce, although stage coating has been shown to minimise this effect (Kamiya et al., 2004). Deviation from log-normality has frequently been reported with cascade impactor data including the ACI (Malton et al., 1982). This has been attributed to the bimodal distribution in size of the particles being analysed. However, it has also been suggested that the non-ideal design of the ACI potentiates this phenomenon.

Significant differences were seen between the deposition profile of Seretide obtained using the ACI, MSLI and NGI. Differences were observed in a number of parameters, including the MMAD, GSD and FPF of SX and FP. However, changes in the values generally followed similar patterns.

5. Conclusions

In the present study, the ACI, MSLI and NGI were compared using two combination formulations containing SX and FP aerosolised at two flow rates of 30 and Q L min^{-1}. The results obtained showed that there were significant differences in the particle size distributions derived from the different impactors although many trends were broadly similar.

The ACI was found to be less suitable for DPI testing at flow rates which were considerably higher than 28.3 L min^{-1}, due to

the significant overlap between the cut-off efficiency curves of the pre-separator and stage 0. In contrast, this was not a problem associated with use of the MSLI. However, the level of detail regarding the aerodynamic particle size distribution of the test formulation obtained using the MSLI is limited by the relatively small number of separation stages particularly when testing carrier-based DPI formulations. The MSLI, nevertheless, represents a viable alternative to the ACI and NGI for inhaler testing although its manipulation for washing and analysis procedures is not straightforward. The NGI was also found to be readily operable across the flow rates tested. The problem of particle bounce can be solved with appropriate stage coating. The combination of good resolution and minimal inter-stage overlap makes the NGI the impactor of choice for DPI testing.

The use of a combination formulation in comparing impactors was particularly useful as it gave added confidence to the findings and allowed for cross-comparison between results obtained using each drug. Despite there being some similarities between aerodynamic deposition profiles obtained using the three impactors, there were also significant differences. Conclusions regarding deposition of drugs, if based on data obtained from different impactors must, therefore, be drawn with caution.

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