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RESEARCH PAPER

Measurement of Drug in Small Particles from Aqueous Nasal Sprays by Andersen Cascade Impactor

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ABSTRACT

Purpose To determine if cascade impactor (CI) measurement of drug in small particles from aqueous nasal sprays, described in FDA's 2003 draft Nasal Bioavailability/Bioequivalence Guidance, can be optimized to reduce measurement variability. To examine the influence of flow rate configurations and number of impactor stages on CI deposition and explore the importance of inlet volume.

Methods A total of eight assemblies and manual *vs.* automatic actuation were tested for deposition on the sum of all stages of the CI, and for Group 2 total drug mass per the Guidance. Mean deposition and variance about the mean were determined for each assembly.

Results The path length for a spherical 1 l inlet was too short to allow adequate aerosol formation. Data variance was reduced by a factor of two or more by using an automatic actuator relative to manual actuation. Impactor assembly modification did not improve variance over the standard assembly.

Conclusions Use of a spherical inlet (≥2 l volume) and automatic actuation are recommended for comparative measurements of drug in small particles arising from aqueous nasal sprays. The standard (8-stage) 28.3 lpm CI flow rate configuration is recommended when using the Andersen Cascade Impactor (ACI), as no other assembly showed a distinct advantage.

KEY WORDS analytical chemistry · cascade impaction · drug delivery system · nasal spray

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ABBREVIATIONS

ACI	Andersen cascade impactor
API	active pharmaceutical ingredient
CV	coefficient of variation
LC	label claim
LOQ	Limit of quantitation
lpm	liters per minute
RSD	relative standard deviation

INTRODUCTION

The Center for Drug Evaluation and Research, FDA, issued a draft guidance (1) providing recommendations for *in vitro* equivalence of locally acting aqueous nasal sprays. Recommendations include two tests relating to droplet or particle size distribution. One is Droplet Size Distribution by Laser Diffraction. The other is Drug in Small Particles/Droplets by Cascade Impactor. The first of these tests is intended to assure that test and precursor aqueous nasal sprays exhibit the same volume median diameter based on the volume-weighted droplet size distribution. The second is intended to assure that the amount of drug contained in small droplets is no greater for the test product than for the precursor product. Based on USP Apparatus 1 (2), small droplets are defined for the purposes of this study as those under 9.0 μm. Pulmonary deposition for particles with aerodynamic diameter greater than 9 μm is essentially zero upon nasal breathing (3–5). Studies with aqueous nasal sprays have not observed pulmonary deposition (6–8), and mass median droplet diameters for each of these three studies were reported to be greater than 50 μm. However, the potential for pulmonary deposition increases as particles decrease in size below an aerodynamic diameter of 9 μm. Thus, if volume median droplet or particle diameter from a nasal product were relatively low and/or the span were relatively wide, there is the possibility of a small proportion

of small particles in a test product reaching the lungs. Nasopharyngeal deposition may also be decreased at low flow rates, thus potentially increasing pulmonary deposition (9). Pulmonary deposition has been reported from a powder inhaler fitted with a nasal adaptor (10) and from a nasal nebulizer (6), demonstrating that particles may reach the lungs via nasal inhalation if the emitted dose contains droplets/particles that are sufficiently small. As noted in the April 2003 draft guidance (1), an excess of small droplets from a test aqueous nasal spray relative to a reference nasal spray might deliver excipients with possible adverse pulmonary effects to regions beyond the nose. The test is thus intended to address a potential safety concern in which the drug serves only as an *in vitro* marker of excipient delivery.

Cascade impaction devices are used to measure the aerodynamic particle size distribution of metered dose inhalation aerosols (MDIs) and inhalation powders (dry powder inhalers, DPIs). These devices fractionate an aerosolized sample on the basis of the aerodynamic size of the droplets or particles (11). The Andersen Cascade Impactor (ACI) is widely used to measure particle size in accordance with pharmacopeial or regulatory specifications. This impactor has 8 stages, labeled 0 through 7. In the standard assembly, Stage 0 is nearest the actuator, has the slowest linear flow, and collects the largest particles. Stage 7 is at the far end of the air stream, and collects the smallest sized particles. A filter after stage 7 collects all smaller particles. The size and number of the holes in each jet plate provide specific cut-points for standardized volumetric flow rates. Regulatory recommendations for CMC documentation of MDIs and DPIs include characterizing a full profile of the dose through the serial multistage impactions (12), based on single or multiple actuations. *In vitro* aerodynamic PSD studies to support bioequivalence for ANDAs of MDIs and DPIs are also based upon profiles. Specifications to assure inter-batch similarity and release of MDIs and DPIs may be based on drug in appropriate groupings of stages and/or accessories. As explained below, groupings are recommended for aqueous nasal sprays assessed comparatively by cascade impaction.

Imprecision and bias are major problems with cascade impaction measurements (13). Large variances are found in measurements taken across laboratories, personnel and instruments. This large variation makes it difficult to distinguish potential differences in small drug particles between test and reference aqueous nasal spray drug products, particularly because these products typically deliver less than 1% of their total dose in the form of particles/droplets less than 9 μm in size. Within the ACI, small particles are distributed over seven stages plus the filter, so the amount of active pharmaceutical ingredient (API) per stage may be near or below the limit of quantitation (LOQ), even with multiple actuations. In the June 1999 draft Nasal BA/BE Guidance (1), the 8 ACI stages were divided into three groups to minimize this issue. Group 1 was defined as all deposition on or above the upper stage of the ACI, Group 2 as deposition on the stage immediately below the upper stage (i.e., 5.8–9.0 μm) and Group 3 as all deposition below Group 2, including the filter. This leads to poor precision for measurement, with coefficients of variation (CVs) typically 20–40% and as high as 100% for Groups 2 and 3. Shen and coworkers sought to overcome the quantitation problem by using only three stages of the ACI (0, 7, and filter), but saw no statistically significant difference between that assembly and the full ACI for the spray weight normalized fractions of drug particles less than 9 μm (14). Another approach, outlined by Garmise and Hickey (15), extended the upper range of the ACI to 16.5 μm by operating the 90 lpm version (16) at 15 lpm. The effect of these changes to configuration and flow rate on the deposition of small particles (< 5 μm) was not studied but this technique did allow quantitation of particles above the 13.6 μm limit normally associated with the 90 lpm ACI flow configuration operated at 28.3 lpm.

The present study was initiated following the issuance of the 1999 Guidance, hence the selection in the short stack ACI of the top stage, the stage immediately following, and the last stage. However, following issuance of the April 2003 draft Nasal BA/BE Guidance, the study was revised to identify conditions that would result in more reproducible measurements of total drug mass deposited on all stages and filter below the top stage of the ACI. In this approach, Group 2 would represent the sum of Groups 2 and 3 of the earlier draft guidance. This study was designed to determine the impact of the influence of flow rate configurations and number of impactor stages on drug deposition in Group 2 as defined in the 2003 Guidance. It was hypothesized that reducing the number of stages used in the ACI would increase the amount of API per stage, thus reducing the variability and allowing more accurate measurement of differences between test and reference drug products. It is recognized that differences in the internal volume of the 8-stage *vs* the 3-stage ACI may lead to differences in deposition patterns between these assemblies. To support *in vitro* bioequivalence where only one assembly would be used for all measurements, this potential difference is not a concern. It was also expected that use of the 90 lpm configuration, due to the increased cut-point sizes when operated at a flow of 28.3 lpm, would result in a greater fraction of the drug mass in the ACI and thus in Group 2 resulting in reduced measurement variability.

MATERIALS AND METHODS

Materials

Drug Product

Beclomethasone dipropionate (Beconase AQ, GlaxoWellcome, Inc., Research Triangle Park, NC, lots 1G606, 1K605, 2A712, 2A714) was used as the test compound. All drug lots were used within their expiry dates. Beconase AQ is labeled to deliver 42 μg beclomethasone dipropionate in 100 mg suspension per actuation.

Reference Standard

Beclomethasone dipropionate (BDP) (USP 4850, Lot J).

Cascade Impactor

An Andersen 1 ACFM Mark II Non-Viable Cascade Impactor (ACI) was used. The standard assembly includes stages 0 through 7, followed by a filter that catches any remaining small particles. For the 90 LPM configuration tests, stages prior to stage 0 were added, and identified with negative numbers. However, the ACI is never used with more than 8 stages, so if stage −1 were added, stage 7 was removed, and so on. The eight assemblies employed in this study are described in Table I. Fig. 1 shows the physical arrangement for these assemblies.

The cut-point or cutoff diameter (17) (the median diameter of the droplets or particles assuming spherical geometry which impact on each plate) is shown in Table II for the standard (28.3 lpm) and 90 lpm configurations, each operated at 28.3 lpm.

For every assembly, the extent of loss of API to the ACI walls was assessed. In all cases, the wall loss was >5% of the total amount delivered into the ACI, thus wall loss was included (see USP <601>).

Table I Assemblies Examined (Flow=28.3 lpm for all)

Assembly No.	Code	Stages (+ Filter)	Inlet (liters)
A[a]	8S 28.3-2L	0 → 7	2
B[a]	8S 28.3-5L	0 → 7	5
C[b]	8S 90/28.3-2L	−2 → 5	2
D[b]	8S 90/28.3-5L	−2 → 5	5
E[c]	3S 28.3-2L	0, 1,7	2
F[c]	3S 28.3-5L	0, 1,7	5
G[d]	3S 90/28.3-2L	−2, −1, 5	2
H[d]	3S 90/28.3-5L	−2, −1, 5	5

[a] 8-stage, 28.3 lpm configuration

[b] 8-stage, 90 lpm configuration

[c] 3-stage, 28.3 lpm configuration

[d] 3-stage, 90 lpm configuration

Inlets

Single-neck, glass round-bottomed flasks of two sizes were used as inlets to the ACI (see additional detail in the Methods Section).

Actuation

For most data reported in this study, an automatic actuator (NSx, Proveris Scientific) was used (see additional detail in the Methods Section).

Methods

For all assemblies, the airflow was maintained at 28.3 (±4%) liters per minute (lpm) through the impactor, and 10 actuations were made per run to raise the level of API on each stage above the LOQ. Across all assemblies, most results are based on 10 replicate runs but, in a few cases, as few as six runs were made.

The original protocol included the use of a 1 l inlet. When using the 1 l flask as the inlet, it was necessary for each of the 10 sprays to be targeted at a slightly different point on the wall of the flask to minimize the amount of suspension that ran into the preseparator. When a 2 l or larger inlet was employed, a substantial aerosol cloud was observed. The cross-chamber distance was 13 cm for the 1 l flask *vs.* 17 cm for the 2 l flask. The 2 l flask used for these experiments is shown in Fig. 2 where the arrow indicates the entry port.

The cascade impactor consists of a series of jet plates; close below each jet plate is the associated collection or "impaction" plate. The combination of jet plate and impaction plate is referred to as an impaction stage. To determine wall losses (i.e., the amount of drug found on the jet plates) for each assembly, ten actuations were made consecutively, then the impactor was disassembled and all impaction plates were rinsed into a common vessel. Jet plates were treated similarly. Analysis for API in the wash solutions by HPLC showed that more than 5% of the sample was lost to the walls. For the study, rinsing of each jet plate and associated impaction plate together was performed per USP guidelines (2). To confirm mass balance, the amount of API deposition on the sampling chamber and all accessories was determined, in addition to each individual stage and the filter.

Preliminary runs using Assembly **B** (8 Stages, 0–7, operated at 28.3 lpm with a 5 l inlet) were made without using the preseparator. Although there was no effect on the amount of drug entering the ACI, there was a slight increase in wall loss and decrease in precision (relative to runs made with the same assembly but with the preseparator). For this

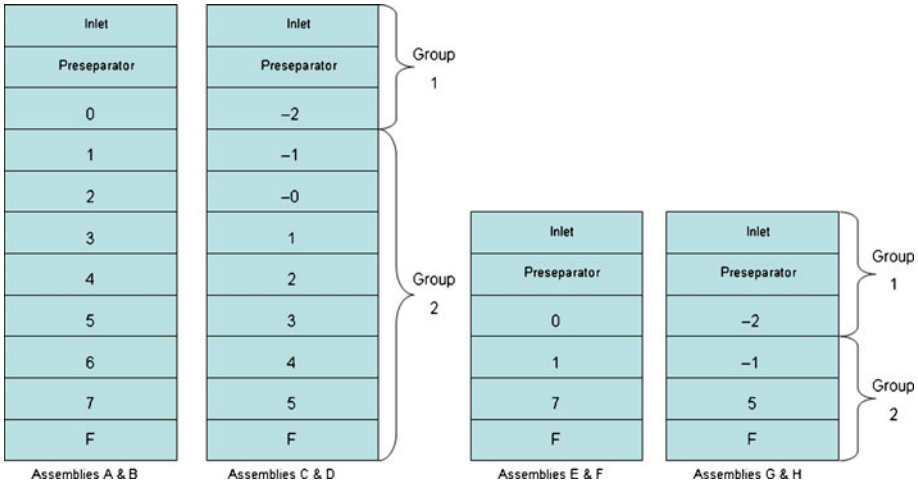

Fig. 1 Stage groupings for the eight assemblies studied.

reason, the preseparator was used for all experimental runs reported here.

Assembly **A** (8 Stages, 0–7, operated at 28.3 lpm with a 2 l inlet) was evaluated using two analysts (n_1=6 runs, n_2=10 runs) making manual actuations with the observed result that the shot weight (determined by weighing the device before and after actuation), the total drug amount in the ACI and the total recovered weight (total drug amount in the ACI plus inlet and preseparator) were significantly different (95% confidence limit) between analysts. Because of this difference, the NSx automatic actuator was used for all subsequent actuations (again making use of two analysts). The results for both analysts, as well as the NSx, are compared in Table III.

Analysis for BDP was performed using a published HPLC method (18). A Symmetry ODS 5 µm, 250 mm×4.6 mm column (Waters Corp., Milford, MA) was used, with a mobile phase of acetonitrile/water (60:40) and a flow rate of 1.8 ml/min. Column temperature was maintained at 20°C and a detection wavelength of 241 nm was used. An injection volume of 50 µl was used, with an expected retention time of 7.7–8.7 min. The method was linear (R^2=1.00) from 0.01 to 20.0 µg/ml with a limit of quantitation of approximately 0.01 µg/ml (RSD=~10) and limit of detection of approximately 0.001 µg/ml. For statistical treatment of the data from these studies, chromatographic peaks that were observed visually but were sufficiently small that the chromatographic software was unable to reliably integrate them were treated as missing data rather than as zero since the goal was to describe the amounts collected on the stages as a function of the ACI assembly.

For each assembly, the percentage of total mass as "small" particles ("SPM%") (i.e., Group 2, the mass of particles

Table II Cut-Points (µm) for Andersen Non-viable 8-Stage Impactor Operated at 28.31 lpm

Standard (28.3 lpm) Configuration		90 lpm Configuration	
Stage	Cut-point	Stage	Cut-point
0	9.0	−2	13.6
1	5.8	−1	11.8
2	4.7	0	9.2
3	3.3	1	6.1
4	2.1	2	5.1
5	1.1	3	3.1
6	0.7	4	1.9
7	0.4	5	0.98

Fig. 2 8-Stage ACI with preseparator and 2 l inlet.

accumulated below the top stage of the ACI) and percentage of total mass in the cascade impactor (i.e., SPM% plus mass on the top stage; identified as "ACI mass%") were examined. Each assembly was the combination of three variables, flask size, flow rate configuration, and number of stages. Flask had two levels: 2 l and 5 l; flow rate configuration had two levels: 28.3 lpm and 90 lpm; number of stages had two levels: 3 and 8.

Specifically, for the response variables, percentage of small particles and percentage of total mass in the impactor, the following questions were of interest:

- Was there a statistically significant difference between the 28.3 lpm configurations (A, B, E and F) and the 90 lpm configurations (C, D, G and H)?
- For the 28.3 lpm configurations, was there a statistically significant difference between the 8-stage assemblies (A and B) and the 3-stage assemblies (E and F)?
- For the 90 lpm configurations, was there a statistically significant difference between the 8-stage assemblies (C and D) and the 3-stage assemblies (G and H)?
- Without regard to flow configuration (28.3 lpm *vs.* 90 lpm), was there a statistically significant difference between the 8-stage assemblies (A, B, C and D) and the 3-stage assemblies (E, F, G and H)?
- Was there a statistically significant difference between the 2 l size of the flask (A, C, E and G) and the 5 l size of the flask (B, D, F and H)?
- Did any assembly provide a statistically significant improvement in variability of the data?

An Excel spreadsheet was employed to facilitate calculations using the log-probit model (19).

Statistical Methods

Statistical analyses were carried out using linear models. The response variable (Y) was SPM% or ACI mass%. The independent fixed factors were flask, flow rate configuration, and stages. Interactions between factors are denoted as flow rate*flask for flow rate configuration and flask, and so on.

Table III Comparison of Analyst Actuation

	Analyst 1		Analyst 2		Auto-actuator	
Part or Stage	Mean	RSD	Mean	RSD	Mean	RSD
	(*n*=6)		(*n*=10)		(*n*=7)	
Inlet	442.21	2.6%	413.76	2.3%	428.29	1.1%
PreSeparator	0.77	20.0%	1.47	12.6%	1.27	45.7%
0	0.35	17.0%	0.71	11.7%	0.41	23.8%
1	0.81	16.3%	1.74	7.7%	1.01	16.1%
2	0.54	16.2%	0.94	11.8%	0.55	11.3%
3	0.49	20.7%	0.90	17.0%	0.47	12.5%
4	0.16	28.4%	0.27	33.9%	0.12	23.0%
5	0		0.01	316.2%	0.01	0.0%
6	0		0.03	167.8%	0	
7	0		0.02	316.2%	0	
Filter	0		0.20	27.2%	0.41	47.1%
Avg. Shot wt. (mg)	105.36	2.0%	99.72	2.5%	104.66	0.7%
Total Recovered	445.33	2.5%	420.04	2.3%	432.54	1.0%
%Label Claim[a]	100.6		100.3		98.4	
Total ACI[b]	2.35	17.3%	4.82	6.6%	2.98	7.2%
ACI mass%[c]	0.5%	19.7%	1.1%	7.7%	0.7%	7.4%
Group 1 total mass[d]	443.33	2.6%	415.93	2.1%	429.96	1.0%
Group 2 total mass	2.01	19.5%	4.11	6.5%	2.57	8.4%
Group 2 as percent of total (SPM%):	0.45%	22.0%	0.98%	11.6%	0.60%	8.68%

[a] Corrected for shot weight

[b] Stages 0 through filter

[c] Total in ACI as percent of total recovered

[d] Sum of inlet, preseparator and stage 0

The linear model analyzed was

$$y_{ijkl} = \mu + X\beta_0 + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma_k + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk} + \varepsilon_{ijkl} \begin{cases} i,j,k = 1,2 \\ l = 1,2,\ldots,m_{ijk} \end{cases}$$

where y_{ijkl} was the observation at the i^{th} flask, j^{th} flow rate, k^{th} stage, and l^{th} replicate, μ was the grand mean, X was total recovered amount, β_0 was the change of y_{ijkl} per unit of X after adjusting the other factors, α_i was the effect of i^{th} flask, β_j was the effect of j^{th} flow rate, $(\alpha\beta)_{ij}$ was the effect of the interaction between i^{th} flask and j^{th} flow rate. The γ_k was the effect of k^{th} stage, and ε_{ijkl} was the unknown random error at the i^{th} flask, j^{th} flow rate, k^{th} stage, and l^{th} replicate.

After the data were fitted to the model, the statistically important factors and interactions were identified. The interaction term was removed from the model when it was not significant at the 0.05 level. One contrast (a comparison involving two or more factor level means) was constructed to compare the difference between the 28.3 lpm configurations (A, B, E and F) and the 90 lpm configurations (C, D, G and H). One contrast was constructed to compare the difference between the 8-stage assemblies (A and B) and the 3-stage assemblies (E and F) for the 28.3 lpm configurations. One contrast was constructed to compare the difference between the 8-stage assemblies (C and D) and the 3-stage assemblies (G and H) for the standard (90 lpm) configurations. One contrast was constructed to compare the difference between 2 l (A, C, E, and G) and 5 l (B, D, F and H).

To study the effects of the same independent factors (flask, flow rate configuration, and number of stages) on the variability of CI results, the linear model was again applied but with the standard deviation (s) of SPM% or s of ACI mass% as the dependent variable (Y).

RESULTS AND DISCUSSION

A number of variables can influence the mean and variance in deposition of drug on the individual cascade impactor stages and in shot weight and total recovered. These variables are related to actuation technique, and include actuation force, insertion depth into the flask, and actuator nozzle angle relative to the entry port of the flask. This variation can be minimized by using an automatic actuator. With respect to Group 2 total mass and SPM%, Table III shows the automatic actuator yielded approximately equal or better precision than obtained by either analyst when manual actuation was employed.

Based on RSD values (Table III), use of the automatic actuator provided more than a 2½-fold improvement in precision for both shot weight and total recovered. Precision of total mass in the ACI was approximately as good or better than with manual actuation. Even for data only partially dependent on actuation technique (e.g., amount in group 1), more than a 2-fold improvement in precision was realized when the automatic actuator was used.

When a 1 l flask was used as inlet, the error (RSD) in the amount found in Group 2 was very high (72% inter-run variability, n=3). This may be partially explained by the necessity of moving the nasal device between actuations (see Methods Section). For this reason, only the 2 l and 5 l flasks were used in the present study. Mean data for the eight assemblies are shown in Table IV and summarized below. As was observed in a preliminary study, the use of the three stage, 90 lpm configuration (stages −2, −1 and 5) yielded

Table IV Summary of Cascade Impaction Data: Automatic Actuator

Assembly No.			Percent mass in ACI (ACI mass%)		Percent mass in Group 2 (SPM%)		Shot weight (g)		Percent label claim[a]		
	Stages + Filter	Flask	Mean	RSD	Mean	RSD	Mean	RSD	Mean	RSD	N[b]
A	0 → 7	2	0.69	7.4%	0.60	8.7%	104.7	0.7%	98.4	0.6%	7
B	0 → 7	5	0.71	22.9%	0.60	22.4%	106.2	0.5%%	99.6	0.8%	10
C	−2 → 5	2	0.69	33.9%	0.67	35.6%	106.3	3.3%	95.6	3.8%	10
D	−2 → 5	5	0.82	15.2%	0.79	14.3%	104.5	0.2%	99.6	0.4%	10
E	0, 1, 7	2	0.49	38.9%	0.42	34.7%	104.8	1.9%	97.0	2.3%	10
F	0, 1, 7	5	0.70	35.0%	0.57	32.7%	105.9	3.3%	100.9	0.9%	6
G	−2, −1, 5	2	0.31	13.2%	0.30	11.8%	104.0	0.8%	96.8	2.0%	9
H	−2, −1, 5	5	0.37	9.8%	0.33	10.6%	104.2	0.2%	98.8	0.1%	7

[a] Percent label claim values have been corrected for shot weight

[b] Number of replicate CI runs

Table V Contrasts Examined

Contrast	Assemblies	*P*-value SPM%	*P*-value ACI mass%
28.3 lpm *vs* 90 lpm configurations	ABEF *vs* CDGH	0.4551	0.2424
28.3 lpm configuration 8-stage *vs* 3-stage	AB *vs* EF	0.0361*	0.0621
90 lpm configuration 8-stage *vs* 3-stage	CD *vs* GH	<0.0001*	<0.0001*
8-stage *vs* 3-stage[a]	ABCD *vs* EFGH	<0.0001*	<0.0001*
2 l *vs* 5 l flask	ACEG *vs* BDFH	0.4488	0.3248

A Statistically Significant Difference ($p < 0.05$) is Indicated by an Asterisk

[a]Without differentiating between 28.3 lpm and 90 lpm configurations

good precision, but not better than that obtained using the standard eight stage, 28.3 lpm configuration (20).

Table IV summarizes the results obtained for the eight assemblies, which reflect two flow rate configurations (28.3 lpm *vs* 90 lpm), two stage number assemblies (8 stage *vs* 3 stage) and two inlets (2 L *vs* 5 L). Results of various statistical contrasts made among these eight variants are shown in Table V, which summarizes the statistical contrasts made between the various assemblies for both metrics (SPM% and ACI mass%). For the factors studied which describe the eight assemblies (inlet, number of stages, and flow rate configuration), neither flow rate configuration (28.3 lpm *vs* 90 lpm) nor inlet (2 l *vs* 5 l) showed statistically significant effects for either metric. However, the values that were observed for both metrics were strongly dependent on the number of stages.

Although speculative, had we conducted our study at a controlled high humidity, decreased measurement variability and increased Group 2 total mass (SPM%), both of which would have been desirable study outcomes, may have occurred (21).

In Table V, regardless of flow rate configuration or inlet, deposition was generally significantly different for assemblies having three stages when compared to those with eight stages, with higher deposition for eight stages (e.g., assembly D *vs* H). This was true for both SPM% and mass ACI%. The higher deposition was accompanied by larger variance, but only for the 90 lpm configurations. For the 28.3 lpm configurations, an increase in variance was observed when going from eight stages to three (e.g., assembly A *vs* E).

A review of Table IV shows that the greatest deposition (ACI mass%) occurred for Assembly D but with more than twice the variance of Assembly A. Assembly H also shows low variance for ACI mass%, but deposition is 55% lower than that observed for Assembly D. Similar trends were observed for percent of total mass as "small" particles (SPM%).

ANALYSIS OF VARIABILITY

Analysis of the model with standard deviation as the dependent variable did not show any statistically significant differences ($p < 0.05$) between the eight assemblies (Table VI).

CONCLUSIONS

The first step was to determine proper inlet size. Use of a 1 l inlet provided a path length that was too short to allow adequate aerosol formation. We therefore recommend a spherical inlet of at least 2 l in volume.

For most summary data, the relative standard deviation was typically reduced by a factor of two or more when an automatic actuator was used compared to that observed for manual actuation. That this relationship was not observed for all measured parameters in all test cases indicates that there are factors in addition to the actuation of the nasal pump which affected results obtained using the ACI (22). Although the use of two analysts undoubtedly led to increased overall variability, for the majority of metrics evaluated here, use of an automatic actuator resulted in lower variability relative to manual actuation, and thus its use is recommended.

Differences in internal volume represented a possible source of variance in the ACI assemblies (23). These differences may have produced different aerosol dynamics leading to variation in deposition patterns, but such effects would be expected to be small relative to differences in the device pumps themselves (24). It is well known that stages preceding any stage of particular interest affect its collection efficiency (25) and thus help explain deposition differences observed between CI assemblies. In addition, although specifically addressing use of CI to assess nebulizer performance, evaporative effects of aqueous solutions and suspensions are known (26). These evaporative effects may

Table VI Tests of Effects for Analysis of Standard Deviation

Source	SPM% *F*-value	SPM% *P*-value	ACI mass% *F*-value	ACI mass% *P*-value
flask	0.0045	0.9506	0.0870	0.7873
flow rate configuration	0.4294	0.5591	1.3044	0.3363
number stages	0.4421	0.5537	0.0828	0.7922
configuration * # stages	6.2803	0.0872	7.3471	0.0731

= number (i.e., number of stages)

*means this is the interaction term for configuration and number of stages

be greater with a larger ACI internal volume, and this may have been a factor as well.

Based on our study results, the 8-stage assemblies showed deposition of a significantly greater percentage of total mass, both total within the ACI (ACI mass%) and as "small" particles (SPM%). Although, of the four 8-stage assemblies, the 28.3 lpm configuration with a 2 l inlet tended to exhibit the lowest percentage deposition, its use also produced the best precision (Table IV), but no contrast offered a statistically significant advantage with respect to standard deviation (Table VI). Thus, our overall recommendation for the ACI is to use the standard 8-stage, 28.3 l per minute configuration with a 2 l inlet and automatic actuation (Assembly A in Table I).

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The findings and conclusions in this article have not been formally disseminated by the Food and Drug Administration and should not be construed to represent any Agency determination or policy.

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