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COMMUNICATIONS

The influence of sampling chamber dimensions on aerosol particle size measurement by cascade impactor and twin impinger

K. FULTS, T. D. CYR*, A. J. HICKEY, *Department of Pharmaceutics (M/C 880), College of Pharmacy, University of Illinois at Chicago, Box 6998, Chicago, IL 60680, USA and *Bureau of Drug Research, Ottawa, Canada*

Abstract—The influence of the sampling chamber dimensions upon particle size estimation by cascade impaction has been investigated and compared with measurements by the twin impinger. Aerosols of salbutamol and disodium fluorescein (DF) were generated from pressurized metered dose inhalers. The mass median aerodynamic diameter (MMAD) by the Andersen Impactor for salbutamol ranged from 2·0 to 2·8 μm with geometric standard deviations (g.s.d.) of 1·7 to 2·4. These observations were independent of the distance travelled to the first impaction surface of the impactor and volume of the sampling chamber. The DF MMAD ranged from 5·0 to 6·9 μm with g.s.d. values of 1·7 to 1·8. Changes in droplet size within the sampling chamber may cause significant differences in particle size estimates as indicated by the cascade impaction data for DF. The respirable fraction of the salbutamol samples was similar whether determined by impaction or using the impinger. The latter device has previously been indicated to give clinically relevant estimates of respirable fraction for commercial inhalation aerosol devices.

Particle size measurement requires a knowledge of the factors which affect the performance of sizing instruments. Few compendial standards for particle sizing of medicinal inhalation aerosols have been established, yet their particle size exerts a major effect on lung deposition. Particles of 1 to 5 μm in aerodynamic size are appropriate for deposition in the lower airways (Gonda 1981; Stahlhofen et al 1983). The British Pharmacopoeia (1988) employs two alternative two-stage impinger devices for the aerodynamic determination of aerosol particle size. The glass version is the twin impinger, which has been described in detail (Hallworth & Westmoreland 1987). The upper stage impinger has a mean cut-off size of 6·4 μm at an airflow rate of 60 L min^{-1}. The second stage of this impinger thus collects respirable particles or droplets and has been shown to correlate with bronchodilator activity for specific drug inhalers (Padfield et al 1983). The United States Pharmacopeia (1985) currently employs a crude static method of particle sizing based on microscopic counting of impacted spray droplets. This method will soon be superceded by dynamic methods (Srinivasan 1991). Recently the Food and Drug Administration, Division of Bioequivalence (1989) recognized the usefulness of cascade impaction by introducing an informal standard for its use. Cascade impactors, unlike the twin impinger, are not marketed as a 'complete' system for evaluating medicinal aerosols. The inlet chamber employed may be varied in size, in practice, and influence the impactor collection efficiency. The present study was undertaken to determine the effect of the inlet chamber dimensions on the performance of an impactor, particle size distribution and respirable fraction of aerosols of disodium fluorescein (DF) and salbutamol from pressurized metered dose inhalers, as measured by cascade impactor and twin impinger. Portions of this work have previously been presented in abstracts (Hickey et al 1989; Fults et al 1990).

Correspondence: A. J. Hickey, Department of Pharmaceutics (M/C 880), College of Pharmacy, University of Illinois at Chicago, Box 6998, Chicago, IL 60680, USA.

Materials and methods

Dry DF was jet milled using a Trost Gem-T opposing Air Jet Mill (Garlock Plastomer Products, Newtown, PA) to a particle size of 3 to 5 μm and formulated in propellents 11, 12, and 14 (Dupont de Nemours Co., Wilmington, DE) in a 1:2:1 ratio using sorbitan trioleate (Span 85, Atlas Chemical Inc, USA) as a suspending agent (DF:Span = 1:1·4) (Pamasol Small Scale Aerosol Packaging Equipment, Pfaffikon, Switzerland) (Dalby & Byron 1988; Hickey et al 1988; Hickey 1990a,b).

Four salbutamol inhalers were submitted for testing by three companies and were used as supplied, with their own actuator. These samples have been coded from 1 to 4.

DF aerosol was generated from a 25 μL valve with an IN-1 actuator (Valois, BLM Associates, Inc., Greenwich, CT). The aerosol was actuated once before collection followed by five actuations into the inlet chamber above the Andersen Sampler or into the mouth piece adaptor of the twin impinger. The airflow through the impactor was started 5 s before the first actuation and there was a 2–3 s pause between actuations. The impactor was allowed to run for 10 s following the last actuation to allow complete deposition of particles from the last actuation.

The Andersen 1 CFM Non-Viable Ambient Sampler (Andersen Samplers, Inc., Atlanta, GA) is an eight-stage cascade impactor and was used with the preseparator and a 0·22 μm final filter. According to the manufacturer the aerodynamic cut-off sizes at which particles are collected with 50% efficiency are 9·0, 5·8, 4·7, 3·3, 2·1, 1·1, 0·7, and 0·4 μm for stages 0 to 7, respectively, at the standard airflow of 28·3 L min^{-1} (Andersen 1958, 1966; Vaughan 1989). These values were adopted for the interpretation of data in these studies.

The Andersen Impactor was used as a representative device to examine the effects of inlet chamber size on inlet (A1) and three inlet devices which differed in the distance to the first impaction plate and in volume (Table 1). Inlets A2, A3 and A4 were modified round-bottomed flasks with 25 mm internal diameter necks each presenting the aerosol at an angle of 30–45° to the axis of the impactor (Fig. 1).

The fraction of DF deposited at each stage was measured by immersion of collection plates in known volumes of phosphate buffer pH 7·4 and subsequent analysis at 486 nm using a Spectronic 20 (Bausch and Lomb, Rochester, NY). Phosphate buffer was prepared using dibasic sodium phosphate (0·084 M, Fisher Scientific, Itasca, IL) solution and monobasic potassium phosphate (0·067 M, Fisher Scientific, Itasca, IL) solution mixed in the ratio of 1·97:8·03, respectively (Diem 1968). Salbutamol

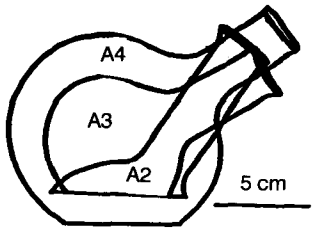

FIG. 1. Diagrams of sampling chambers A2, A3, and A4.

Table 1. Sampling inlet configurations for the Andersen Impactor.

Inlet	Distance to impact surface (cm)	Volume (mL)
A1[a]	5	40
A2	10	70
A3	10	160
A4	18	1000

[a] Standard impactor inlet.

Table 2. Estimates of difluorescein particle size[a] (MMAD, μm), distribution[a] (g.s.d.), and respirable fraction (Andersen < 5·8 μm, twin impinger < 8·1 μm).

Sampling method	MMAD	g.s.d.	Respirable fraction
A1	5·0 (0·2)[b]	1·7 (0·0)	6·9 (1·7)
A2	6·6 (0·5)	1·7 (0·1)	18·0 (6·5)
A3	6·5 (0·5)	1·7 (0·0)	17·8 (2·2)
A4	6·9 (0·4)	1·8 (0·1)	20·7 (2·2)
Twin impinger[c]			27·0 (7·8)

[a] Based on fraction of total output entering impactor. [b] Ranges for MMAD, g.s.d. and respirable fraction are in parentheses, n = 3. [c] Twin impinger operated at 10 L min^{-1}.

was dissolved in known volumes of 0·01 M sodium hydroxide solution and analysed using a Perkin-Elmer Lambda 3B UV/VIS spectrophotometer (Perkin-Elmer, Oakbrook, IL).

The mass of "drug" in the inlet chamber and the upper and lower portions of the inertial devices was determined separately. For the Andersen Impactor the respirable fraction was that collected below the stage with a cut-off size of 5·8 μm. The fraction of the aerosol collected in the lower portion of the twin impinger was considered the respirable fraction (Padfield et al 1983; Phillips et al 1990). Assuming a log-normal statistical fit to the size data the mass median aerodynamic diameter (MMAD) and geometric standard deviation (g.s.d.) were derived for the fraction of the aerosol in the impactor (Hinds 1982).

Results and discussion

The respirable fraction of DF increased with distance to the first impaction surface from 6·9 to 20·7% (Table 2). The two inlets A2 and A3, which have similar transit distances to the first impaction surface, gave similar respirable fractions. This suggests that the distance travelled rather than the volume for evaporation is of greater significance for this particular aerosol. The corresponding twin impinger result was 27% which was higher than values obtained by cascade impaction. This can be adequately explained in terms of the difference in particle cut-off diameter.

The MMAD values derived for DF increased with the sampling chamber distance and volume (Table 2). The range of particle size (MMAD) observed was 5·0 to 6·9 μm. The largest inlet allowed time for a reduction in inertia, so large particles could enter the impactor resulting in an upward shift in MMAD with no apparent change in g.s.d. The results for salbutamol exhibit no appreciable change in MMAD with an increase in inlet size by cascade impaction (Table 3). The differences in g.s.d. are also not significant. Therefore, DF alone exhibited within-sample variation due to the sample chamber configuration.

The salbutamol respirable size fractions measured by the Andersen Sampler and twin impinger methods are compared in Table 4. The twin impinger yields the highest values for each of the salbutamol samples, which can be explained by the different cut-off diameters of the impactor and impinger. The A4 inlet configuration consistently resulted in a lower estimate for respirable fraction, yet loss of droplet inertia (by reducing chamber impactional loss) and increased evaporation should increase the respirable fraction. Turbulence in the chamber at the high airflow may be responsible for increased deposition in the large chamber.

Table 4. Percent of salbutamol reaching the impactor or impinger in the respirable size range (impactor < 5·8 μm, impinger < 6·4 μm).

Sampling chamber	Salbutamol number 1	2	3	4
A1	41·6 (2·7)[a]	40·2 (9·4)	36·2 (5·0)	48·2 (8·3)
A2	44·4 (5·8)	41·0 (8·4)	39·8 (4·2)	44·6 (5·8)
A3	34·8 (16·8)	45·5 (6·3)	46·4 (6·3)	49·5 (6·3)
A4	33·0 (20·2)	38·5 (2·9)	46·1 (7·7)	35·9 (1·1)
Impinger[b]	44·6 (23·7)	45·6 (14·3)	47·6 (22·9)	51·9 (11·1)

[a] Ranges for MMAD in parentheses, n = 3. [b] Twin impinger operated at 60 L min^{-1}.

The twin impinger apparently overestimates the percentage of the aerosol output which will reach the airways (Zainudin et al 1989), as does the cascade impactor data presented here for comparison. The cascade impactor data may be expressed as the percentage of the total output collected at or below any desired stage. The stage acting as the cut-off for the respirable fraction, therefore, may be selected for its correlation with in-vivo data.

In conclusion, the physicochemical behaviour of aerosol droplets in the sampling chamber appeared to be influenced by turbulence, inertial deposition and evaporation. The extent to

Table 3. Estimates of salbutamol particle size (MMAD, μm) and size distribution (g.s.d.) derived from the fraction of the total output entering the Andersen Sampler.

	Salbutamol number 1		2		3		4	
Sampling chamber	MMAD	g.s.d.	MMAD	g.s.d.	MMAD	g.s.d.	MMAD	g.s.d.
A1	2·4 (0·0)*	1·9	2·8 (0·7)	1·9	2·1 (0·2)	1·8	2·0 (0·3)	1·9
A2	2·5 (0·2)	2·1	2·1 (0·6)	2·0	2·3 (0·3)	2·2	2·0 (0·2)	2·0
A3	2·4 (0·1)	1·8	2·5 (0·4)	1·7	2·2 (0·2)	1·8	2·0 (0·1)	1·8
A4	2·7 (0·8)	2·4	2·4 (0·4)	2·4	2·2 (0·1)	2·1	2·2 (0·1)	2·3

* Ranges for MMAD in parentheses, n = 3.

which each of these factors contributes to the deposition in the sampling chamber inlet, and ultimately in the inertial impactor, depends upon the actual particle or droplet size of the aerosol. It may not be possible, using this method in a single configuration, to obtain a definitive particle size estimate. Indeed, it may never be possible to achieve this aim. Specifications for the dimensions of inlets to inertial devices are necessary. Beside the need to specify sampling chamber dimensions, the impactor type may also be important due to differences in transit times to impaction on the plates and wall losses for different models (Hickey 1990b). The approach to establishing appropriate inlet dimensions should follow that previously taken for the twin impinger, wherein the data obtained can be shown to have clinical significance for a particular drug.

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The effect of the uptake of particles on the chemotaxis of polymorphonuclear leucocytes in-vitro

R. HYDE, D. A. LEWIS, *P. W. TAYLOR, *Pharmaceutical Sciences Institute, Aston University, Aston Triangle, Birmingham B4 7ET and* **Research Centre, Ciba Geigy Pharmaceuticals, Horsham, Sussex RH12 4AB, UK*

Abstract—The relationship between the phagocytic uptake of latex microspheres (1·1 μm diam.) and the mobility of rat polymorphonuclear leucocytes (PMNLs) has been investigated in-vitro. The movement of PMNLs was found to be independent of the uptake of particles but about half of the PMNLs were not receptive to the chemo-attractant *N*-formylmethionyleucylphenylalanine. However, the uptake of particles was greater when particle/cell ratios were high and the greatest number of particles were carried into a cellulose nitrate filter by chemotaxis at the highest particle/cell ratio of 30:1.

We are investigating the possibility of using polymorphonuclear leucocytes (PMNLs) as carrier vehicles to target drug-loaded microspheres in-vivo. PMNLs leave the blood vessels and accumulate in tissues in various infectious conditions and in non-infectious conditions such as rheumatoid arthritis, myocardial infarction and some malignant tumours (Reba & Chandeysson 1980). Boggs (1974) transfused PMNLs successfully into patients with neutropenia for the treatment of infection. This suggests the possibility of loading PMNLs by phagocytosis in-vitro with drug loaded particles and transfusing the cells into patients, where, by the process of chemotaxis, they may accumulate at sites of disease. The effect of the phagocytosis of particles by PMNLs on their directed mobility (chemotaxis) is unknown. In this communication we have examined this relationship in-vitro.

Correspondence: D. A. Lewis, Pharmaceutical Sciences Institute, Aston University, Aston Triangle, Birmingham B4 7ET, UK.

Materials and methods

Microspheres. Fluoresbrite carboxylated polystyrene latex microspheres (2·5% solids-latex) with a batch diameter of 1·1 μm (s.d. = 0·02) were purchased from Polysciences Inc., Warrington, PA, USA. Particles were dispersed by immersion in a sonic bath for 10 s before dilution and subsequent use. Particle suspensions were diluted with Hank's balanced salt solution (HBSS). Manufacturer's particle size was checked by electron microscopy.