Comparison Between Pharmacopeial Testing and Testing Based on Mixing Inlet Methodology with Application to Nebuliser Testing: Part 2 – Main Study

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Summary

The compendial approach to determine fine particle dose (FPD) of preparations for nebulisation when using a simulated patient breathing pattern, is to combine the results from two pharmacopeial methods. The delivered dose (DD) is collected on the inhalation filter during breathing simulation, while the fine particle fraction (FPF) is determined using the cooled Next Generation Impactor (NGI) operated at 15 L/min. FPD is then calculated as the product of DD and FPF. A recent advance in mixing inlet lung simulation (MILS) allows cascade impactor assessments to be made at constant flow rate whilst operating the nebuliser using a varying flow profile associated with breath simulation. The purpose of this study was to investigate the feasibility of the MILS approach for direct determination of $FPD_{<5\mu m}$ using a vibrating membrane nebulizer (eFlow® Rapid) delivering aqueous salbutamol sulphate with an adult tidal-breathing pattern (inspiratory:expiratory ratio = 1:1, tidal volume = 500 mL, 15 bpm, peak inspiratory flow rate (PIFR) = 24 L/min) as the model system. $FPD_{<5\mu m}$ obtained with the MILS was 72% of that obtained using the compendial method, showing that the two procedures were not equivalent for this particular comparison, although MILS methodology may be more pertinent. Whether this conclusion holds for other nebulizer types, drug products or alternate breathing patterns remains to be investigated. Regardless of the outcome of those studies, the present finding raises an important question as to which of the two methods provides the $FPD_{<5\mu m}$ that is more representative of the lung dose received by a patient.

Introduction

The pharmacopeial method for the evaluation of preparations for nebulisation to establish the aerodynamic particle size distribution (APSD) in general and fine particle dose in particular is based on sampling the aerosol by the cooled NGI at a constant flow rate of 15 L/min [1,2]. When studying the nebuliser performance during breathing simulation, measurements of drug delivery rate and delivered dose are made separately with the nebuliser-on-test attached to a breathing simulator, mimicking the selected tidal breathing pattern [1,2]. The FPD, with cut-off typically chosen to be 5 μm ($FPD_{<5\mu m}$), is subsequently calculated as the product of DD and $FPF_{<5\mu m}$. The need to undertake two separate tests to establish $FPD_{<5\mu m,}$ which is the critical quality attribute for product performance, is cumbersome and increases the potential for error. Moreover, one can question whether the value of $FPF_{<5\mu m}$ determined at a constant flow rate is fully representative of that obtained using breathing simulation when only the inhaled portion of the generated aerosol is entering the impactor. Furthermore, in the context of developing more clinically appropriate testing methods, it would be useful to be able to observe changes in $FPD_{<5\mu m}$ at the same time as nebuliser performance is being evaluated at different breathing patterns reflective of patient age categories and obstructive lung disease state. A recent advance in MILS [3] methodology has made it possible for cascade impactor assessments to be made at the required constant flow rate whilst operating the nebuliser using a continuously varying flow profile associated with breath simulation [4]. The purpose of the present bench study was to evaluate the potential of MILS methodology as a direct, more robust and potentially clinically more relevant alternative to the current approach combining two pharmacopeial methods for determination of APSD when the nebuliser is operated at a varying flow rate. As model system for the evaluation a vibrating membrane device was selected as a representative of the nebuliser class of orally inhaled products (OIPs). In this initial assessment the standard adult tidal breathing pattern was studied because the focus was on investigating if the new and more realistic MILS method provided equivalent outcomes to the combination of compendial test procedures. It was hypothesized that $FPD_{<5\mu m}$ obtained by the MILS-based procedure would be statistically equivalent with the corresponding measure derived from the separate determinations of DD and $FPF_{<5\mu m}$.

Materials and Methods

Three nebulizer units (eFlow® Rapid, PARI GmbH, Starnberg, Germany) were tested with a single power unit and evaluated with salbutamol sulphate nebuliser solution (Ventolin® 5 mg/2.5 mL (GSK plc, Middlesex, UK)). Figure 1 illustrates the three test configurations that were studied.

For the reference compendial methods, DD was determined capturing the droplets emitted by the nebulizer-on-test when connected to a breathing simulator (model F-SIG 6300, FIA AB, Södra Sandby, Sweden) set to operate with the following adult tidal-breathing waveform: inspiratory:expiratory ratio = 1:1, tidal volume = 500 mL, 15 bpm, PIFR = 24 L/min) [BS-procedure]. A filter (Respirgard II, model 303EU, Vital Signs, US) captured during the inhalation phase the emitted droplets at the mouthpiece of the nebuliser. In a parallel study $FPF_{<5\mu m}$ was determined, sampling the emitted droplets into an NGI (MSP Corp., St. Paul, MN, USA), equipped with a USP/Ph.Eur. induction port and operated at a nominal flow rate of 15 L/min [NGI-procedure].

The NGI and induction port were cooled in a refrigerator at 5°C for at least 90 minutes before testing. Following sampling, the mass of salbutamol sulphate deposited in the throat, on stages 1-7 and on the MOC was determined in each instance by a validated HPLC procedure.

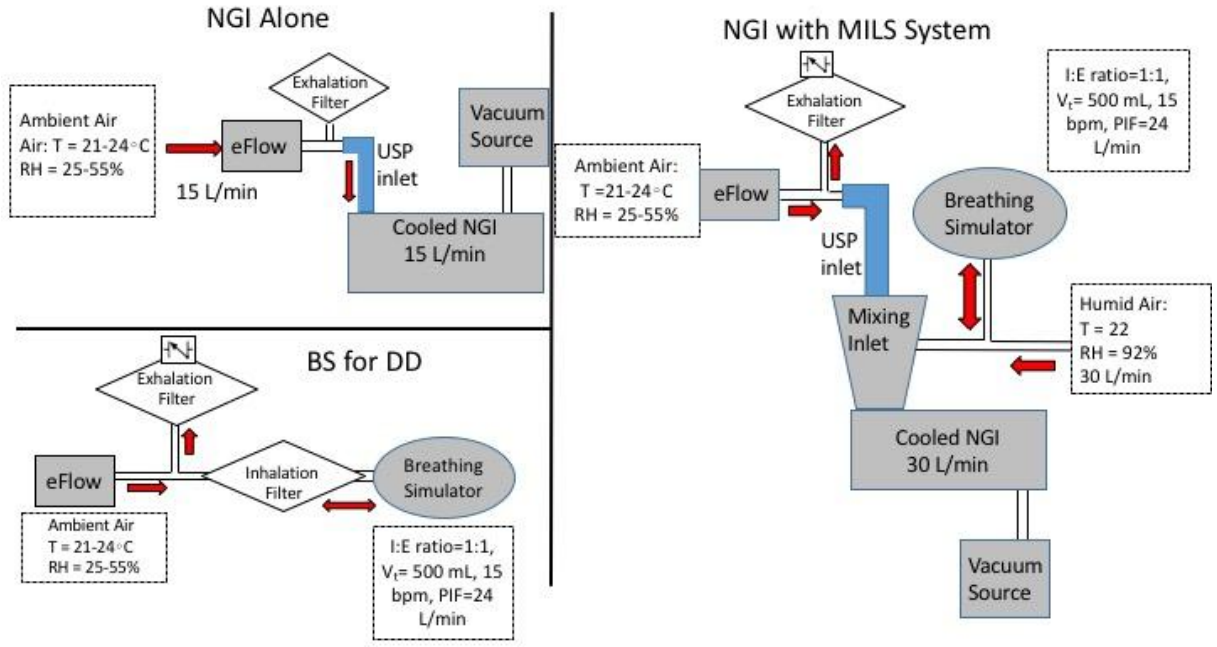

Figure 1 Configurations for the Compendial Procedures (BS for DD and NGI Alone) and for the NGI with MILS Methodology

$FPD_{<5\mu m}$ for the MILS procedure was determined directly from the NGI stage depositions measured with the nebuliser-on-test connected via the Nephele MILS mixing inlet (RDD OnLine, Richmond, VA, USA) to both the breathing simulator and to cascade impactor, as shown in Figure 1. Note that the flow rate through the NGI for the MILS procedure had to be increased to 30 L/min (the lowest apart from 15 L/min, for which archival calibration data are available [5]), because it was necessary that the constant flow through the impactor be higher than the PIFR of the selected breathing pattern (24 L/min). The need to run NGIs at different flow rates for the compendial and alternate MILS procedures was a cause of concern. For this reason a pilot study (Part 1) was undertaken, investigating four different configurations, each operating the NGI at a flow rate of 30 L/min but either with the impactor cooled or at room ambient temperature, as well as examining the use of dry or humid supplementary air feeding the mixing inlet [6]. This investigation demonstrated that the difference between these alternate configurations and the compendial method was minimized when the impactor was cooled and the pressurized air to the mixing inlet was humidified.

Table 1 shows the design and test sequence for the entire 4-day study, balanced in terms of test order within day using each of the three test procedures and undertaken by a single operator. The study uses a paired design so that the MILS/(BS+NGI) $FPD_{<5\mu m}$ ratio could be calculated for each combination of day and nebuliser unit; these ratios were used for calculation of the desired confidence interval (CI).

Table 1 Parallel Study Design Allowing Determination of MILS/(BS+NGI) $FPD_{<5\mu m}$ Ratio for Each Day and Nebuliser Unit

Day No.	Nebulizer unit No.	Test Order			Day No.	Nebulizer unit No.	Test Order		
		BS	NGI	MILS			BS	NGI	MILS
1	1	2	3	1	3	1	20	21	19
1	2	4	5	6	3	2	22	23	24
1	3	9	7	8	3	3	27	25	26
2	1	11	12	10	4	1	29	30	28
2	2	15	13	14	4	2	33	31	32
2	3	16	17	18	4	3	34	35	36

The protocol stated that equivalence between the investigated MILS method and the combined pharmacopeial BS+NGI method could be claimed if the obtained 90% confidence interval for the MILS/(BS+NGI) $FPD_{<5\mu m}$ ratio was completely within the acceptance interval 85% - 118%. The study was sized (n = 12 ratios) based on the pilot study so the obtained CI for the mean ratio would have a length of at most 15%.

Results

The key measures obtained from the investigation are summarised in Table 2.

Table 2 Performance Measures Obtained from the Vibrating Membrane Nebulisers Evaluated by Compendial (BS and NGI) Procedures and the Alternate MILS Method

Parameter	Procedure	N	Mean	SD	RSD	Range
DD from BS	Separate Compendial	12	1009 μg	57 μg	5.7 %	926 – 1127 μg
$FPF_{<5\mu m}$ from NGI		12	47 %	3.0 %	6.3 %	43 – 52 %
$FPD_{<5\mu m}$ from BS+NGI		12	475 μg	50 μg	10.6 %	424 – 572 μg
$FPD_{<5\mu m}$ from MILS	Combined Approach	12	340 μg	29 μg	8.5 %	297 – 392 μg

The primary end-point for assessing the equivalence between the compendial BS+NGI methods and the MILS method was $FPD_{<5\mu m}$. This metric obtained by MILS testing was expressed as a percentage of $FPD_{<5\mu m}$ calculated as DD x $FPF_{<5\mu m}$ from the data derived from the compendial BS and NGI methods, respectively. Individual values of this ratio (Figure 2) varied from 59 to 83%, with the overall average being 72%. The 90% CI for the MILS to BS+NGI FPD ratio was 68.9% to 75.2%. The length of the obtained CI was 6.3%, much shorter than the expected length of 15%; this outcome was a consequence of the SD of the ratio being much smaller than in the pilot study (6.6% vs. 15.5%).

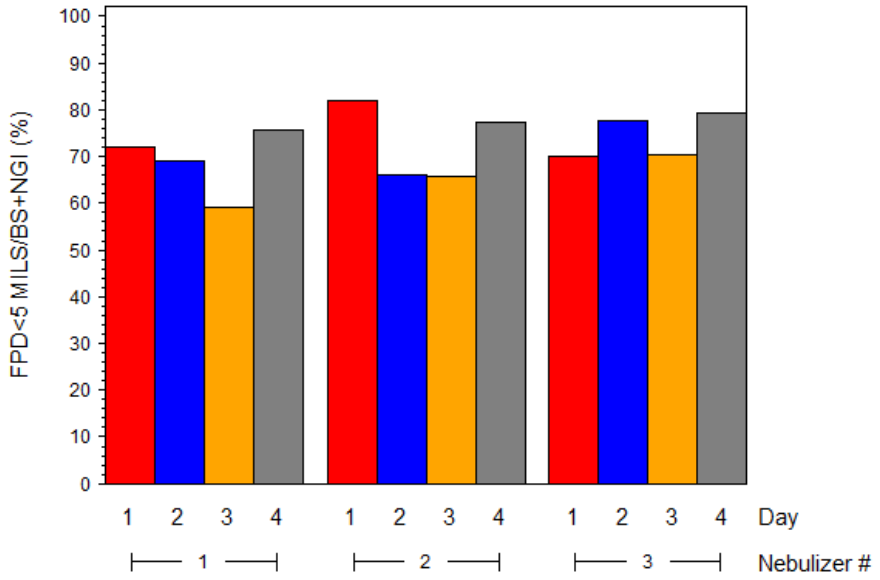

Figure 2 Values of $FPD_{<5\mu m}$ by MILS Procedure Expressed as a Percentage of $FPD_{<5\mu m}$ by BS + NGI Compendial Procedures for Each Nebuliser Tested on Each Day of the Investigation

Discussion

The outcome from the investigation disproved the hypothesis, since the MILS procedure resulted in a significantly smaller $FPD_{<5\mu m}$, compared with the existing combination method. Importantly, there were no obvious trends in the data associated with a particular nebulizer, nor did the order of testing indicate any systematic drift in the ratio (Figure 2). The droplet size distributions from the NGI normalised to impactor-sized mass and expressed as cumulative distribution functions (CDFs) were largely equivalent (Figure 3) between the two configurations, indicating that evaporation/condensation-related changes in the MILS configuration compared with the compendial set-up had not taken place to any appreciable extent. However, the raw data for absolute mass salbutamol sulphate recovered (mean ± SD) revealed substantially less drug entered the NGI in the MILS configuration (728 ± 50 μg) than using the compendial method (1724 ± 84 μg). This outcome was expected, since the NGI-MILS only collected the ”inhaled” dose from the nebuliser, whereas all the emitted aerosol was captured by the NGI when operated in the compendial configuration.

Finally, 577 ± 58 µg salbutamol sulphate was recovered from the exhalation filter in the MILS set-up, greater than 449 ± 42 µg when the nebulisers were evaluated for DD. This small but significant difference is explicable because slightly more drug particles exited the measurement apparatus via the induction port during exhalation phase than was the case with the simpler set-up for determination of DD during breathing simulation.

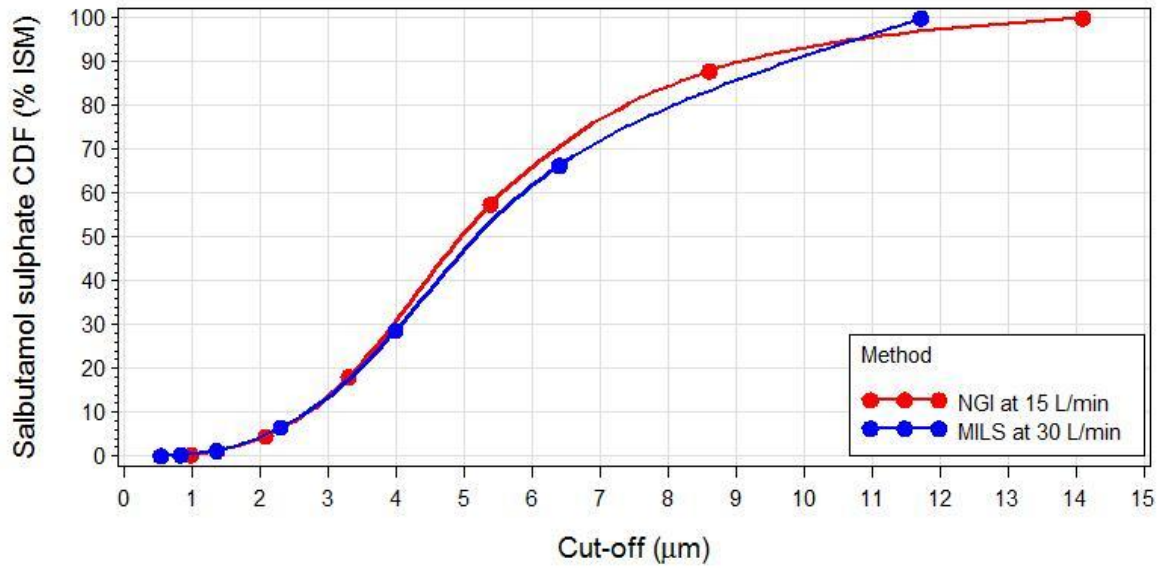

Figure 3 Cumulative Size Distributions for Salbutamol Sulphate as Percentage of Impactor Sized Mass (ISM = Sum of Drug Depositions on S2 to the MOC) by Compendial (NGI) and MILS Configurations for the eFlow® Rapid Vibrating Membrane Nebuliser

Conclusions

The MILS-based procedure evaluated, allowing direct measurement of $FPD_{<5\mu m}$ during breath simulation by inserting a mixing inlet between the nebulizer and cascade impactor, is not equivalent to the determination of this metric by the existing compendial procedures involving separate determinations of DD and $FPF_{<5\mu m}$. The $FPD_{<5\mu m}$ determined with the MILS configuration was found to be 72% of that found using the current pharmacopeial approach, and the associated 90% CI for the FPD ratio was 69% - 75%. Whether similar deviations will be found for other nebuliser types, drug products, FPD size limits, or alternate breathing patterns remains to be investigated. Regardless of the outcome from those studies, the present finding raises the important question, which of the two approaches provides an FPD that is more representative of the lung dose received by a patient. In response, it is suggested that the MILS-based approach, despite its increased complexity, may provide a more accurate answer to this question than the current pharmacopeial methods.

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