

Comparison of efficacies of full and abbreviated cascade impactors in aerosol characterization of nebulized salbutamol sulfate produced by a jet nebulizer

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Abstract

The properties of aerosols generated from salbutamol sulfate solution (1 mg/mL) using an air-jet nebulizer were evaluated using Next Generation Impactor (NGI), a full cascade impactor, and Fast Screening Impactor (FSI), an abbreviated impactor measurement (AIM). Both impactors were operated under the same experimental conditions. The samples were recovered and assayed using validated high performance liquid chromatography (HPLC). The study investigated AIM-Human Respiratory Tract (HRT) concept by comparing key parameters of aerosolization i.e. fine particle dose (FPD) and fine particle fraction (FPF) measured using FSI, with NGI as baseline. The results showed that FSI yielded different but comparable values for FPD and FPF, indicating that it is alternative impactor to NGI. Despite the fact that FSI could not replace NGI, it may be used as an alternative impactor for simple and rapid aerosol characterization of formulations in some pharmaceutical development and quality control processes.

Keywords

Aerosol characterization, Fine particle dose (FPD), Fine particle fraction (FPF), Impactor, Salbutamol sulfate

Introduction

Aerodynamic Particle Size Distribution (APSD) is extensively recognized as a Critical Quality Attribute (CQA) in the *in vitro* characterization of orally-inhaled and nasal drug products (OINDP) (Nichols et al. 2013; Fishler and Sznitman 2017; Roberts and Mitchell 2019). The effectiveness of nebulizer delivery is usually dependent on two key parameters: fine particle dose (FPD) and fine particle fraction (FPF). Fine particle dose (FPD) refers to particle mass below 5 μm , i.e., the ‘respirable/therapeutically useful’ dose of the drug, while FPF

is the fraction of the emitted dose less than 5 μm (%) (Guo et al. 2013; Ohar et al. 2020).

The gold standard for measuring the APSD of inhaled products for regulators, pharmacopoeias and researchers is the cascade impactor. Cascade impactors consist of a series of collection plates or cups in order of decreasing jet diameters with stage number (Kuhli et al. 2009; Rissler et al. 2009). They separate a formulation into various fractions based on inertial impaction which is a function of their aerodynamic diameters, which in turn, depend on size and particle density (Marple et al. 2003; Mitchell and Nagel 2003; Nichols et al. 2013; Roberts and Mitchell 2019).

The Next Generation Impactor (NGI) was proposed by the pharmaceutical industry to meet all pharmacopoeia requirements, especially for OINDPs testing, and it is listed in European Pharmacopoeia as Apparatus E (Mitchell and Nagel 2003). It (NGI) has seven collecting cups/plates and a glass microfibre filter placed at the final stage (Micro-Orifice Collector; MOC) (Marple et al. 2003) However, the use of multi-stage impactors is extremely time-consuming and labour-intensive, especially in quality control and early stage of product development (Mitchell et al. 2010; Mohan et al. 2016; Fishler and Sznitman 2017). Moreover, quantitative chemical analysis for formulations with low drug contents or potent drugs is still challenging when the nebulized drug is deposited across all the stages of NGI (Mitchell et al. 2009a; Nimmano et al. 2018). The pharmaceutical industry and instrument manufacturers are currently working to develop faster methods of APSD determination. Thus, much attention is being paid to the concept of Abbreviated Impactor Measurement (AIM).

Abbreviated impactor measurements (AIMs) have recently been developed to speed up the process (Tougas et al. 2009). An example is the Fast-Screening Impactor (FSI). The FSI only has two stages which divide particles into coarse particle mass (CPM) and fine particle mass (FPM), with a cut-off diameter of 5 µm between stages (Johal et al. 2013; Mohan et al. 2016). The coarse particles are non-inhalable and would likely be deposited in the upper airways i.e. trachea and bronchi, while the fine particles would be deposited in the lower airways i.e. bronchioles and alveoli (Johal et al. 2013). This makes FSI suitable for application in AIM- Human Respiratory Tract (HRT). However, the determination of properties of nebulized formulations using FSI instead of full resolution cascade impactor, has received very little attention. Up until now, only one study reported that FSI was found to be a useful alternative impactor to the NGI for *in vitro* determination of the aerosol properties of liposomes containing hydrophobic drugs (erlotinib and genistein) (Nimmano et al. 2018).

Nebulizers are categorized into three main types: air-jet, ultrasonic and vibrating-mesh. The air jet nebulizer is the most common inhaler device. It is connected to an air compressor using a tubing. Air is compressed causing the

air to flow at high velocity through the liquid formulation, thereby turning it into an aerosol suitable for inhalation.

The proposed approach using AIM concept may require further evaluation through testing of various pharmaceutical formulations. This is to make for a better understanding of how abbreviated impactor measurement can be applied. Consequently, this study was undertaken to investigate if FSI can be used interchangeably with NGI for aerosol characterization of a salbutamol sulfate delivered from a jet nebulizer.

Materials and methods

Materials

Salbutamol sulfate was sourced from Micro Technologies, UK. Acetonitrile (99.9%, gradient grade for HPLC, HPLC-grade water and trifluoroacetic acid (TFA, >99.0% pure) which were solvents and reagents were used in HPLC analysis, were purchased from Sigma- Aldrich (Pool, UK).

A PARI LC® Sprint Reusable Nebulizer (PARI Medical Ltd, Byfleet, UK) was used to deliver aerosol of salbutamol sulfate. The aerosol properties of salbutamol sulfate were characterized with NGI and FSI. Both impactors were acquired from Copley Scientific, UK.

Determination of time taken to nebulize water to dryness and aerosol mass output

Various volumes of HPLC-grade water i.e., 2, 2.5, 4 and 8 mL were put into an air-jet nebulizer reservoir which was connected to a PARI Boy air compressor (PARI Medical Ltd, Byfleet, UK) via a tube. The nebulizer was tapped intermittently when the aerosol produced became less apparent, so as to maximize the efficiency of aerosol output. The time (min) taken to nebulize the water to dryness was measured after aerosol generation ceased completely. The weight of the nebulizer was measured before and after nebulization, and the output efficiency (%) was calculated as shown in the equation below:

$$\text{Output efficiency (\%)} = \frac{\text{Weight difference of nebulizer before and after nebulization}}{\text{Weight of fluid placed into the nebulizer reservoir}} \times 100\%$$

Quantitative analysis of salbutamol sulfate

Salbutamol sulfate was assayed at 276 nm using an HPLC system equipped with an autosampler and UV/VIS detector (Agilent 1100 Series, USA). A phenyl column was used, with isocratic elution using mobile phase of 0.1% v/v

trifluoroacetic acid (TFA) in HPLC grade water and 100% v/v acetonitrile (ACN) (80:20 v/v) at a flow rate of 1 mL/min. Each run was set for 6 min at 30 °C, with an injection volume of 10 µL. The HPLC method was validated for analysis of salbutamol sulfate according to the ICH Q2 guideline. The lower limit of quantification (LOQ) was 3.53 µg/mL, while the limit of detection (LOD) was 1.17 µg/mL

Aerodynamic particle size characterization using the Next Generation Impactor (NGI)

The NGI was set up according to the procedures described in the European Pharmacopoeia, after chilling the impactor at 5 °C for 90 min to reduce water evaporation due to heat from the impactor (Adorni et al. 2019). A PARI LC® Sprint nebulizer filled with 4 mL of salbutamol sulfate was nebulized for 9 min and directed towards the NGI induction port. A mouthpiece adaptor was used to connect the nebulizer to the induction port. The NGI

was attached to a vacuum pump (Copley Scientific, UK) and the air-flow rate through the impactor was controlled at 15±5% L/min using a flow meter (Copley Scientific, UK).

After 9-min nebulization, salbutamol sulfate were collected from the nebulizer reservoir, mouthpiece, mouthpiece adaptor, induction port, collecting cups and the back-up filter using HPLC grade water. All collected samples were analysed using validated HPLC analysis. Triplicate measurements were made. The aerosolization parameters in this study were calculated as follows (Nimmano et al. 2018):

$$\text{Mass balance(\%)} = \frac{\text{Mass of drug collected from nebulizer to filter}}{\text{Mass of drug initially placed into the nebulizer}} \times 100$$

$$\text{Emitted Dose (\%ED)} = \frac{\text{Mass of drug collected from induction port to filter}}{\text{Mass of drug collected from nebulizer to filter}} \times 100$$

Fine Particle Dose (FPD) = Drug mass less than 5 µm determined from the plot of cumulative mass of active substance versus cut-off diameter.

Fine Particle Fraction (FPF) = Percentage cumulative fraction of drug less than 5 µm from the plot of cumulative fraction of active substance versus cut-off diameter.

Mass Median Aerodynamic Diameter (MMAD) = The diameter in which 50% of the aerosol droplets are larger or smaller than the stated size, derived from the plotted graph of log cumulative fraction of salbutamol sulfate versus stage cut-off diameter.

Geometric Standard Deviation (GSD) = The variability of the aerosol particle size distribution.

Aerodynamic particle size characterization using the Fast Screening Impactor (FSI)

A two-stage abbreviated impactor, FSI (Copley Scientific, UK) was kept in the fridge at 5 °C for at least 90 min before each experiment, so as to ensure comparability between the two impactors (Tservistas et al. 2010; Nimmano et al. 2018). The impactor had an additional insert calibrated at 30 L/min, which was modified by alternately covering

three of the six nozzles with a wet glass microfiber filter to give a cut-off for 5 µm at a flow rate of 15 L/min. The flow rate of the vacuum pump was set at 15±5% L/min. Measurements were made in triplicate.

The FSI was also used with the air-jet nebulizer filled with 4 mL of salbutamol sulfate solution under the same operating conditions as the NGI. The salbutamol sulfate deposited in the induction port, extra insert of FSI housing, glass microfiber filter, and any salbutamol sulfate remaining in the nebulizer, were recovered after nebulization by rinsing with HPLC-grade water. The recovered salbutamol sulfate was made up to reasonable volume. The samples were analysed using HPLC, to determine the amount deposited at each stage.

Analysis of aerosolization parameters i.e., Emitted Dose (ED), Fine Particle Dose (FPD) and Fine Particle Fraction (FPF).

Fine Particle Dose (FPD) for FSI is the amount/mass of salbutamol sulfate deposited on the lower stage of FSI, while Fine Particle Fraction (FPF) was calculated as follows:

Fine Particle Dose (FPD) for the FSI is the amount/mass of salbutamol sulfate deposited on the lower stage of the FSI, while Fine Particle Fraction (FPF) can be calculated as follows:

$$\text{Fine Particle Fraction (FPF)} = \frac{\text{Mass of drug from the filter}}{\text{Mass of drug from deposited drug on upper and lower stage of the FSI}}$$

Statistical analysis

Statistical analysis of all measurements was carried out using IBM SPSS Statistic 22 Software. The results are presented as mean ± standard deviation (SD). Comparison between the two groups was done using Student's *t*-test. A value of *p* < 0.05 was considered as indicative of statistically significant difference.

Results and discussions

Effect of nebulization fill volume on time to nebulize to dryness and aerosol output

Longer times for aerosolization to dryness were required with increase in fill volumes of water from 2 to 8 mL in the air-jet nebulizer. Nebulization periods between 5 and

10 min are recommended, and an extended nebulization time may reduce patient adherence (Jevon and Ewens 2001; Chan et al. 2011). Moreover, in order to achieve the most efficient treatment through nebulization, the percentage of nebulization at any time should be measured.

In this study, it was observed that 2 mL was the least fill volume for this specific device. However, approximately 55% was released as aerosol. Increasing fill volume from 2 to 8 mL significantly increased the percentage of aerosolization from 55 to 87%, respectively. Time taken to dryness between fill volumes of 4 and 8 mL of HPLC water was increased two-fold, from 9 min to 20 min, respectively. Consequently, nebulization of 4 mL formulation for 9 min was considered the most appropriate experimental setup throughout this study. These results are shown in Fig. 1A, B.

Assessment of aerosol properties using the NGI and the FSI

Fig. 2A is a bar chart showing amounts of salbutamol sulfate deposited on each stage of NGI. The highest depositions of salbutamol sulfate occurred on stages 2, 3, 4 and 5, whereas stages 1, 6, 7 and 8, and induction port had the lowest depositions. The optimum particle size required for drug deposition in the lungs is in the range of 2–6 μm . Therefore, these data are consistent with expected results since the cut-off diameters operated at flow rate of 15 L/min were 14.1, 8.61, 5.39, 3.30, 2.08, 1.36, 0.98 and 0.70 μm from stages 1 to the last stage of NGI.

For FSI, the distribution of drug mass as seen in Fig. 2B shows no difference between those collected from upper and lower stages (fraction below 5 μm) of the impactor.

The values of % mass balance for all runs were within the European Pharmacopoeia acceptance limit of 75–125% (Wyka et al. 2007). It is challenging to achieve 100% mass balance due to technical errors during transferring and rinsing steps prior to HPLC analysis. The MMAD and GSD of the APSD could not be determined for FSI because of the requirement for plotting of multiple data points and interpolation

of data which were not available with FSI (Guo et al. 2013; Nimmano et al. 2018). The MMAD for NGI was approximately 5 μm , which allows for good deposition in the lungs. The poly-dispersal of aerosols is measured through GSD ($\text{GSD} > 1.2$). In this study, FPD and FPF were derived from interpolation from the graph for cumulative mass/fraction of salbutamol sulfate under 5 μm . The results for aerosol parameters in both NGI and FSI are presented in Table 1.

There was no significant difference in emitted dose (ED) between the two impactors. This is a reflection of

Table 1. Aerosol parameters of salbutamol sulfate nebulized into the impactors at the flow rate of 15L/min ($n=3$, mean \pm S.D.).

| Aerosolization parameters | NGI | FSI |
|---------------------------|------------------|------------------|
| ED (%) | 71.56 \pm 1.35 | 69.54 \pm 4.58 |
| FPD (mg) | 1.97 \pm 0.11 | 0.94 \pm 0.18 |
| FPF (%) | 68.6 \pm 2.5 | 43.0 \pm 1.2 |
| MMAD | 5.02 \pm 0.35 | - |
| GSD | 2.12 \pm 0.03 | - |

comparable values for the key aerosolization parameters, particularly FPF and FPD (Nimmano et al. 2018). The NGI gave higher values of FPD and FPF, when compared with FSI. Statistical analysis revealed significant difference between NGI and FSI with respect to FPD and FPF ($p < 0.05$). Nebulized aerosols travel across the longer path of NGI, while FSI is comprised of only two stages (Fig. 3A, B). Moreover, the NGI has relatively larger internal volume of 1245 cm^3 , relative to FSI. These could lead to more evaporation of droplets, and reduction in droplet size, resulting in the higher values of FPD and FPF for NGI (Kuhli et al. 2009; Nimmano et al. 2018).

The different FPD and FPF could also be due to the different way of determining the FPD and FPF of the full resolution and abbreviated cascade impactor. The FSI used a simple approach as the mass/fraction of salbutamol sulfate less than 5 μm can be found in the lower stage of the impactor, whereas the NGI required interpolation from data collected on the mass/fraction of drug deposited on each stage of the impactor (Nimmano et al. 2018).

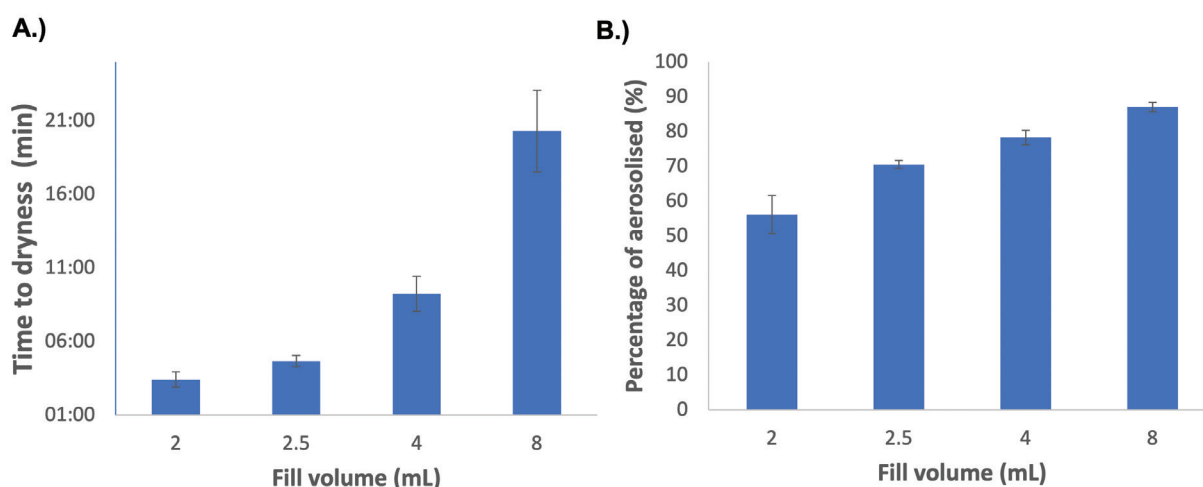


Figure 1. Effect of fill volumes of water on (A) dryness time and (B) percentage of nebulized ($n=3$, mean \pm S.D.).

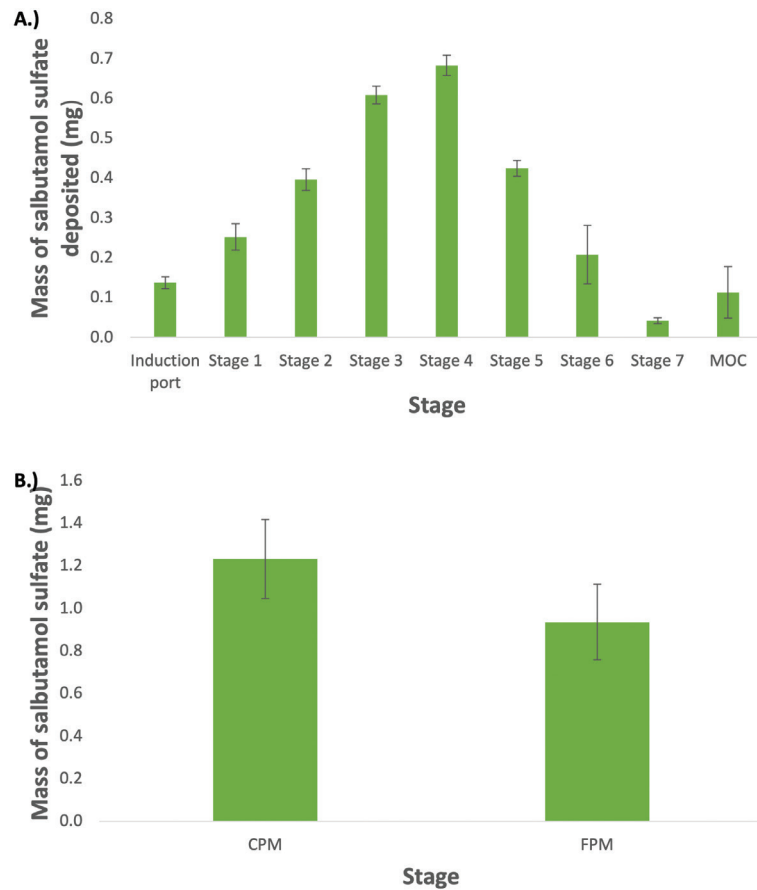


Figure 2. Mass of salbutamol sulfate deposited at each stage of (A) the NGI and (B) the FSI (n=3, mean± S.D.).

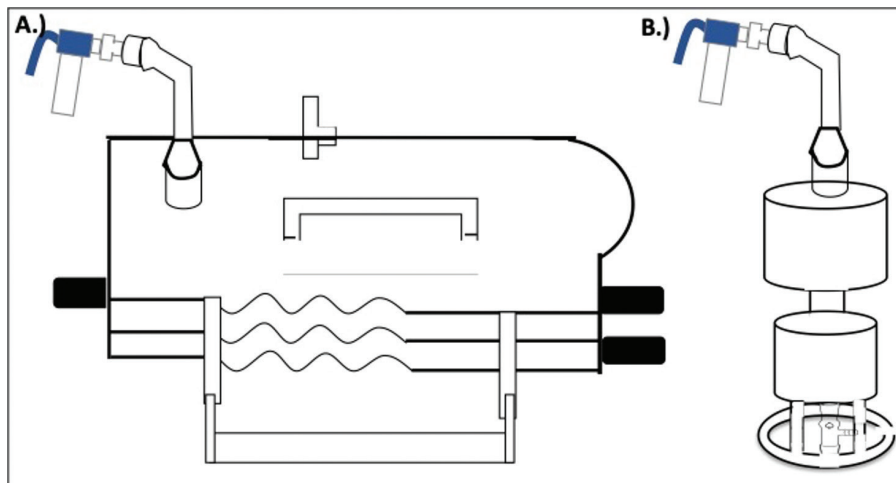


Figure 3. Structure of (A) the NGI and (B) the FSI.

The lower values of FPD and FPF obtained with the FSI may suggest that large volumes of solution were nebulized, not to dryness. The results obtained in this study are in a good agreement with previous report of co-loaded liposomes, reflecting no effect of formulation approaches on the performance of the NGI and FSI.

Few studies have demonstrated equivalent values of FPF for pMDIs and DPIs when the performance of the full

cascade impactor and abbreviated impactor were compared (Mitchell et al. 2009a, 2009b). Significant difference in FPD but not in FPF was observed for 2 out of 3 DPIs when using the NGI against the abbreviated impactor (Mohan et al. 2016). These observations could possibly be due to different inhalation products and/or formulations.

FSI allowed for a faster determination of properties of nebulized aerosol compared to NGI. Each run of

NGI can take up to an hour for completion. The use of FSI took an average of 30 minutes thus can potentially halve the amount of time taken per determination. The NGI is also more complex and require more care and attention during the analysis as compared to the FSI. The low loss carryover of FSI can help reduce any operator-induced variation from dissimilar operational habit or technique such as in sample recovery from impactor. In addition, aerosolized drug is deposited across eight stages in the NGI thus may lead in analytical quantification problem due to low levels of active substance deposition on some stages. The FSI is consequently useful in overcoming the situation which may be an issue with smaller fill volume of nebulizer solution or formulations with low total amount of active substance (Nimmano et al. 2018).

Other future research could evaluate the effect of changes in formulation i.e. change in viscosity or surface tension of nebulizer liquid, in the discriminatory ability of the cascade impactor methods due to the limitation of current results. The study could also test for level of variations from multiple operators to check for sensitivity of both impactors.

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Conclusion

The FSI is a suitable faster alternative impactor to the NGI, giving different but comparable values of the key parameters of aerosolization. The study on the effect of physicochemical properties of the formulation i.e. surface tension and viscosity, inhalation products i.e. pMDIs, DPIs and nebulizers as well as the formulation approaches i.e. emulsions, solutions, niosomes etc. on the performance of the two impactors should also be further evaluated. Despite the FSI is unable to provide all the information that a full resolution cascade impactor can such as MMAD and GSD and could not replace the NGI for all studies, it may be beneficial to use the FSI interchangeably with the NGI in some pharmaceutical development and quality control process, and the inclusion of the FSI within the Pharmacopoeia may be justified.

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