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Research Article

Optimized Inhalation Aerosols. II. Inertial Testing Methods for Particle Size Analysis of Pressurized Inhalers

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Pressurized metered dose inhaler (MDI) output from three different albuterol formulations was characterized using three inertial separation devices. Results were compared for the Delron six-stage cascade impactor (DCI6), the Andersen Mark II eight-stage impactor (ACI8), and Copley's twin-stage liquid impinger (LI). None of the devices tested in this study was ideal in all respects. All devices could differentiate between formulations in terms of respirable doses (albuterol amount with aerodynamic diameters <5.5 through 6.4 μm). Only the high-flow rate LI could differentiate among all three formulations when data were presented in terms of respirable percentage (RP) of drug collected. Values for RP were in excellent agreement for the independently calibrated impactors when the same evaporation chamber was used atop the impactors. The LI appeared to overestimate values for RP *in vivo*. Results are discussed in light of the debate surrounding the revision of USP aerosol testing requirements. Rigorous specifications for evaporation chambers and methodologies are necessary for meaningful inter- and intra-laboratory comparison of results when any of these devices are used.

KEY WORDS: aerosols; metered dose inhaler; inertial testing; particle size analysis; inhalation; pressurized inhaler.

INTRODUCTION

An increase in the usage of pressurized MDIs and the development of generic formulations has prompted the USP Committee of Revision to reexamine the testing requirements for aerosols. One of the mostly hotly debated topics is that of particle size measurement. Many devices have been employed to determine particle size distribution of drug escaping from MDIs. Some are designed to collect and then physically examine the particles (1–5), while others are intended to observe and size the contents of the plume without disturbing its contents (6,7). Because aerosolized drug particles have potential to impact and sediment at various points on their journey to the site of action in the lung, it is thought that particle size determination based on mass and inertia is most appropriate (8). The cascade impactor (CI) (9) sizes aerosolized particles based on their inertia and has been found to give the most representative *in vitro* measurement of particle size resembling *in vivo* conditions (10). It also has the potential to size the whole aerosol output. In the FDA's guidance (11) for comparative testing, the cascade impactor is mentioned, however, no specific impactor or methodology is cited. Many CIs are available commercially but these are not marketed as closed systems, even though scientists prefer to use them as such. To close the system a chamber is placed atop the impactor connecting it to the outlet of the actuator. Obviously, if the size or shape of this chamber is changed, then different particle size distributions would be expected. Another sizing technique based on liquid impingement, a technique similar to impaction in theory, has become one of the accepted European Compendial methods for determining the deposition of emitted dose (12). It has been recommended (13) for inclusion within the USP General Chapter, Aerosols ⟨601⟩, along with a requirement for a Deposition of Emitted Dose test similar to that employed in the BP (12).

Particle size measurement is also important for optimization of suspension formulations. To do this different formulations must be compared and the testing method should be accurate and reliable. The purpose of this study was to determine whether cascade impaction or liquid impingement is better able to detect differences between formulations, in both a comparative and an absolute sense. We have compared the aerosol output of two model albuterol formulations (albuterol A and B) versus the marketed product, Ventolin (albuterol C). One of the model formulations (albuterol A) was subjected to accelerated stability testing at elevated temperature and relative humidity. MDIs were actuated into an identical evaporation chamber to compare the results for particle size distribution and drug output as determined by a six-stage Delron and eight-stage Andersen Mark II CI. In addition to comparing the twin-stage LI to the CIs, this study was undertaken to determine the collection efficiency and dose determination of the LI relative to the USP sampling apparatus (14).

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MATERIALS AND METHODS

Preparation of Aerosol Units

A series of suspension aerosols was prepared using micronized albuterol suspended in a 70:30 blend by weight of propellant 12 (P-12) and P-11 (Dupont, Wilmington, DE). Oleic acid (Fluka AG, Ronkonkoma, NY) was employed as suspending agent using a technique and conditions similar to that described previously (4). Briefly, micronized albuterol was levigated to a smooth, uniform paste with oleic acid. Aliquots of the resulting slurries were transferred to 20-ml containers and fitted with 65 μl/actuation inverted metered dose valves. Propellant was added through the valve. Valve crimping and propellant filling were performed using small scale aerosol pressure packaging equipment (Pamasol, Pfaffikon, Switzerland). Each assembled aerosol unit was mixed for 1 hr using a wrist action shaker.

Five of these model formulations (albuterol A) were held for 13 weeks at 40°C and 65% relative humidity (RH). Five other containers (albuterol B) were stored for the same duration under ambient conditions. A further five containers of the Innovator albuterol product (albuterol C; Ventolin, Glaxo Lot Z5678DA, stored under ambient conditions) were tested for purposes of comparison.

Cascade Impaction

Each of the 15 canisters (fitted with identical actuators) was alternately shaken and fired five times to prime the metering valves. The outlet of the actuator was then fitted into the aerosol inlet port of an evaporation chamber (which serves to enable both droplet evaporation and preselection of large propellant droplets) located atop a calibrated Delron DCI-6 cascade impactor (Delron Research Model DCI-6, Powell, Ohio) through which air was drawn at 12.45 liters/min. The connection between the actuator and the inlet of the evaporation chamber was made airtight by an adapter (Tygon tubing). The adapter, actuator, evaporation chamber, and first stage of the cascade impactor are described with their critical dimensions in Fig. 1. The aerosol was discharged manually 10 times, with 2–3 sec between actuations (as was the case with all three sampling devices). The actuator, evaporation chamber, each slide, and the terminal filter of the impactor were washed with a known volume of 0.1 *M* sodium hydroxide (NaOH) solution. The washings were immediately analyzed spectrophotometrically (Varian DMS 100S, Varian Instrument Division, Palo Alto, CA) at 243 nm for albuterol. Calibration curves were rectilinear and unaffected by the presence of the surfactant. The aerodynamic particle diameters, which are collected with 50% efficiency at each stage of the DCI6, are 11.2, 5.5, 3.3, 2.0, 1.1, and 0.5 μm at stages 1 through 6, respectively (15). An absolute glass-fiber filter was placed below stage six to collect particles less than 0.5 μm.

The above procedure was repeated using the Andersen 1ACFM Nonviable Ambient Particle Sizing Sampler (Mark II, Andersen Samplers, Inc., Atlanta, GA). The Andersen consists of a preseparator, eight stages, and an absolute filter. Each stage has a number of orifices (96 to 400) of different diameters arranged in a radial pattern on each stage. Beneath the series of air inlet orifices, at each stage of the sampler, are 8.255-cm stainless-steel plates which collect droplets or particles. The aerodynamic sizes, which are collected with 50% efficiency at each stage of the sampler, are 9.0, 5.8, 4.7, 3.3, 2.1, 1.1, 0.7, and 0.4 μm at stages 0 through 7, respectively (16). The preseparator was employed to accord with normal practice for the ACI8 prior to entry of the aerosol into the sampler to remove particles greater than 10 μm in diameter. An identical adapter and evaporation chamber to that shown in Fig. 1 was located atop the ACI8 and its preseparator. Samples were drawn through the impactor at a volumetric flow rate of 28.3 liters/min.

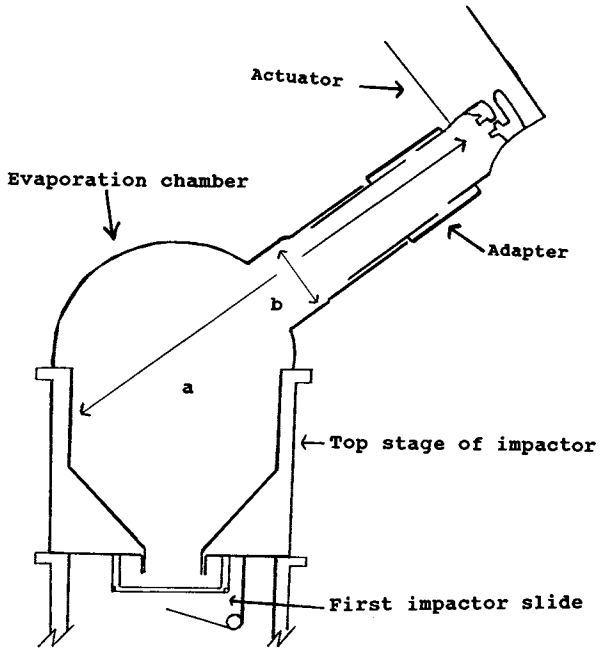

Fig. 1. Scale diagram of the actuator, adapter, evaporation chamber, and first stage of the DCI6. The volume of the evaporation chamber was 250 ml and its diameter was 8.4 cm. The distance between the actuator nozzle and the opposite inner wall of stage 1 (dimension a) was 21.5 cm, while the diameter of the evaporation chamber inlet (dimension b) was 2.5 cm. The evaporation chamber, originally described in Ref. 4, was not intended to mimic *in vivo* oropharyngeal dimensions.

Liquid Impingement

MDI output was also sized using the LI (Copley Instruments Ltd., Nottingham, England). Five milliliters of 0.1 *M* NaOH was placed in the upper stage of the impinger and 20 ml in the seconed stage to act as collection fluid (1). Air was drawn through the impinger at a flow rate of 60 liters/min for 5 sec before inserting the actuator into the throat inlet and actuating 10 times with 2- to 3-sec intervals. The glassware was then disassembled and washed with known volumes of 0.1 *M* NaOH to collect drug deposited in the throat, first stage, and lower impinger (second stage) prior to spectrophotometric assay for albuterol at 243 nm.

Each canister was tested once on each of the sampling devices. For each canister the amount of drug in the actuator, in the nonrespirable size and respirable size range, was determined. Generally, particles less than 5 μm are believed to be capable of penetrating to the pulmonary regions

(17,18). The terms "nonrespirable" and "respirable size range" used here are based on the aerodynamic diameter of the drug aerosol being greater or less than 5.5 through 6.4 μm. These terms are used to simplify the presentation of the results. Table I summarizes the respirable cutoff diameters for the three sampling devices along with the sampling flow rates for each.

RESULTS AND DISCUSSION

The ratio of drug to surfactant in Ventolin (albuterol C) was unknown to us. Examination of the particulate output from each formulation by optical microscopy after collection using the USP method (19) revealed little visual difference between albuterol B and albuterol C, however, the particulate output from albuterol A showed pronounced crystal growth, suggesting that this formulation was physically degraded by 13 weeks storage at elevated temperature and humidity (20). These MDI formulations were employed to assess the differences between two well-known CIs and the twin-stage LI.

Table II shows the respirable doses, RDs, as amounts of drug deposited with aerodynamic diameters <5.5, 5.8, or 6.4 μm (Table I) following actuation and collection of each formulation in the three devices. Table III shows the results alongside those for actuator retention and nonrespirable aerosol output (aerosol with aerodynamic diameters >5.5, 5.8, or 6.4 μm) in terms of percentage of the total recovered from each apparatus, actuator, evaporation chamber, and adapter. In all of these experiments the adapter formed an airtight seal between the mouthpiece of the MDI and the inlet port of the sampling device. Analysis of variance (21) demonstrated no significant difference, at the 5% level of significance between the mean RPs for formulations A, B, and C in either the DCI6 or the ACI8. However, Table III and Fig. 2 indicate that Ventolin (albuterol C) had a much higher RP than the other two formulations when liquid impingement was employed. The apparent inability of the impactors to discriminate among formulations A, B, and C when data are presented in percentage terms (Table III) is almost certainly due to a combination of factors. Among these are the dissimilar geometries of the impinger and the CIs, differences in the collection efficiencies of the devices, the various flow rates employed, and the different cutoff diameters. The differences between formulations when data were presented in terms of the RD (Table II) showed that all three devices were capable of discriminating between some of the formulations in terms of the mean amounts of drug in the "respirable" size range (e.g., A vs C, null hypothesis rejected, $P < 0.05$). However, only the LI could detect a difference ($P < 0.05$) between the mean RD of albuterol B and that of albuterol C.

The ability of the CIs to discriminate between RDs but not RPs could be due to a difference in the total amount of drug collected (actuator plus sampling device) from each of the formulations. Albuterol A showed a marked decrease in total drug (62.91 μg/actuation), but there was no significant difference between total amounts collected for albuterols B and C when actuated into either the DCI6 or the ACI8.

In comparative testing, the choice between these different particle sizing devices having similar aerodynamic selectivity is not critical provided the data are presented in an appropriate fashion (Table II rather than Table III). From a different point of view, we may question which of these devices, if any, is capable of providing *in vitro* values for RD and RP that in some way indicate *in vivo* lung deposition. While the design of the chamber into which the MDI is actuated will probably define that fraction of the aerosol plume which is prevented from entering any of these devices, calibration of the instrument is also important. The cutoff diameters of the six-statge Delron were assigned by indepen-

Table I. Operating Characteristics of the Three Sizing Devices

	Aerodynamic cutoff diameter corresponding to respirable fraction (μm)[a]	Flow rate (L/min)
DCI6[b]	5.5[e]	12.45
ACI8[c]	5.8[f]	28.30
LI[d]	6.4[g]	60.00

[a] The aerodynamic below which particles are considered to be in the respirable size range.
[b] Delron six-stage CI.
[c] Andersen Mark II eight-stage CI.
[d] Twin-stage LI (1).
[e] By independent calibration (15).
[f] Taken from Ref. 16.
[g] Taken from Table 1 of Ref. 1.

Table II. Comparison of Respirable Doses, RDs (μg per Actuation), Collected for the Three Sizing Devices

	6-stage CI	8-stage CI[b]	LI
Albuterol A	10.8 (1.4)[a]	11.6 (1.1)	19.0 (3.3)
Albuterol B	17.0 (1.3)	19.5 (5.6)	27.5 (1.7)
Albuterol C	18.9 (1.5)	25.8 (5.4)	42.4 (3.8)

[a] Values in parentheses are standard deviations ($n = 5$).
[b] RP (Table III) × total recovered in (DCI6 + actuator + evaporation chamber).

Table III. Comparison of Percentage of Drug Collected for the Three Sizing Devices

	6-stage CI	8-stage CI	LI
Actuator			
Albuterol A	17.96 (1.69)[a]	13.34 (0.85)	14.88 (1.04)
Albuterol B	16.15 (0.58)	13.01 (0.89)	13.84 (1.15)
Albuterol C	17.18 (1.24)	13.28 (2.99)	13.14 (1.29)
Nonrespirable			
Albuterol A	64.59 (3.00)	68.17 (3.19)	62.79 (2.90)
Albuterol B	66.33 (1.49)	66.96 (5.46)	60.07 (1.12)
Albuterol C	64.53 (0.52)	61.71 (4.09)	48.75 (1.88)
Respirable[b]			
Albuterol A	17.45 (2.29)	18.50 (1.64)	22.33 (2.34)
Albuterol B	17.52 (1.31)	20.04 (5.72)	26.09 (1.15)
Albuterol C	18.29 (1.47)	25.01 (4.09)	38.11 (2.44)

[a] Standard deviations in parentheses ($n = 5$).
[b] Respirable percentage, RP.

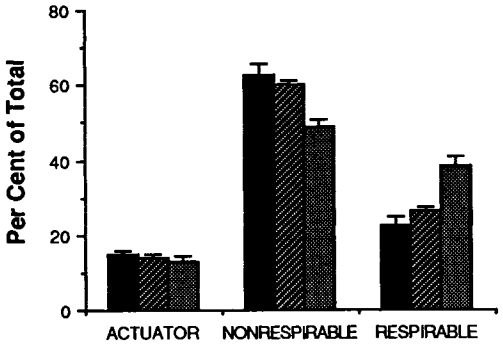

Fig. 2. Percentage drug deposited in the actuator, and as nonrespirable and respirable aerosol, after determination by liquid impingement for (■) albuterol A, (▨) albuterol B, and (▩) albuterol C. Error bars are standard deviations.

Table IV. Comparison of Amount of Drug Leaving the Actuator for Albuterol C[a]

Sampling device	Amount of drug leaving actuator (μg per actuation)	CV (%)[b]
USP sampling apparatus	95.03	5.59
6-stage impactor[c] with adaptor (airtight seal)	90.81	5.43
6-stage impactor without adaptor (not connected airtight)	70.26	6.59
Twin-stage liquid impinger	96.60	3.75

[a] These results were collected on a separate batch of albuterol C.
[b] Coefficient of variation.
[c] DCI6 amounts collected on slides alone. Wall losses were not included in theses determinations (see Materials and Methods).

dent calibration (values for stages 1 through are 11.2, 5.5, 3.3, 2.0, 1.1, and 0.5, respectively; 15) and differ markedly, especially in the first stages, from those quoted by the manufacturer (values quoted for stages 1 through 6 are 16.0, 8.0, 4.0, 2.0, 1.0, and 0.5, respectively). On the other hand, the cutoff diameters quoted by the manufacturer for the Andersen Mark II are in agreement with two independent studies (22,23). To the best of our knowledge, independent calibration data for the marketed LI is unpublished. Also, both CIs demonstrate steeper collection efficiency curves than that which was reported for the original LI (1,15,22).

From the obvious physical differences between these devices (Table I), the much larger values for respirable drug determined in the LI may be due either to the higher flow rate or to an incorrectly assigned aerodynamic cutoff diameter of 6.4 μm (1). Because data from all of these devices are treated as if the cutoff diameters are absolute, the rather broad collection efficiency curve which is published for the LI (1) may also permit assignment of a greater proportion of the aerosol to the respirable component. However, the high flow rate should reduce the opportunity for propellant droplet evaporation and thus decrease RP rather than increase it (Table III). Furthermore, the amount of albuterol as aerosol in the 5.5- through 6.4-μm size range (range of cutoff diameters between devices, Table I) appeared, from our impactor experiments, to be far too small to make up the discrepancy between these devices. Based on an assumption of log-normality, the mass difference between 5.5 and 6.4 μm for albuterol C was approximately 0.5 μg/actuation. However, another major difference between these devices concerns the issue of wall losses. Because all portions of the apparatus must be washed and assayed for drug, these do not exist in the LI. The wall losses associated with the Delron can be estimated from Table IV. The amount of albuterol C leaving the actuator and collected by the six-stage CI with adapter was greater than 95% of that collected by the USP sampling apparatus which operates at an identical flow rate. Thus the DCI6 showed <5% wall losses for albuterol C. In the Andersen, Mitchell *et al.* (22) determined the total wall losses for monodisperse 7.4 and 1.5-μm methylene blue particles to be 10.2 and 5.8% of the mass collected by the impactor, respectively. They also observed that wall losses for small particles were spread through the impactor, whereas most of the wall deposition for the larger particles occurred in Stage 0. Wall losses affect experimental data in two ways: first, not all the aerosol output from the actuator is determined; and second, because losses often occur preferentially for larger particles, the measured size distributions may be biased toward smaller particles. In the present study, preferential loss of large particles would overestimate the percentage of total output made up by small particles. This may explain the higher value for RP for albuterol C ($P < 0.05$) obtained by this CI compared with the DCI6. However, it is not likely that <5% wall losses can be used to explain the differences in RP for albuterol C between the DCI6 and the LI.

To our knowledge, one point which has not been discussed in the literature concerns the intradevice variation to be expected when sizing the output of the same MDI with replicate devices. The closeness of the results for the two CIs reported here supports the assumption that machined metal impactors can be calibrated and then reproduced with a high degree of precision. This may or may not be the case with the largely glass LI. If the cutoff diameter for our impinger were greater than 6.4 μm, this would explain the larger RPs obtained for all three formulations. This may also explain the discrepancies between our results for Ventolin when compared to those of Hallworth and Westmoreland (1) also testing Ventolin. Our value for percentage deposition in stage 2 (RP Table III) is considerably lower than theirs (compare 38.1 to 56.6%), as is the value of 44.7% obtained by Zainudin *et al.* (24). In addition to determining the percentage deposition in the second stage of the LI, these researchers determined percentage deposition in the lung by mixing technetium-labeled Teflon particles with albuterol in a MDI formulation. After inhalation, six subjects, five of whom were asthmatics, were seated in front of a gamma camera and radioactivity in the lung was measured. Interestingly, the mean percentage deposition in the lung was 12.7%, a value considerably less than that determined by liquid impingement. Indeed, it is even debatable whether 60 liters/min is an appropriate flow rate with which to sample pressurized MDI output, given that instructions for use generally restrict inhalation to <30 liters/min.

Whether CI or LI results can be used to determine "potency" (dose of drug leaving the actuator) is examined in Table IV for albuterol C. This demonstrates that the twin-

stage impinger is just as capable as the USP sampling apparatus to determine "potency" as defined by the FDA Division of Bioequivalence (11). The slightly higher amount detected by the LI is consistent with the reduced actuator retention associated with a 60-liter/min flow rate versus one of 12.5-liter/min in the USP sampling apparatus. Table IV demonstrates another important point. In the absence of an airtight seal between the actuator and the evaporation chamber, only 70 μg of drug was sampled in the DCI6, as compared to approximately 91 μg in the presence of an airtight seal. To determine correctly the dose leaving the actuator by employing the LI, all portions of the apparatus must be washed and assayed for drug content. In our hands, this process is as time-consuming as a determination using cascade impaction and can be subject to greater operator variability. To expedite experimentation, it has been proposed (13) to assay only that amount deposited in the second stage and express it as a fraction of the total drug collected using the USP sampling apparatus in order to determine the deposition of emitted dose. Because it has been demonstrated that volumetric flow rates affect the deposition of drug in the actuator (note that increases in flow rate causes decreased actuator retention, Table III), to express that fraction of drug collected at 60 liters/min in terms of the total drug collected at 12.5 liters/min (USP sampling apparatus) would be erroneous. It would seem more logical, if the LI were being used as a particle sizing device, to analyze for drug content in the liquid impinger as originally intended and then to sum the amounts to satisfy the USP requirements for "potency." This study demonstrates that the LI is just as efficient as the USP apparatus and can offer more information on the nature of the formulation.

CONCLUSION

At present, no inertial particle sizing device provides validated measures of *in vivo* deposition from pressurized metered dose inhalers. While throat deposition can be simulated *in vitro* (25), the throat models available are presently not validated with respect to *in vivo* applicability (26). Nevertheless, the formulator must choose a sizing device that can provide preclinical information on the physical stability of pressurized suspension formulations. Also, a rigorous method is required to perform routine comparative testing of formulations in both laboratory and regulatory settings. The values most often sought in comparative testing are respirable doses and respirable fractions. Not surprisingly, none of the devices tested in this study was ideal in all respects.

The twin-stage liquid impinger was capable of discerning differences between three albuterol formulations in terms of respirable percentage and respirable doses. Whether these differences are clinically important is unknown. Also, because of its high collection efficiency, the twin-stage liquid impinger could replace the USP sampling apparatus to determine the dose leaving the actuator of MDIs. The LI does provide higher estimates for *in vivo* deposition of drug than the two CIs tested. This could be attributed to a number of factors, the most important of which may be the high operational flow rate and the different chamber geometry used to attach the MDI. Although 60 liters/min may be appropriate for testing dry powder inhalers (which require high flow rates to function), it is questionable whether 60 liters/min is the optimal flow rate for testing formulations intended for use by asthmatics. Also, the question of reproducibility of results between LIs remained unanswered. Nevertheless, liquid impingement may well be appropriate for comparative testing of MDI formulations.

The CIs tested were capable of discriminating between formulations when results were expressed in terms of respirable doses. It is possible to compare results between impactors employing different flow rates and stage numbers as long as they have similar aerodynamic selectivity, the connection between the actuator outlet and the impactor is identical in geometry and the seal is airtight. The USP General Chapter on Aerosols ⟨601⟩ should include rigorous specifications for evaporation chambers and methodology in impaction tests to ensure meaningful inter- and intralaboratory comparisons.

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REFERENCES

1. G. W. Hallworth and D. G. Westmoreland. The twin impinger: A simple device for assessing the delivery of drugs from metered dose pressurized aerosol inhalers. *J. Pharm. Pharmacol.* 39:966–972 (1987).
2. G. W. Hallworth and U. G. Andrew. Size analylsis of suspension inhalation aerosols by inertial separation methods. *J. Pharm. Pharmacol.* 28: 898–907 (1976).
3. G. W. Hallworth and R. R. Hamilton. Size analysis of metered suspension pressurized aerosols with the Quantimet 720. *J. Pharm. Pharmacol.* 28:890–897 (1976).
4. R. N. Dalby and P. R. Byron, Comparison of output particle size distributions form pressurized aerosols formulated as solutions or suspensions. *Pharm. Res.* 5:36–39 (1988).
5. P. R. Byron, R. N. Dalby, and A. J. Hickey. Optimized inhalation aerosols. I. The effects of spherical baffle size and position upon the output of several pressurized nonaqueous suspension formulations. *Pharm. Res.* 6:225–229 (1989).
6. D. Hathaway. Particle size measurement of an aerosol deodorant using laser holographic microscopy. *Aerosol Age* Sept. 28 (1973).
7. K. K. L. Ho, I. W. Kellaway, and R. L. Tredree. The determination of aerosol output during size analysis by laser diffraction. *J. Pharm. Pharmacol.* 39(suppl.):77p (1987).
8. C. S. Kim, D. Trujillo, and M. A. Sackner. Size aspects of metered-dose inhaler aerosols. *Am. Rev. Resp. Dis.* 132:137–142 (1985).
9. K. R. May. *J. Sci Instr.* 22:187–195 (1945).
10. I. Porush. Pharmaceutical products. In H. R. Shepherd (ed.), *Aerosols: Science and Technology,* Interscience, New York, 1961, pp. 387–408.
11. FDA Division of Bioequivalence. Guidance for the *in Vitro* Portion of Bioequivalence Requirements for Metaproterenol Sulfate and Albuterol Inhalation Aerosols.
12. *British Pharmacopoeia,* p. 875, Appendix XVII C, A204–A207 (1988).
13. P. A. Haywood, M. Martin-Smith, J. M. Padfield, I. J. Smith, and R. N. Woodhouse. Establishing more meaningful specifications and tests for metered-dose pressurized inhalers formulated as suspensions. *Pharm. Forum.* 15(3):5193–5202 (1989).
14. USP XXII, Physical tests and determinations, p. 1556 (1990).

15. I. Gonda, J. B. Kayes, and F. J. T. Fildes. Characterization of hygroscopic inhalation aerosols. In N. G. Stanley-Wood and T. Allen (eds.), *Particle Size Analysis,* Wiley-Heyden, London, 1981, pp. 31 et seq.
16. *Operating Manual for Andersen 1ACFM Non-Viable Ambient Particle Sizing Samplers,* Fourth Revision, Andersen Samplers, Atlanta, GA, November 1985.
17. P. R. Byron. The prediction of drug residence times in regions of the human respiratory tract following aerosol inhalation. *J. Pharm. Sci.* 75:433–438 (1986).
18. S. P. Newman. Deposition and dose determination from pressurized metered dose inhalers. *Respiratory Drug Delivery II,* Colorado, April 1990.
19. USP XXII, Metaproterenol sulfate inhalation aerosol, p. 836 (1990).
20. N. C. Miller. The effects of water in inhalation suspension aerosol formulations. In P. R. Byron (ed.), *Respiratory Drug Delivery,* CRC Press, Boca Raton, FL, 1990, pp. 249–257.
21. G. W. Snedecor and W. C. Cochran. *Statistical Methods,* 7th ed., Iowa State University Press, Ames, 1980.
22. J. P. Mitchell, P. A. Costa, and S. Waters. An assessment of an Andersen Mark II cascade impactor. *J. Aerosol Sci.* **19**:213–221 (1988).
23. N. P. Vaughan. The Andersen impactor: Calibration, wall losses and numerical simulation. *J. Aerosol Sci.* **20**:67–90 (1989).
24. B. M. Z. Zainudin, S. E. J. Tolfree, M. Biddiscombe, M. Whitaker, M. D. Short, and S. G. Spiro. An alternative to direct labelling of pressurised bronchodilator aerosol. *Int. J. Pharm.* **51**:67–71 (1989).
25. G. P. Martin, A. E. Bell, and C. Marriott. An *in vitro* method for assessing particle deposition from metered pressurized aerosols and dry powder inhalers. *Int. J. Pharm.* **44**:57(1988).
26. P. R. Byron. Aerosol formulation, generation and delivery using metered systems. In P. R. Byron (ed.), *Respiratory Drug Delivery,* CRC Press, Boca Raton, FL, 1990, p. 197.