

Cascade Impactor Mensuration – An Assessment of the Accuracy and Precision of Optical Measurement Systems

Frank Chambers*, Jolyon Mitchell[†] and Christopher Shelton[†]
on behalf of the European Pharmaceutical Aerosol Group (EPAG)

*AstraZeneca plc, Charnwood, Loughborough, UK

[†]Trudell Medical International, London, Canada

[†]PPD Inc., Middleton, Wisconsin, USA

Summary

Multi-stage cascade impactors (CIs) are the preferred measurement technique for the assessment of aerodynamic particle size distribution of an inhalable aerosol. Stage mensuration using optical image analysis is the recommended compendial method for monitoring CI “fitness for purpose” within a GxP environment. The EPAG Impactor Sub-Team has undertaken an inter-laboratory study to assess the accuracy and precision of a range of currently used mensuration systems. Measurement of two Andersen cascade impactor “reference” stages confirmed that these systems were capable of reproducible jet measurement. In most cases, overall measurement error was found to be around 1% of nominal jet diameter. Observed small, but consistent differences between ‘dots’ and ‘spots’ of a calibrated chromium-plated reticle were attributed to treatment of partially-lit pixels along the circumference.

Introduction

The measured aerodynamic particle size distribution (APSD) of an inhalable aerosol is a key performance parameter for assessing the ability of an inhaler to deliver a therapeutic dose of the medication to the patient lung (1, 2). Multiple stage cascade impactors (CIs) provide the pharmaceutical industry with several apparatuses that can measure the aerodynamic particle size distribution of a pharmaceutical aerosol with direct traceability to the mass of active drug substance (3). These devices operate by inertial size-separation of particles passing through a plate containing one or more nozzles (jets) with an impaction surface located at a fixed distance from the jet-plate. Jet size (diameter for circular jets) is therefore a critical attribute defining the link between CI aerodynamic performance and the accuracy of the measured APSD (3).

Periodic measurement of the diameter or area of each jet of each stage (stage mensuration) is preferred as the means of routinely assuring CI aerodynamic performance (4) to individual stage calibration by particles of known aerodynamic size. This procedure is cited in the pharmacopoeia as the primary method for monitoring the “fitness for purpose” of CIs used within a GxP environment (5, 6). Current guidance has focussed on the achievable tolerances in impactor manufacture, whilst making the concession that alternative mensuration limits can be justified on a case-by-case basis.

Typically semi-automated optical measuring systems utilizing software-driven image analysis recognition capability are employed to measure jet diameters. While the algorithms associated with the software for analysis of the image obtained for each jet are not well understood, notable differences in reported jet diameters between several commercial systems have been reported for 8-stage impactors, especially involving stages with smaller nozzles (7). Furthermore, a recent survey of members of the Impactor Sub-Team of the European Pharmaceutical Aerosol Group (EPAG) revealed that a variety of different optical systems and associated calibration procedures are currently in use (8). As a result, the sub-team has undertaken industry-wide collaborative round-robin studies aimed at assessing separately the measurement precision and accuracy of current mensuration systems with a view to establishing appropriate in-use limits for CI stage mensuration.

Experimental

The optical mensuration systems that were assessed in both the precision and accuracy studies are summarized in Table 1. Nine participating sites assessed the inherent variability in stage mensuration data as the precision component of the study (the data from 7 is included here). Two “reference” stages of an Andersen Cascade Impactor (ACI) were provided by one of the study participants (Westech Instruments, Bedfordshire, UK). The stages chosen (stage 2 – 400 jets, nominal jet diameter 0.914 ± 0.0127 mm and stage 7 - 201 jets, 0.254 ± 0.0127 mm) represent the extreme measurement conditions in terms of numbers of jets/stage and jet size for the ACI.

On receipt at each site, the stages were cleaned using a procedure recommended by the stage provider and measured in triplicate in a pre-determined order (Stage 2, 7, 2, 7, 2, 7). Each participant calibrated their mensuration system using their standard in-house procedure prior to analysis, and provided data for some or all of the following parameters (depending on instrument capabilities):

- Number of jets measured per stage
- Jet diameter
- Calculated jet area from diameter
- Measured Jet area

- Calculated Jet diameter from area

Table 1 Optical mensuration systems evaluated – Precision and Accuracy Studies

System Name	Site Number (Precision Data)	Site Number (Accuracy Data)
Andersen Visual Inspection System (AVIS)	1	1
AVIS	2	
Mondo	3	
Mondo	4	
Mitutoyo QV404 (4)	4	
Mitutoyo QV404 (5)	5	5
Mitutoyo QV404 (6)		6
RAM Optical Instrumentation (ROI) Data Star 100	6	6
RAM Optical Instrumentation (ROI) Omis II	7	7
Optical Gaging Products Smart Scope FLASH		7

The mensuration data generated for the reference stages by each participant were forwarded to the Study Co-ordinator in the form of a MS Excel spreadsheet, along with a brief description of the calibration procedure used. The mean, median, range and % RSD for each stage were subsequently calculated and the individual jet measurement data assessed statistically using the SAS statistical evaluation package (SAS Institute Inc, Cary, NC, USA).

The measurement accuracy study was undertaken by five of the original participating sites in the mensuration precision investigation to provide some insight into the source of the measurement differences observed in the first phase. Since each optical mensuration instrument has different proprietary algorithms for treatment of partially lit pixels at the edge of the image produced by the impactor jet circumference, the potential exists for systematic differences in jet edge detection. In addition, physical properties of the stage nozzles themselves, e.g. jet depth, may be treated differently by each instrument type.

For this experiment, a calibrated glass reticle, containing a series of 2-dimensional, chromium-imaged circles, was evaluated as a reference standard along with a set of ring gauges which served as control models. The reticle (National Physical Laboratory (NPL), Middlesex, UK), a certified length-traceable, two-dimensional standard, was comprised of 9 chromium-on-glass and 9 glass-on-chromium circular spots arranged in a parallel pattern, ranging in diameter from 0.254 mm to 5.5 mm. The glass reticle allowed for assessment of instrument measurement accuracy without interference from other image artefacts. The three calibrated ring gauges, representing 1.0, 2.5 and 4.0 mm nominal diameters (Alpha Gauging Ltd, UK), were selected as a control system since the gauge aperture would be more representative of the exit plane of impactor jet orifices than the opaque or fully transparent circles comprising the NPL reticle.

The study protocol consisted of making ten replicate measurements of each circle/aperture on three different days, resulting in 30 (n=30) data points for each element. The measurement accuracy for mean reported diameter was calculated for each calibrated circle and ring gauge as a percent of the reported value from the associated certificate of analysis. Analysis of these standards was conducted according to each sponsor's internal operating procedures, which included calibration of the instrument with separate reference standards prior to sample analysis. The raw data generated by each site were collected in MS Excel format and referred back the study co-ordinator where the values of % accuracy were calculated along with 95% confidence intervals based on the nominal circle diameter.

Results and Discussion

A complete description of each of the various instrument systems is not presented here, but in general, each system comprises a CCD camera and lens to focus on an individual stage jet and collect an image at the exit plane for analysis. The camera is mounted above a platform upon which an inverted stage is placed for inspection. Each instrument has its own software program that controls movement of the platform or camera, depending on design, to sequentially manoeuvre each jet beneath the camera for inspection and processes the collected image for each jet. Reported diameter can be determined from either the image circumference or the total number of pixels (area) illuminated in the camera, or both, depending on the instrument. Notably, the design of the AVIS system, where the light source is located beneath the sample tray, limited the instrument to analysis of the ring gauges and the glass spots of the reticle since the image produced by chromium-on-glass 'dots' flooded the CCD light sensors. All of the instruments referenced in this study are fully automated systems with the exception of the Mondo instrument, where positioning of the nozzle under the camera lens and subsequent image processing were completed manually.

The mean jet diameter for the reference stages determined by each system was within the current USP tolerance limits. However, wider confidence intervals were observed with the fully manual Mondo system, most likely due to subjectivity imposed by the analyst when processing the resulting image for each nozzle in this fully manual system. Evaluation of the maximum differences observed in the diameter data (Table 2), show that reproducibility of site-to-site measurements was acceptable, given

the difference in local calibration procedures for the systems evaluated. The maximum difference observed was equivalent to 3 % of the nominal jet diameter for stage 2. Bias was not isolated to particular systems, given that the maximum deviation observed with stage 2 was between the AVIS and the Omis systems, whilst for stage 7 the maximum difference was observed between two AVIS instruments. Examination of the jet area data (not shown) showed that the maximum observed difference in area was related to the systems that have the most dissimilar operating systems (Mitutoyo and AVIS); this difference was investigated further in the accuracy phase of the study.

Table 2 Maximum Deviations in Jet Diameters for Reference ACI Stages 2 and 7

Stage 2 Means (mm)			
Site Number	Mean	Lower Confidence limit	Upper Confidence Limit
1	0.903637	0.903474	0.903800
7	0.923309	0.923146	0.923472
Stage 2 Difference (mm)			
Difference	Difference	Lower Confidence limit	Upper Confidence Limit
Site 1 – Site 7	-0.019673	-0.019903	-0.019442
Difference (as % of Nominal Jet Diameter)	2.16	2.19	2.14
Stage 7 Means (mm)			
Site Number	Mean	Lower Confidence limit	Upper Confidence Limit
1	0.252073	0.251822	0.252324
2	0.259549	0.259297	0.259800
Stage 7 Difference (mm)			
Difference	Difference	Lower Confidence limit	Upper Confidence Limit
Site 1 – Site 2	-0.007476	-0.007832	-0.007120
Difference (as % of Nominal Jet Diameter)	2.94	3.08	2.80

Reported diameters of each reticle element were within 3% of theoretical value across all systems, based on the accuracy results with the glass reticle (Table 3), and the Mitutoyo, AVIS and Smartscope systems reported diameters within 1% of theoretical value.

Table 3: Accuracy Data for Glass Reticle

Chromium Spot on Glass								
Spot No.	Certified value (mm)	Percent Difference versus Certified Value (%): NM= not measured						
		QV404 (5)	QV404 (6)	Omis II	Data Star	OGP Smart Scope	Omis II Repeat	
1	0.2540	99.76	99.86	99.00	99.81	99.84	98.61	
2	0.3430	99.82	99.92	99.25	99.97	99.90	98.89	
3	0.5330	99.84	99.93	99.46	100.02	99.95	99.25	
4	0.7110	99.88	99.88	99.55	100.14	99.83	99.37	
5	0.9140	99.85	99.91	99.62	100.14	99.87	99.47	
6	1.8900	99.91	99.93	99.51	99.74	99.93	99.99	
7	2.5500	99.92	99.94	99.59	99.86	99.94	100.19	
8	4.5000	99.96	NM	99.61	NM	99.86	100.25	
9	5.5000	99.99	NM	99.95	NM	99.97	100.02	
Glass Spot on Chromium								
Spot No.	Certified value (mm)	Percent Difference versus Certified Value (%): NM= not measured						
		QV404 (5)	QV404 (6)	Omis II	Data Star	AVIS	OGP Smart Scope	Omis II Repeat
1	0.2540	100.15	99.81	102.75	100.60	99.99	100.09	102.09
2	0.3430	99.92	99.88	102.02	100.63	100.04	100.08	101.48
3	0.5330	99.94	99.90	101.22	100.69	100.00	100.06	100.88
4	0.7110	99.95	99.86	100.74	100.72	100.00	100.05	100.35
5	0.9140	99.92	99.90	100.20	100.70	100.00	100.06	100.15
6	1.8900	99.98	99.97	99.90	100.54	100.00	100.10	100.34
7	2.5500	99.98	99.98	99.85	100.46	100.00	100.14	100.44
8	4.5000	100.00	NM	99.82	NM	100.00	100.08	100.68
9	5.5000	100.00	NM	100.28	NM	100.00	100.05	100.41

Analysis of Variance (2-way with replication – MS Excel 2000) of accuracy results for both instrument and day factors associated with the measurement data revealed statistical significances existed for both factors. However, this outcome was not unexpected, due to the large number of measurements made, combined with the high measurement precision (<0.05% RSD for n=10 replicates) observed for most

instruments. Also the diameter of chromium spots on a glass background ('dots') tended to be underestimated in diameter whereas glass spots on a chromium background ('holes') tended to be overestimated in diameter (Table 3). This difference is most likely due to be incorrect assignment of partially lit pixels around the image circumference by the image analysis software. The RAM Omis II system reported the largest differences between 'dots' and 'holes', most apparent as image size decreased to the smallest size measured. This finding is indicative that the image resolving power of this system is lower than that of the other commercially available systems evaluated.

Diameter measurements reported for the smallest ring gauge tended to be slightly more underestimated than the results obtained for the reticle glass spot of similar size (Table 4). This effect likely originates from the 3-dimensional profile of the gauges that creates shadows affecting edge-contrast detection. The magnitude of the error observed when evaluating this smallest gauge with all of the mensuration instruments is indicative of an increase in measurement bias associated with decreasing jet size for CI stage measurements. Given the similarity in visual appearance of the ring gauges compared to impactor jets, which both have similar (3-dimensional) structure, it is reasonable to conclude that calibrated ring gauges may provide a more realistic calibration target than 2-dimensional glass reticles.

Table 4 Summary of Measurement Accuracy Data

Calibrated Ring Gauges								
Gauge No.	Certified value (mm)	Percent Difference versus Certified Value (%): NM = not measured						
		QV404 (5)	QV404 (6)	Omis II	Data Star	AVIS	OGP Smart Scope	Omis II Repeat
1	1.0097	98.97	98.92	98.38	99.37	99.04	98.81	99.20
2	2.4995	100.04	100.11	100.11	100.42	100.02	99.98	100.43
3	4.5001	100.00	NM	100.16	NM	100.00	100.17	100.39

Conclusions

The measurements of the two ACI reference stages confirmed that all systems reported jet diameters that were within the limits defined in the US Pharmacopeia. Evaluation of the calibrated standards showed that inter-mensuration instrument variability encompassed most of the interval between the maximum and minimum diameter values obtained for each stage, and small, but consistent, differences were observed between instruments with area- and diameter-based algorithms. The accuracy study also revealed a size-related bias in one of the diameter-based mensuration systems that was attributed to the instrument's boundary recognition algorithm. The magnitude of this bias was confirmed by a replicate set of measurements made after the main study. In the worst case, the overall measurement error for the stage mensuration systems was not more than 3 % of the smallest nominal jet diameter, demonstrating that the currently available techniques are suitable for confirming CI system suitability.

References

1. United States Federal Drug Administration (FDA). 1998. Draft Guidance: Metered dose inhaler (MDI) and dry powder inhaler (DPI) drug products chemistry, manufacturing and controls documentation. United States Federal Drug Administration, Rockville, MD, USA. Docket 98D-0997.
2. European Medicines Agency (EMA). 2006. Guideline on the pharmaceutical quality of inhalation and nasal products. European Medicines Agency, London, UK: Ref: EMEA/CHMP/QWP/49313/2005 Final.
3. Mitchell, J.P. and Nagel, M.W; Cascade impactors for the size characterization of aerosols from medical inhalers; Their uses and limitation. *J. Aerosol Med.* 2003;16, 341-376.
4. Christopher D, Curry P, Doub B, et al. Considerations for the development and practice of cascade impactor testing including a mass balance failure investigation tree. *J Aerosol Med.* 2003;16:235-247.
5. European Pharmacopeia; Section 2.9.18 – Preparations for inhalation: aerodynamic assessment of fine particles. European Pharmacopeia: 5th Edn., Council of Europe, 67075 Strasbourg, France, 2005;2799-2811.
6. United States Pharmacopeia; USP 30-NF 25; Chapter 601 – Physical tests and determinations: Aerosols. United States Pharmacopeia, Rockville, MD, USA., 2007; 220-240.
7. Shelton, C. M. Comparison of Optical Systems for Mensuration of 8-Stage Cascade Impactor. In: *Drug Delivery to the Lungs-15*, The Aerosol Society, London, UK, 2004: 133-136.
8. Chambers, F., Arp, J., Asking, L. European Pharmaceutical Aerosol Group (EPAG): The role of the impactor sub-team. In: *Drug Delivery to the Lungs-18*, The Aerosol Society, Edinburgh, UK, 2007;68-70.