AAPS PharmSciTech, Vol. 11, No. 3, September 2010 (© 2010)
DOI: 10.1208/s12249-010-9473-1

Research Article

Relative Precision of Inhaler Aerodynamic Particle Size Distribution (APSD) Metrics by Full Resolution and Abbreviated Andersen Cascade Impactors (ACIs): Part 2—Investigation of Bias in Extra-Fine Mass Fraction with AIM-HRT Impactor

Jolyon P. Mitchell,[1] Mark W. Nagel,[1] Cathy C. Doyle,[1] Rubina S. Ali,[1] Valentina I. Avvakoumova,[1] J. David Christopher,[2] Jorge Quiroz,[2] Helen Strickland,[3] Terrence Tougas,[4] and Svetlana Lyapustina[5,6]

Received 15 March 2010; accepted 11 June 2010; published online 10 July 2010

Abstract. The purpose of this study was to resolve an anomalously high measure of extra-fine particle fraction (EPF) determined by the abbreviated cascade impactor possibly relevant for human respiratory tract (AIM-HRT) in the experiment described in Part 1 of this two-part series, in which the relative precision of abbreviated impactors was evaluated in comparison with a full resolution Andersen eight-stage cascade impactor (ACI). Evidence that the surface coating used to mitigate particle bounce was laterally displaced by the flow emerging from the jets of the lower stage was apparent upon microscopic examination of the associated collection plate of the AIM-HRT impactor whose cut point size defines EPF. A filter soaked in surfactant was floated on top of this collection plate, and further measurements were made using the same pressurized metered-dose inhaler-based formulation and following the same procedure as in Part 1. Measures of EPF, fine particle, and coarse particle fractions were comparable with those obtained with the ACI, indicating that the cause of the bias had been identified and removed. When working with abbreviated impactors, this precaution is advised whenever there is evidence that surface coating displacement has occurred, a task that can be readily accomplished by microscopic inspection of all collection plates after allowing the impactor to sample ambient air for a few minutes.

KEY WORDS: AIM-HRT; impactor; inhaler; measurement bias; plate coating.

INTRODUCTION

In Part 1 of this series of two papers (1), the relative precision of two different abbreviated cascade impactors (CIs) was compared with that of the benchmark full-resolution Andersen eight-stage non-viable impactor (ACI). The system intended to provide size fractions that might potentially be pertinent for the human respiratory tract (so-called AIM-HRT impactor) was observed to overestimate the extra-fine particle fraction (EFF) <1.1-μm aerodynamic diameter by a significant amount. In contrast, substantially equivalent values of fine particle and coarse particle fractions (FPF and CPF, respectively) were obtained with the boundary size set at 4.7-μm aerodynamic diameter. A slight overestimation of EPF based on an upper limit of 1.0 μm compared with the corresponding metric obtained from a full-resolution ACI had been observed in a previous and separate investigation using a slightly different abbreviated CI (2). In that study, precautions had been taken to mitigate particle bounce and re-entrainment by coating all stages with a surfactant layer to provide a tacky impact surface, in accordance with current good practice (3). The cause of the slight anomaly was therefore interpreted in terms of differing mass transfer of the small amount of active pharmaceutical ingredient associated with non-recovered particles that are deposited on the internal surfaces of either CI. In view of the high degree of agreement achieved with all other metrics based on mass fractions described in Part 1 of the study (1), further investigation was felt to be justified with the objective of seeking out and remedying the cause of the bias associated with EPF.

MATERIALS AND METHODS

The experiment was undertaken as a follow-on investigation to the study described in Part 1 (1) for assessing the relative precision that took place at the same location (Trudell Medical International, London, Ontario, Canada), utilizing the same methodology as far as was possible. Thus, the same operators performed the same specific tasks contributing to a given complete measurement sequence in order to minimize the risk of introducing operator-linked

[1] Trudell Medical International, London, Ontario, Canada.
[2] Merck, Kenilworth, New Jersey, USA.
[3] GlaxoSmithKline Inc., Zebulon, North Carolina, USA.
[4] Boehringer Ingelheim Pharma Inc., Ridgefield, Connecticut, USA.
[5] Drinker, Biddle & Reath, 1500 K Street, NW, Suite 1100, Washington, District of Columbia 20005-1209, USA.
[6] To whom correspondence should be addressed. (e-mail: svetlana.lyapustina@dbr.com)

bias. However, the pressurized metered-dose inhaler (pMDI) canisters used in the original study had been exhausted by the time the present investigation took place, and so different canisters from the same batch were used. The measurement equipment was operated under tightly controlled ambient conditions as in the main experiment (1), with temperature and relative humidity confined to the ranges 21.0±1.0°C and 45±5% RH, respectively.

The AIM-HRT system was the same as that used in the main experiment described in Part 1 (1). Thus, the apparatus was configured to evaluate simultaneously the values of coarse (CPM), fine (FPM), and extra-fine (EPM) particle mass, with the boundary size for the first stage defining FPM and CPM set at 4.7-μm aerodynamic diameter. The second boundary demarcating the upper limit for EPM was 1.1-μm aerodynamic diameter. As in the main experiment (1), a dummy stage (stage 0 from a full-resolution ACI, but without its collection plate) was inserted between the inlet cone and first impaction stage in order to maintain a comparable internal volume to that of the ACI before size separation took place. The collection surfaces excluding the glass microfiber backup filter (product 934-AH, Whatman Inc., Florham Park, NJ) were coated with the same surfactant (Brij-35 polyoxyethylene 23 lauryl ether, Fisher Canada, Nepean, ON) applied in a solution of a volatile solvent. The applied coating was prepared from 3 g Brij dissolved in 20 mL methanol, from which 1 ml solution was removed and mixed thoroughly with glycerol (5 g) to thicken.

Before any measurements took place with the active drug substance, the HRT impactor was operated at 28.3 L/min±5% without coupling an inhaler to the Ph.Eur./USP induction port for a similar length of time used to gather the samples in the original experiment (2.5 min). The collection surfaces of both impaction stages were subsequently examined by optical microscopy to see if there had been visible changes to the expected uniform coating that had been observed in previous validation studies.

Following the microscopic examination (see "RESULTS"), five replicated measurements with the AIM-HRT system were made using a currently marketed pMDI-delivered HFA-albuterol (salbutamol) as the test product. As in the main experiment (1), five actuations from the pre-primed inhaler were delivered at 30-s intervals per measurement. However, on this occasion, the collection surface for the lower impaction stage of the apparatus was modified to include a glass microfiber filter (90 mm cut to 82.6-mm diameter) of the same type used as a backup filter, saturated with surfactant solution from which the methanol had been allowed to evaporate (Fig. 1). Afterwards, the mass of albuterol recovered from each component was assayed by the same validated high-performance liquid chromatography–ultraviolet spectrophotometric method based on the US Pharmacopeial monograph for albuterol (4) as had been employed in the precision study (1).

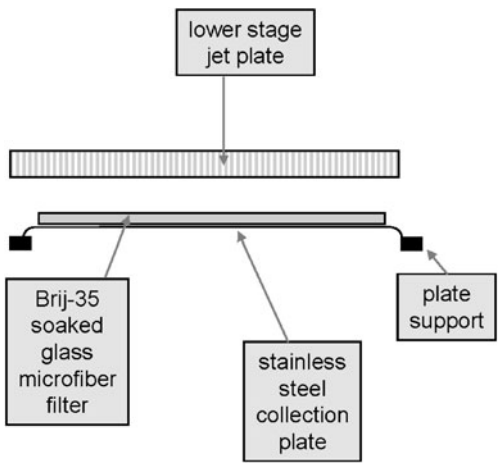

Fig. 1. Configuration for the lower impaction stage of the AIM-HRT CI to eliminate bias from particle bounce and re-entrainment

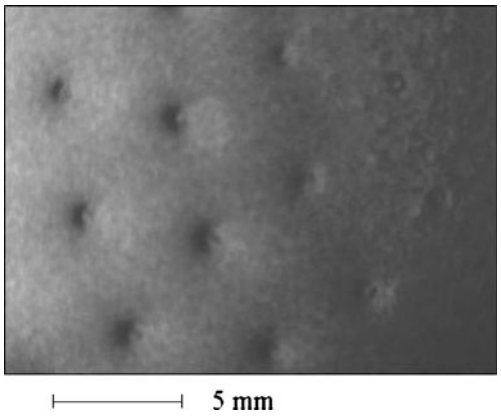

Fig. 2. Photomicrograph of displaced Brij-35 surfactant on collection plate for the second impaction stage of the AIM-HRT system)

RESULTS

Photomicrographs of the surfactant-coated stainless steel collection plate located beneath the second nozzle plate and operated at 28.3 L/min without actuating the inhaler revealed that depressions in the plate coating had formed beneath the nozzles (Fig. 2). The resulting cumulative mass weighted size distribution data for the original and repeated measurements with the Brij-saturated filter located on top of the collection plate of the lower stage (Table I) demonstrated close agreement between EPM, as well as with FPM and CPM determined by the modified AIM-HRT system using the ACI-derived data taken from the main study as benchmarks. This improvement in agreement for EPF is also illustrated in the cumulative APSD data based on the total mass per actuation entering the induction port to be captured in either of these configurations (Fig. 3). Comparison with the original AIM-HRT data also illustrates the magnitude of the reduction in EPF following the modification to the lower collection stage of this CI.

DISCUSSION

The discovery that particle bounce and re-entrainment had not been entirely eliminated by the application of a surfactant coating for the lower stage of the AIM-HRT impactor was a surprise, given the established practice of using such types of coating for accurate measurements for the full-resolution ACI (5-7) as well as pharmacopeial recommendations for their use (8,9). This finding is of importance since apart from the bias in EPF, internal deposition of particles on CI surfaces other than those intended for collection (so-called wall or inter-stage losses) can also be

Table I. Metrics Measured by Modified AIM-HRT Impactor Compared with Equivalent Metrics from the Main Precision Study Using Full-Resolution ACI

Impactor/study (sample size)	CPM (μg) mean (SD)	FPM (μg) mean (SD)	EPM (μg) mean (SD)
ACI Main study (1) (n=18)	44.08 (3.18)	35.43 (1.78)	2.21 (0.86)
HRT Main study (1) (n=18)	45.17 (3.21)	35.00 (2.01)	7.93 (2.03)
HRT Current study Brij-soaked filter (n=5)	43.6 (1.88)	35.0 (2.01)	2.1 (0.30)

affected since particles that are not deposited at the correct location are more susceptible to adhere to all types of surfaces with which they come into contact (10). It follows that it is desirable to avoid particle bounce altogether.

These findings indicate that merely coating the plate with a thin layer of surfactant may be insufficient to eliminate particle bounce from the second stage of this type of abbreviated impactor system. Interestingly, there appears to be no need to take this precaution for the uppermost stage as measures of FPF and CPF, both of which might also be anticipated to have been affected, were comparable for the benchmark ACI data and the unmodified AIM-HRT system (Table I). However, in support of this observation, there was no visible evidence of surfactant displacement with the first-stage collection plate in the preliminary testing of the AIM-HRT system undertaken without actuating the inhaler. Furthermore, such behavior has not, to the authors' knowledge, been observed with the full-resolution ACI, and its cause is obscure. However, examining the aerodynamic characteristics of the full ACI (11), it is apparent that the flow Reynolds numbers (Re_{stage}) between stages 2 and 5 are similar, but all values are below the lower limit of the range from 500 to 3,000 (Table II), recommended by Marple and Liu (12) for ideal size-separating characteristics in laminar flow through the 400 nozzles of each stage. Furthermore, cross-flow induced by air exiting the nozzles near the center of the nozzle plate and flowing outward past other air jets located near the periphery of the nozzle cluster can prevent the air jets near the edge of the cluster from reaching the impaction plate with multi-nozzle designs (13). Although values of the cross-flow parameter (X_f) for stages 3 to 5 were well below the recommended maximum of 1.2 (12), X_f for stage 2 is at this limit. Both sets of criteria therefore indicate that the aerodynamic size-separating behavior based on flow through the individual stages of concern within the ACI may not be ideal so that the anomalous effect on the coating media seen in the present study when stages 3 and 4 were removed to create the HRT impactor might be expected. It is therefore conjectured that the missing stages 3 and 4 from the full-resolution ACI in the original AIM-HRT configuration created conditions where the flow exiting each nozzle of the stage above displaced the surfactant layer radially. This flow exited the nozzles initially perpendicular to the collection plate before diverging to exit parallel to the upper surface of the plate. This behavior resulted in a lack of surfactant coverage where particles would normally impact. Visual inspection of the collection plates after several actuations of the inhaler into the original AIM-HRT system confirmed that although most of the deposits remained embedded in the remaining surfactant, some faint streaking indicative of particle bounce was present.

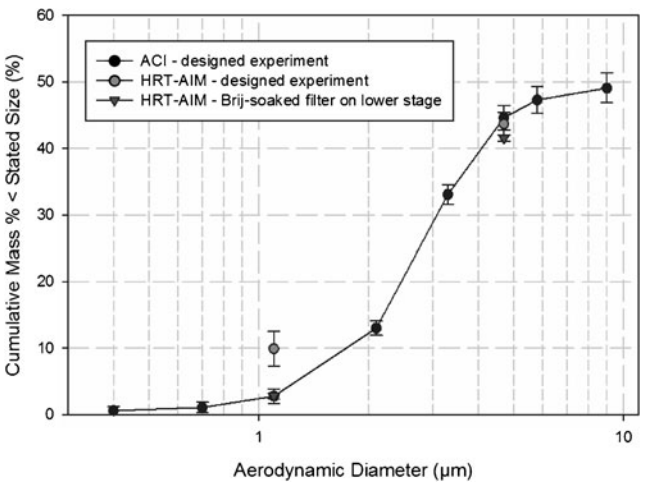

Fig. 3. Comparative measures of impactor-sized sub-fractions by QC and HRT abbreviated systems with ACI: Original and repeated measurement sets with the HRT impactor

The outcome from this follow-on study is that surfactant displacement can be prevented by fixing it within the glass microfiber matrix of a filter. At the outset, it is important to realize that by coating the filter with surfactant, any residual open porosity was likely not present to the extent that aerodynamic size separation for the stage is significantly impaired (14). However, confirmation that this is the case will require a separate study comparing the calibration of the stage with and without the coated filter, using reference particles of known aerodynamic size, and is outside the scope of the present remedial investigation. It is believed that the proposed modification to the collection surface also increases the depth of the tacky media within which the particles can penetrate on arrival. The excellent agreement in EPF from the improved HRT configuration and the ACI is also indirect proof that the slight reduction in the nozzle-to-plate distance brought about by insertion of the filter on top of the collection plate was unimportant. Finally, it is appreciated that this investigation did not examine other types of coating, such as silicone oil, that are widely used (15) and which may be more durable especially if the viscosity is high. However,

Table II. Aerodynamic Characteristics for Selected Stages of the ACI

ACI stage	Re_{stage} (11)	X_f	Configuration in HRT impactor
2	110	1.2	Present
3	141	0.93	Removed
4	188	0.69	Removed
5	292	0.44	Present

the solution that was found is easy to implement and appears to resolve the bias entirely. Furthermore, recovery of many different small-molecule APIs is well established with Brij-35 in glycerol as coating material. Interestingly, the findings of the present study are in agreement with the observations of Dunbar *et al.* (16) who could not eliminate particle bounce in their ACI when the collection plates were coated with a thin layer of vacuum grease, but succeeded when they inverted the plates so that they could be fitted with 20-μm pore glass fiber filters saturated in water.

CONCLUSIONS

A follow-on investigation of bias in measures of EPF by the AIM-HRT system traced displacement of the surfactant coating used on the lowermost collection plate of this impactor as the source of the problem; once precautions were taken to eliminate particle bounce caused by the absence of a sufficient covering of surfactant, substantial agreement was obtained between this abbreviated impactor and the full-resolution ACI. This finding, taken together with the absence of bias in metrics associated with the AIM-QC and ACI, and the comparable precision for all three systems described in Part 1 (1) confirm that such abbreviated impactors should be able to be substituted for the full-resolution impactor when appropriate. However, the unanticipated discovery of bias associated with uncontrolled particle bounce and re-entrainment with the AIM-HRT system serves as a reminder to potential users of AIM-based CIs that careful validation of the chosen technique against the full-resolution impactor data used to characterize the drug product in the first place should always be a part of the preparatory work before abbreviated impactor measurements are implemented on a routine basis. Inspection of the data for bias caused by particle bounce and re-entrainment should form a key component of method validation, and the use of a surfactant-coated filter as an alternative substrate to a thin-coating of surfactant may be an appropriate remedy if this non-ideal behavior is evident.

ACKNOWLEDGMENTS

The authors wish to acknowledge feedback received during the preparation of this manuscript from the other members of the IPAC-RS Cascade Impactor Working Group and also from discussions with members of the European Pharmaceutical Aerosol Group (EPAG) Impactor Sub-Team. The helpful comments of the anonymous reviewers were also appreciated.

REFERENCES

1. Mitchell JP, Nagel MW, Doyle CC, Ali RS, Avvakoumova VI, Christopher JD, *et al.* Relative precision of inhaler aerodynamic particle size distribution (APSD) metrics by full resolution and abbreviated Andersen cascade impactors (ACIs): Part 1. AAPS PharmSciTech. 2010;11:843–51. doi:http://dx.doi.org/10.1208/s12249-010-9452-6.
2. Mitchell JP, Nagel MW, Avvakoumova V, MacKay H, Ali R. The abbreviated impactor measurement (AIM) concept: Part 1—Influence of particle bounce and re-entrainment—evaluation with a "dry" pressurized metered dose inhaler (pMDI)-based formulation. AAPS PharmSciTech. 2009;10(1):243–51. doi:10.1208/s12249-009-9202-9.
3. Christopher D, Curry P, Doub B, Furnkranz K, Lavery M, Lin K, *et al.* Considerations for the development and practice of cascade impaction testing including a mass balance failure investigation tree. J Aerosol Med. 2003;16:235–47.
4. United States Pharmacopeial Convention. US Pharmacopeia 32/National Formulary 27, Rockville, MD. Monograph for albuterol.
5. Dolovich M. Measurement of particle size characteristics of metered dose inhaler (MDI) aerosols. J Aerosol Med. 1991;4:251–63.
6. Rao AK, Whitby KT. Non-ideal collection characteristics of inertial impactors—single stage impactors and solid particles. J Aerosol Sci. 1978;9:77–86.
7. Rao AK, Whitby KT. Non-ideal collection characteristics of inertial impactors—cascade impactors. J Aerosol Sci. 1978;9:87–100.
8. European Directorate for Quality in Medicines (EDQM). European pharmacopeia 6.8, monograph 2.9.18. Preparations for inhalations: aerodynamic assessment of fine particles. Strasburg: EDQM; 2010.
9. US Pharmacopeial Convention. United States Pharmacopeia 33/National Formulary 28, Chapter <601> Aerosols, nasal sprays, metered-dose inhalers, and dry powder inhalers. Rockville, MD; 2010.
10. Mitchell JP, Nagel MW. Cascade impactors for the size characterization of aerosols from medical inhalers: their uses and limitations. J Aerosol Med. 2003;16(4):341–77.
11. Marple VA, Olson BA, Miller NC. The role of inertial particle collectors in evaluating pharmaceutical aerosol delivery systems. J Aerosol Med. 1998;11S1:S139–53.
12. Marple VA, Liu BYH. Characteristics of laminar jet impactors. Environ Sci Technol. 1974;8:648–54.
13. Fang CP, Marple VA, Rubow KL. Influence of cross-flow on particle collection characteristics of multi-nozzle impactors. J Aerosol Sci. 1991;22:403–15.
14. Huang C-H, Lin Y-C. Influence of substrate porosity and thickness on the collection efficiency of an impactor stage. J Aerosol Sci. 2010;41(4):364–72.
15. Sethuraman VV, Hickey AJ. Evaluation of pre-separator performance for the 8-stage nonviable Andersen impactor. AAPS PharmSciTech. 2001;2(1):article 4. Available at: http://www.aapspharmscitech.org/.
16. Dunbar C, Kataya A, Tiangbe T. Reducing bounce effects in the Andersen cascade impactor. Int J Pharm. 2005;301(1–2):25–32.