ELSEVIER

International Journal of Pharmaceutics 186 (1999) 71–79

international journal of pharmaceutics

www.elsevier.com/locate/promis

Aerodynamic particle size of metered-dose inhalers determined by the quartz crystal microbalance and the Andersen cascade impactor

Tsi-Zong Tzou [1]

Inhalation Drug Delivery Laboratory, Building 260-4N-12, 3M Pharmaceuticals, St. Paul, MN 55144, USA

Received 1 November 1998; received in revised form 4 January 1999; accepted 23 February 1999

Abstract

Given the rapid sizing capability and high sensitivity, the quartz crystal microbalance (QCM) cascade impactor has been evaluated for the size determination of metered-dose inhaler (MDI) aerosols. The effects of surfactants present in MDI formulations, crystal coating, particle bounce and crystal overloading on the QCM cascade impactor are investigated. To reduce particle bounce, it is necessary to coat the crystals and use new coated surfaces for each measurement. Mass median aerodynamic diameters (MMADs) obtained from the QCM cascade impactor are compared to those from the commonly used Andersen cascade impactor. For MDI formulations containing little or no surfactants, MMADs obtained from the QCM and Andersen cascade impactors are comparable. For MDI formulations containing a significant amount of surfactant (or any non-volatile excipients), the QCM cascade impactor measures the combined size distribution of the drug and non-volatile excipients. A technique is devised in this study to deduce the drug-only size distribution from the QCM impactor for surfactant-containing MDI formulations and show comparable results to the Andersen cascade impactor except for high drug load Intal. The QCM impactor has proved to be a useful tool for rapid size measurement of MDI formulations.

Keywords: Andersen cascade impactor; Loading effect; Metered-dose inhaler (MDI); Particle size distribution; Particle bounce; Quartz crystal microbalance (QCM) cascade impactor

1. Introduction

Metered-dose inhalers (MDIs) are one of the most efficient techniques to deliver drugs to the lungs. The most important factor influencing the deposition of drugs in the lung is the particle size. A particle size of 1–5 μm is generally considered to be desirable to reach the lower respiratory tract. The size distribution of pharmaceutical aerosols is commonly measured by the Andersen Mark II cascade impactor (Andersen Samplers, Inc., Smyrna, GA). Since the Andersen cascade impactor test is time-consuming, it is of great

E-mail address: stzou@mmm.com (T.-Z. Tzou)

[1] Present address: 3M Unitek, 2724 S. Peck Road, Mailstop 118, Monrovia, CA 91016, USA.

0378-5173/99/$ - see front matter
PII: S0378-5173(99)00139-8

interest to use the real-time PC-2 quartz crystal microbalance (QCM) cascade impactor (California Measurements, Inc., Sierra Madre, CA) to determine the size distribution of MDI aerosols. The QCM impactor is often used to monitor the size distribution of dilute environmental aerosols (Hering, 1987; Horton et al., 1992). Brice et al. (1988) have reported comparable MMADs between the QCM and Andersen cascade impactors when testing environmental aerosols. Unlike environmental aerosols, MDIs deliver a unit dose rapidly with drug particles exiting at high velocities and require different sampling techniques. Currently, there are few literature references to the particle size determination of pharmaceutical aerosols using the QCM impactor. Chiang (1990) determined the size distribution of four MDI products using the QCM impactor, but comparisons with the Andersen cascade impactor were not made.

The QCM cascade impactor determines particle size by inertial classification. The impaction plates employ piezoelectric quartz crystals as mass sensors. Each of the ten stages has a sensing crystal and a reference crystal. The upper sensing crystal collects particles and the lower reference crystal compensates for frequency shifts due to temperature and humidity changes. The net frequency changes from each crystal pair are proportional to the mass of deposited particles. The Sauerbrey equation (Sauerbrey, 1959) relates the frequency change, Δf (Hz), of the QCM to the change in mass, Δm (g):

$$\Delta f = -(2.3 \times 10^{-6}) f_0^2 \frac{\Delta m}{A}$$

or:

$$\Delta m = -(1.4 \times 10^{-9}) \Delta f$$

where f_0 is the resonant frequency of the unloaded quartz (MHz) and A is the area of the electrode (cm^2). The average mass sensitivity of the electrode is 1.4 ng per Hz when particles are deposited across the entire electrode. As reported by Sauerbrey (1959), the mass sensitivity across the electrode is not linear and follows a Gaussian distribution with the highest sensitivity in the center of the electrode. Thus stages with larger jets are less sensitive to deposited aerosols, and each stage has a different mass sensitivity constant. The microcomputer connected to the QCM impactor takes into account the different mass sensitivity for each stage and calculates the mass on each stage accordingly.

The Andersen cascade impactor determines particle size based on inertial classification, followed by UV/high-performance liquid chromatography (HPLC) analysis of the deposited drug. A comparison between the Andersen and QCM cascade impactors is shown in Table 1. The QCM impactor can determine the particle size of MDIs from single actuations given the high sensitivity of the crystals, whereas the Andersen cascade impactor often requires multiple actuations due to analytical limitations. Only recently have studies been conducted on MDI particle size from single actuations using the Andersen cascade impactor and HPLC with electrochemical detection (Nasr, 1993; Nasr et al., 1997). The QCM impactor measures the size distribution of the drug together with any non-volatile excipients, whereas the Andersen cascade impactor determines the drug size distribution using drug specific assay. While the drug mass balance is available for the Andersen cascade impactor, the QCM impactor does not have the total drug mass available. Unlike the Andersen cascade impactor that requires external UV/HPLC analysis, the QCM impactor can generate particle size results within 1 min of sampling. Only recently has an automated An-

Table 1
Comparison between the Andersen and QCM cascade impactors

QCM	Andersen
Ten stages	Eight stages including filter
0.24 l/min	28.3 l/min
Sensitive, single actuation	Often multiple actuations
'Real-time' signal	'External' UV/HPLC analysis
Rapid	Time-consuming; throughput of about 30 samples per day using automated Andersen impactor
Not drug specific	Drug specific
Total mass of drug is not available	Mass balance available

Table 2
MDI formulations evaluated

Drug productPRIVATE	Nominal delivered dose (μg)	Formulation	Propellant(s) /cosolvent	Amount of non-volatile excipient(s)
A: QVAR	80	Solution	HFA134a/ethanol	None
B: Proventil HFA	108	Suspension	HFA134a/ethanol	Low; <0.05% w/w
C1: Maxair Press-and-Breathe	200	Suspension	CFC12/CFC11	High; >0.3% w/w
C2: Maxair Autohaler				
D: Intal	800	Suspension	CFC12/CFC114	High; >0.3% w/w
E: Aerobid-M	250	Suspension	CFC11/CFC12/CFC114	High; >0.3% w/w

dersen cascade impactor (Novi Systems, Ltd., 1998) become available to give a throughput of about 30 samples per day.

Particle bounce from the impaction surface can be a serious problem with inertial impactors in general. It can result from elastic collisions between particles and impaction surfaces and between particles themselves. Dry hard particles readily bounce from uncoated impaction surfaces. Particles that bounce off and deposit on subsequent stages cause a bias towards a smaller mass median aerodynamic diameter (MMAD). To obtain a non-distorted size distribution, it is important that particles stick to the impaction surfaces and that particles stick among themselves. This is especially critical for the QCM impactor where the mass added to the crystal must be genuinely held by the crystal in order for the Sauerbrey equation to hold. For particles to stick to impaction surfaces, high-viscosity grease coatings have been used (e.g. Rao and Whitby, 1978a,b). As particles accumulate on the surfaces, the efficiency of grease coatings decreases rapidly with particle loading (Reischl and John, 1978; Turner and Hering 1987; Pak et al., 1992). Particle build-up on impaction surfaces may also affect the flow stream such that smaller particles get collected prematurely, resulting in a larger MMAD (Chiang, 1994; Nasr et al., 1997). In addition, for the QCM impactor, the Sauerbrey equation breaks down when particles overload the crystals.

In this work, the QCM cascade impactor has been used to measure the aerodynamic particle size of MDI aerosols. The effects of surfactants present in MDI formulations, crystal coating, particle bounce and crystal overloading on the QCM cascade impactor are investigated. Size distributions obtained from the QCM cascade impactor are compared to those from the Andersen cascade impactor.

2. Materials and methods

2.1. Drug products

The five MDI products evaluated in this study are listed in Table 2. Both hydrofluoroalkane (HFA) and chlorofluorocarbon (CFC) products were included: (A) QVAR™ (beclomethasone dipropionate) (3M), (B) Proventil®–HFA (albuterol sulfate) (Schering-Plough), (C) Maxair™ (pirbuterol acetate) (3M), (D) Intal® (disodium cromoglycate) (Fisons) and (E) Aerobid®-M (flunisolide hemihydrate) (Forest). HFA products A and B had little or no surfactant. CFC products C, D and E contained a significant amount of surfactants (relative to drug). Product D contained a large amount of drug where drug loading effect could be studied. Product E had a non-volatile flavoring agent, menthol, in addition to the surfactant. Product A was a solution formulation and products B, C, D and E were suspen-

sions. Placebos for surfactant-containing products B, C, D and E were also prepared to study the contribution of surfactants to the size distribution when the QCM impactor was used.

2.2. Cascade impactor procedure

For measurements using the QCM impactor, the bulk stream flow rate was set at 28.3 l/min, the same flow rate as sampling in the Andersen cascade impactor. The QCM cascade impactor samples isokinetically at 0.24 l/min from the bulk stream through a metal tube located between the entry port and the QCM cascade impactor; the remainder of the aerosol stream is drawn through a collection filter by a vacuum pump. A schematic diagram of the flow system is shown in Fig. 1. The effect of crystal coating was studied by comparing results from Vaseline coating with those without coating. No coatings were used for the surfactant-containing placebo formulations to collect liquid particles except that placebo for Aerobid-M (placebo E) employed Vaseline coating to reduce bounce of solid particles (menthol). For each measurement, a single actuation was made.

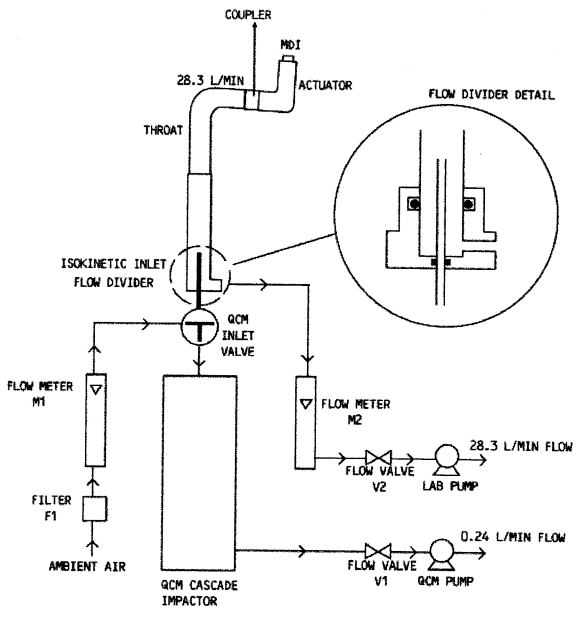

Fig. 1. Schematic diagram of the QCM cascade impactor flow system. Adapted from California Measurements, Inc.

Given a typical frequency drift (noise) of less than 1 Hz/min, single-actuation particle-size determination of MDIs (typical signals of 30–100 Hz on stages with the largest mass) can be easily achieved.

For the Andersen cascade impactor, the flow rate was 28.3 l/min. Ten or 20 actuations were performed for each measurement. Drug assay was performed using UV/HPLC. No coatings were used on Andersen cascade impactor collection surfaces.

A glass throat (1 inch ID, 105° bend) was used as the entry port for both cascade impactors. For each drug product, two or three canisters were tested. Each canister was tested three times using the QCM impactor. With the Andersen cascade impactor, each canister was tested once. Each aerosol canister was shaken and primed five times prior to the test. The actuator used for the beclomethasone and albuterol products (products A and B) was a 3M M3709 actuator (0.010 inch orifice). Other commercial products were tested using their own commercial actuators.

2.2.1. QCM cascade impactor: crystal coating and procedure

Both sides of the sensing and reference crystals for each stage (a total of four surfaces per stage) were coated according to the procedure suggested by the manufacturer (California Measurements, Inc., 1991). The technique was to apply more than enough coating to the crystal surface and gradually wipe off the excess until the frequency drop was between 1.5 and 2.0 kHz (approximately 1-μm thick coating) for each side of the crystal.

The frequency drift of the QCM was checked to be less than 1 Hz/min before a measurement was made. The bulk flow rate was adjusted to 28.3 l/min using a mass flow meter (Top Trac, Sierra Instruments, Monterey, CA) after the vacuum pump was turned on and the flow rate stabilized. For each measurement, a single actuation was made after rotating the inlet valve from filtered air to aerosol sample. After the frequency of the QCM stage stabilized, the inlet valve was rotated back to filtered air again. The mass changes were calculated from the frequency changes by the microprocessor in the QCM impactor. The vac-

uum pump was turned off. This procedure was repeated until three single-shot measurements were made. The impactor was then disassembled and the sensing crystals were flipped to the other side or exchanged with the reference crystals so that a total of four surfaces could be used before cleaning was necessary. Crystal surfaces were cleaned with hexane using a cotton swab. Cleaning and coating four sets of crystal surfaces (ten per set) would take approximately an hour.

2.2.2. Andersen cascade impactor procedure and UV/HPLC analysis

The flow rate was adjusted to 28.3 l/min using a mass flow meter (Top Trac, Sierra Instruments, Monterey, CA) after the vacuum pump was turned on. With a 10-s pause between actuations, ten or 20 actuations were made for each measurement. The vacuum pump was turned off 30 s after the last actuation. The impactor was then disassembled. Drug collected on each stage was rinsed with the appropriate amount of diluent.

The HPLC analyses for beclomethasone, albuterol and flunisolide (products A, B and E) were the same as described previously (Stein and Olson, 1997; Tzou et al., 1997, 1998).

Pirbuterol acetate and disodium cromoglycate (products C and D) were analyzed using a UV/VIS spectrometer (DU-64, Beckman, Fullerton, CA). The extinction coefficient for pirbuterol acetate was 30.4 ml/mg/cm at 294 nm, and the diluent was 0.1 N methanolic HCl. The extinction coefficient for disodium cromoglycate was 44.7 ml/mg/cm at 320 nm, and the diluent was 1% disodium orthophosphate dihydrate buffer with orthophosphoric acid (pH 7.4) in methanol. A scan of the excipient solution at the wavelength of interest indicated no interference from the excipient.

2.3. Particle size calculation

Cut-points provided by the manufacturers (Andersen Samplers, Inc., 1985; California Measurements, Inc., 1991) were used for each of the impactors. The cut-points for the QCM impactor are 35.4, 17.7, 9.05, 4.50, 2.25, 1.13, 0.56, 0.28, 0.14 and 0.07 μm. The cut-points for the Andersen cascade impactor are 9.0, 5.8, 4.7, 3.3, 2.1, 1.1, 0.7 and 0.4 μm. A non-linear regression program, Impactorplot (Nephele Enterprises, White Bear Lake, MN), was used to fit a log–normal distribution to the data to obtain the MMAD and the geometric standard deviation (G.D.S.) for both impactors.

2.4. Deducing the drug-only particle size distribution from the QCM impactor for high-surfactant formulations

For MDI formulations containing a high amount of surfactant, the QCM impactor measures the combined size distribution of drug and surfactant. To obtain the drug-only particle size distribution from the QCM cascade impactor, the combined distribution of the drug and surfactant (or any non-volatile excipients) must be corrected for the contribution from the placebo formulation. A preliminary approach for the non-volatile excipient correction is devised here. Assuming the non-volatile excipient distribution is the same in the drug and the placebo formulations, the drug particle size distribution can be deduced by subtracting the non-volatile excipient contribution from the combined particle size distribution of the drug and non-volatile excipient:

$$\%d_i = \frac{\%D_i - \%p_i W}{\sum_i (\%D_i - \%p_i W)} \times 100$$

where $\%d_i$ is the calculated weight percent of drug only on stage i, $i = 1$–10, $\%D_i$ is the average measured weight percent of drug formulation on stage i, $\%p_i$ is the average measured weight percent of placebo formulation on stage i, W is the weighing factor of non-volatile excipients, $W = w_p/(w_d + w_p)$, and w_p and w_d are the weight percents of non-volatile excipients and drug in the drug formulation, respectively.

3. Results and discussion

The QCM impactor results are presented below to determine: (1) whether surfactants in the formulations alone can stick drug particles to the

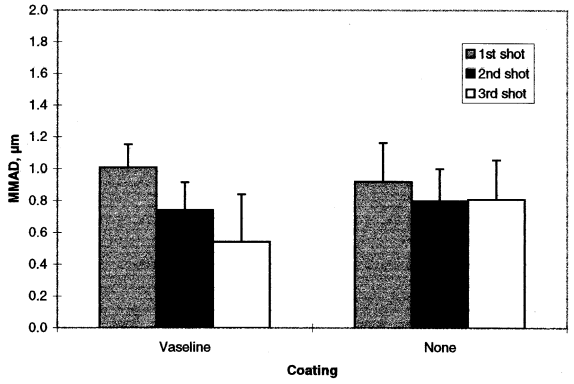

Fig. 2. Effect of coating and loading on MMADs of QVAR (average ± S.D.).

crystal surfaces, i.e. whether coating crystals is necessary for high-surfactant formulations; and (2) whether surfactants in the formulations can stick drug particles upon previously deposited particles, i.e. whether multiple measurements are possible with the same set of coated crystals (loading effect). Table 3 gives the QCM impactor results with and without coatings and the Andersen impactor results. The results are discussed in Sections 3.1 and 3.2.

3.1. Formulations containing little or no surfactants (products A and B)

With little or no surfactants in these formulations, coating the quartz crystals was necessary to reduce particle bounce since the frequency changes, thus the mass changes, with Vaseline coating were greater than those without coating (e.g. twice as large with coating for product A). Multiple measurements using the same set of coated crystal surfaces showed variations in MMAD among the first, second and third shots (product A as an example in Fig. 2) as the coated surfaces became covered with particles. Using new coated crystal surfaces for each measurement was necessary so that incoming particles come into contact with the coated surfaces rather than the collected particles. Only the first measurement from a set of coated crystals (Table 3) was compared to the Andersen results. MMADs obtained from the QCM and Andersen cascade impactors agreed well for these formulations. Vaseline appeared to be effective in reducing particle bounce.

3.2. Formulations containing a significant amount of surfactants (products C, D and E)

Small MMADs observed for these products on the QCM impactor without coatings indicated particle bounce. Even with a significant amount of surfactant in the formulations, coating the quartz crystals was necessary to reduce particle bounce. Multiple measurements using the same set of coated crystal surfaces showed variations in MMAD among the first, second and third shots (product D as an example in Fig. 3). Using new coated crystal surfaces for each measurement was necessary. Only the first measurement from a set of coated crystals (Table 3) was compared to Andersen results.

There is a discrepancy in MMADs between the QCM and Andersen impactors for high-surfactant formulations. Unlike the Andersen cascade impactor with UV/HPLC analysis, the QCM impactor does not measure the drug particle size selectively; both drug particles and non-volatile excipients are collected on the impactor and result in a combined particle size distribution. Note that the placebo formulations show smaller particle size than the drug formulations. In other words, the surfactant particle size may be smaller than the drug size since dissolved surfactants may be incorporated into smaller sprayed droplets which

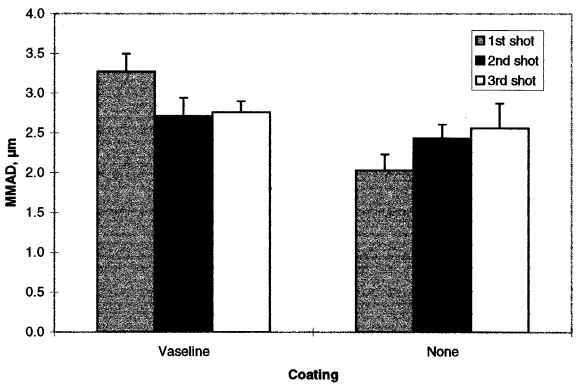

Fig. 3. Effect of coating and loading on MMADs of Intal (average ± S.D.).

Table 3
QCM and Andersen cascade impactor results[a]

Product	QCM cascade impactor (first shots only)						Andersen impactor		Calculated from QCM impactor (Vaseline coating and placebo)	
	No coating		Vaseline coating		Placebo		Drug only		Drug only	
	MMAD (μm)	GSD	MMAD (μm)	GSD	MMAD (μm)	GSD	MMAD (μm)	GSD	MMAD (μm)	GSD
A	0.92 (0.24)	2.82	1.01 (0.14)	2.13	Not applicable		1.15 (0.02)	1.62	Not applicable	
B	Not performed		1.76 (0.14)	2.01	0.66 (0.02)	1.77	1.96 (0.01)	1.59	1.82	1.93
C1	1.45 (0.11)	2.25	2.25 (0.05)	1.93	1.36 (0.10)	2.18	3.33 (0.11)	1.65	3.02	1.56
C2	1.76 (0.11)	1.95	2.55 (0.08)	1.97	1.28 (0.07)	1.96	3.09 (0.08)	1.81	3.10	1.70
D	2.03 (0.20)	2.55	3.27 (0.23)	1.79	2.17 (0.02)	1.92	4.65 (0.05)	1.44	3.85	1.46
E	Not performed		2.99 (0.22)	1.75	1.88 (0.05)	1.98	4.14 (0.06)	1.78	4.15	1.23

[a] Results are given as average (S.D.).

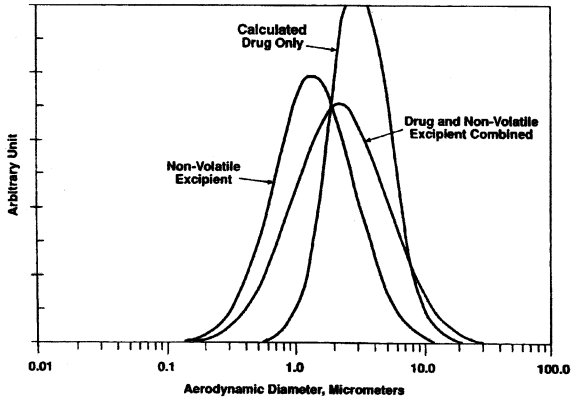

Fig. 4. Calculated drug-only size distribution and measured drug and placebo size distributions for Maxair Press-and-Breathe.

will not hold even a single suspended drug particle. Therefore the combined distribution of drug and surfactant particles from the QCM cascade impactor is biased toward a smaller size due to its inability to distinguish drug and surfactant. The drug only particle size distribution can be calculated by subtracting the non-volatile excipient contribution from the combined size distribution of the drug and non-volatile excipients using the technique described in Section 2.4. Using Maxair Press-and-Breathe as an example, the calculated drug-only distribution and the measured drug and placebo particle size distributions are given in Fig. 4. After the correction is made for the contribution from the placebo formulations, the calculated drug only MMADs from the QCM impactor (Table 3) are comparable to those from the Andersen impactor except for Intal. The drug amount in Intal was much higher than that in other drug products; at this drug level, crystal coatings might not eliminate particle bounce or the large mass might not be intimately felt by the crystals (overloading) even within a single actuation.

4. Conclusion

The QCM cascade impactor has been used to determine the size distributions of MDI aerosols. Even with surfactants present in the formulations, coating the crystals was necessary to reduce particle bounce. Using new coated crystal surfaces for each measurement was necessary; multiple measurements were not plausible using the same set of coated crystals. MMADs obtained from the QCM and Andersen cascade impactors are comparable for MDI formulations containing little or no surfactants. Surfactants or non-volatile excipients present in the formulations have their own size distributions which may differ from those of the drugs. MMADs for high-surfactant formulations obtained from the QCM impactor should be corrected for the contribution from the placebo formulations. A technique has been described to deduce the drug only size distribution from the QCM impactor for MDI formulations containing surfactant. The calculated drug only MMADs from the QCM impactor agree with those from the Andersen impactor except for high drug load Intal. The technique is applicable to formulations containing any non-volatile excipients, including, but not limited to, surfactants. This technique may also be extended to other instruments, where drug particles and other non-volatile excipients are sized simultaneously, e.g., the Aerosizer (Amherst Process Instrument, Hadley, MA).

The QCM impactor has proved to be a useful tool for rapid size measurement of MDI formulations and is particularly useful where HPLC methods for drugs are not available. To obtain mass balance for the QCM impactor, it is recommended to rinse the entry port and the filter that collects the bulk stream (only 0.24 out of 28.3 l/min is not collected) as supplementary testing.

Acknowledgements

The author wishes to thank Judi Nacionales for her technical assistance. Stimulating discussions with Charles Thiel and Nicholas Miller on particle size calculation are greatly appreciated.

References

Andersen Samplers, Inc., 1985. Operating Manual for An-

dersen 1 ACFM Non-Viable Ambient Particle Sizing Samplers. Anderson Samplers, Inc.

Brice, K.A., Fellin, P., Ernst, D.L., 1988. Generation of aerosols containing polycyclic aromatic hydrocarbons (PAH) for testing of air monitoring methods. 19th Annual Meeting of the Fine Particle Society (Aerosols), 20 July, Santa Clara, CA.

California Measurements, Inc., 1991. Instruction Manual Aerosol Particle Analyzer (QCM Cascade Impactor System).

Chiang, W., 1990. Rapid determination of MDI particle size distribution using crystal microbalance cascade impaction. In: Respiratory Drug Delivery II. Interpharm Press, Inc, pp. 725–738.

Chiang, W., 1994. Cascade impactor design and performance. Pharmaceutical Technology Conference, 20 September, Atlantic City, NJ.

Hering, S.V., 1987. Calibration of the QCM impactor for stratospheric sampling. Aerosol Sci. Technol. 7, 257–274.

Horton, K.D., Ball, M.H.E., Mitchell, J.P., 1992. The calibration of a California Measurements PC-2 quartz crystal cascade impactor (QCM). J. Aerosol Sci. 23 (5), 505–524.

Nasr, M.M., 1993. Single-puff particle-size analysis of albuterol metered-dose inhalers (MDIs) by high-pressure liquid chromatography with electrochemical detection (HPLC-EC). Pharm. Res. 10 (9), 1381–1384.

Nasr, M.M., Ross, D.L., Miller, N.C., 1997. Effect of drug load and plate coating on the particle size distribution of a commercial albuterol metered dose inhaler (MDI) determined using the Andersen and Marple-Miller cascade impactors. Pharm. Res. 14 (10), 1437–1443.

Novi Systems, Ltd. 1998. The Automated Andersen. Product brochure.

Pak, S.S., Liu, B.Y.H., Rubow, K.L., 1992. Effect of coating thickness on particle bounce in inertial impactors. Aerosol Sci. Technol. 16, 141–150.

Rao, A.K., Whitby, K.T., 1978a. Non-ideal collection characteristics of inertial impactors I: single-stage impactors and solid particles. J. Aerosol Sci. 9, 77–86.

Rao, A.K., Whitby, K.T., 1978b. Non-ideal collection characteristics of inertial impactors: II. cascade impactors. J. Aerosol Sci. 9, 87–100.

Reischl, G.P., John, W., 1978. The collection efficiency of impaction surfaces. Staub. Reinhalt. Luft. 38, 55.

Sauerbrey, G.Z., 1959. Z. Phys. 155, 206–222.

Stein, S.W., Olson, B.A., 1997. Variability in size distribution measurements obtained using multiple Andersen cascade impactors. Pharm. Res. 14 (12), 1718–1725.

Turner, J.R., Hering, S.V., 1987. Greased and oiled substrates as bounce-free impaction surfaces. J. Aerosol Sci. 18 (2), 215–224.

Tzou, T.-Z., Pachuta, R.R., Coy, R., Schultz, R.K., 1997. Drug form selection in albuterol-containing metered-dose inhaler formulations and its impact on chemical and physical stability. J. Pharm. Sci. 86 (2), 1352–1357.

Tzou, T.-Z., Schultz, R.K., Ross, D.L., 1998. Flunisolide aerosol formulations. US Patent 5,776,433.