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Particle size determination of metered dose inhalers with inertial separation methods: Apparatus A and B (BP), Four Stage Impinger and Andersen Mark II Cascade Impactor

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Abstract

The particle size of pharmaceutical aerosols is the main factor governing their deposition in the human respiratory tract. Of the many methods that are available for particle size analysis of aerosols, inertial methods have been found to give the most representative results, as compared to in vivo conditions. Two devices working on this principle have been included in the British Pharmacopoeia, Apparatus A and Apparatus B. One of their disadvantages is, however, that they only divide the aerosol particles into two fractions and do not yield a particle size distribution. Therefore, a third device, the Multistage Cascade Impactor no. 1, has additionally been taken up in the USP. Apart from Apparatus A and B, two devices that comply with this USP monograph were used in this study. The first was a self-made Four Stage Impinger, the second device being the Andersen Mark II Cascade Impactor with eight stages and a preseparator. The aim of this study was to compare the results of particle size analysis of different test aerosol formulations in metered dose inhalers with these four devices. In the first part of the study, one formulation was analyzed with all four methods. There was excellent agreement between Apparatus A and the Four Stage Impinger on the one hand and between Apparatus B and the Andersen Impactor on the other. In the second part of the study, Apparatus A and the Four Stage Impinger were compared in greater detail by sizing five more aerosol formulations. There was again excellent agreement in the fine particle fractions as determined with the two methods. By comparing the fraction of particles below 2.8 μm additionally, the Four Stage Impinger allowed better distinction between the aerosol formulations than Apparatus A. All in all, each of the four devices turned out to be useful for determining the particle size of an aerosol. Considering the analytical effort necessary and the amount of data generated with each of the devices, the Four Stage Impinger appeared to be the most effective.

Keywords: Aerosol; Metered dose inhaler; Particle size; Inertial separation; Impinger; Impactor; Inhalation

1. Introduction

The particle size of pharmaceutical aerosols intended for inhalation is one of the key factors that governs the site and extent of their deposition in the human respiratory tract. This deposition of the particles on their way into the respiratory tract occurs mainly due to impaction and sedimentation (Newman, 1985). Therefore, of the many methods for particle size analysis of aerosols, inertial separation techniques that de-

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termine the size of aerosol particles based on their mass and inertia are the most appropriate in vitro methods to imitate in vivo deposition (Kim et al., 1985). They are the only methods to date that are generally accepted for particle size analysis of pharmaceutical aerosols.

Of these sizing methods, two have been entered in the British Pharmacopoeia (1993) and three have been included in the US Pharmacopoeia XXII (1994). The two devices for determining the particle size of aerosols that are part of the BP are Apparatus A and Apparatus B. Apparatus A (BP) has also become known as the Twin Impinger (Hallworth and Westmoreland, 1987) or Single Stage Impactor no. 3 in the USP. It is a whole glass apparatus that divides the aerosol into two fractions of particles larger and smaller than 6.4 μm in diameter. Apparatus B (BP), also called Single Stage Impactor no. 2 (USP XXII), is a single stage cascade impactor with a 50% cut-off diameter of 9.8 μm at the same flow rate of 60 l/min. Particles that are smaller than 9.8 μm are retained in a glass fibre filter. USP additionally lists a device called the Multistage Cascade Impactor no. 1. This is a cascade impactor or impinger with several separation stages where the aerosol is split into more than just two fractions. Thus, a more detailed particle size distribution of the aerosol is achieved. The USP Multistage Cascade Impactor no. 1 is not further specified regarding flow rate, number of stages, cut-off diameters or type of impactor. Two devices complying with this monography were tested. The first was a self-made Four Stage Impinger that was designed after modifying the ideas of May (1966) and Bell et al. (1973). This Impinger also worked at a flow rate of 60 l/min and had an inlet port of the same geometry as the Twin Impinger. The 50% cut-off diameters were 15.3, 6.4 and 2.8 μm for stages 1–3. A glass fibre filter in stage 4 retained the fine particles below 2.8 μm. With this Four Stage Impinger it is possible to obtain a particle size distribution that is still very basic, but already more informative than the results of the Twin Impinger. An even more detailed particle size distribution can be obtained by a classical cascade impactor such as the Andersen Mark II Cascade Impactor. This Impactor also complies with the Multistage Impactor no. 1 monograph. It works with a flow rate of 28.3 l/min. Here particles are separated into nine fractions. Particles larger than 10 μm are retained in a preseparator. Following the preseparator, eight stages with 50% cut-off diameters of 9.0, 5.8, 4.7, 3.3, 2.1, 1.1, 0.7 and 0.4 μm separate the aerosol particles.

The aim of this study was to assess the particle size distribution of different aerosol formulations in pressurized metered dose inhalers and to correlate the results of the different methods. In order to do so, a standard aerosol formulation was characterised with the four different devices at first. Then the two devices that turned out to be the most effective regarding both the analytical effort necessary and the resulting data were tested with another five aerosol formulations.

2. Materials and methods

The following chemicals were used: tetrabutylammonium hydrogen sulfate of reagent grade quality (Fluka Chemika, Buchs, Switzerland), potassium dihydrogen phosphate of analytical quality, methanol and acetonitrile both of HPLC quality (Merck, Darmstadt, Germany) and Span 85 (ICI Specialty Chemicals, Essen, Germany). 0.45 μm cellulose acetate filters (Sartorius, Göttingen, Germany) and Whatman GF/A glass fibre filters (Whatman, Maidstone, UK) were used.

2.1. Aerosols

Six different aerosol formulations (A–F) in pressurised metered dose inhalers were used. Aerosols A contained a cromolyn sodium suspension in a mixture of propellants 11, 12 and 114. Aerosol B was a suspension of cromolyn sodium in propellants 12 and 114. Aerosol C, D and E consisted of cromolyn sodium in different nonchlorofluorocarbon propellants. Aerosol F consisted of beclomethasone 17,21-dipropionate in propellants 12 and 114. All aerosols contained Span 85 as surface-active ingredient. The metered dose was 1.0 mg per actuation for cromolyn

sodium and 0.05 mg per actuation for beclomethasone 17,21-dipropionate.

2.2. Assay

The aerosol cans were thermostatted in a water-bath at 25°C. Prior to use they had to be primed by shaking them vigorously for 30 s. After waiting 10 s a puff was released. This procedure was repeated twice. Afterwards aerosol can and actuator were cleaned and completely blow dried. For analysis the actuator was attached to the inlet port of the impactors with a moulded adaptor (except for apparatus B) and sealed air tight. The aerosol cans were then shaken again for 10 s and after waiting 5 s one puff was released into the apparatus. This was repeated nine times to ensure that enough drug for analysis was released into the apparatus. Three determinations were made for each of the devices except for Apparatus A where six determinations were carried out with Aerosol A. For rinsing the drug out of the impactor, demineralized water was used as a solvent for cromolyn sodium and methanol for beclomethasone 17,21-dipropionate.

2.2.1. Apparatus A

The impinger was attached to a suitable vacuum pump that was set at a continuous air flow of 60 l/min. The 50% cut-off diameter at this flow rate was 6.4 μm. The upper stage (part D) of the impinger was filled with 7 ml of solvent. 30 ml were filled in the lower stage (part H). After firing 10 puffs into the apparatus, actuator, throat and the impinger stages were rinsed with solvent. Three solutions were obtained: the first was from rinsing the actuator, the others from stage 1 and 2 of the impinger. Stage 1 washings included those from the throat and from the stage 1 inlet tube (parts B–D). Stage 2 washings included those from the inside and outside of the stage 2 inlet tube and the jet (parts E–H). The washing solutions were then diluted to volume (100.0 ml) and filtered.

2.2.2. Apparatus B

Apparatus B was also attached to a vacuum pump with a continuous air flow of 60 l/min. The 50% cut-off diameter was 9.8 μm. A glass fibre filter was used to retain the fine particle fraction. Because of the narrow inlet tube, the MDI actuator was directly attached to the inlet tube here and 10 puffs were released into the apparatus. Afterwards the actuator, inlet tube, impaction chamber and impaction plate were rinsed with demineralized water and diluted to volume. The impaction chamber also had to be rinsed, since particles had deposited on its wall. The filter was placed in 50.0 ml of demineralized water and put into an ultrasonic bath for 1 min. Altogether five different solutions were obtained here.

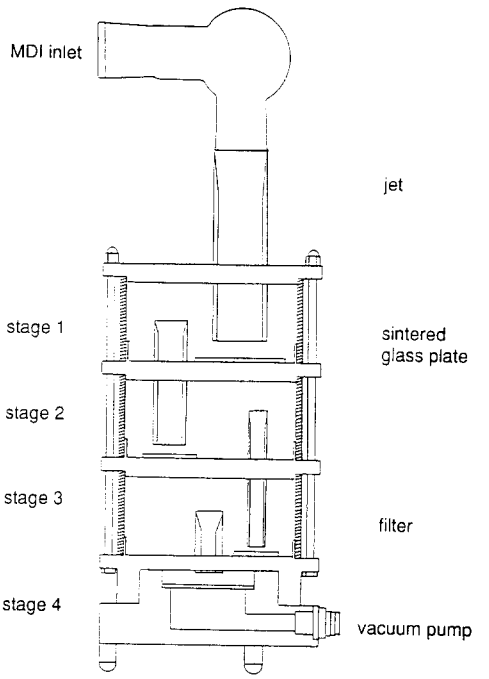

Fig. 1. The Four Stage Impinger.

2.2.3. Four Stage Impinger

The Four Stage Impinger (Fig. 1) was also attached to a vacuum pump that was set at a continuous air flow of 60 l/min. As in Apparatus A, a modified 50 ml round flask served as 'throat'. A glass fibre filter in the last stage was used to retain the fine particle fraction. 50% cut-off diameters were calculated as 15.3, 6.4 and 2.8 μm for stages 1, 2 and 3, respectively. They were

filled with 20 ml of solvent each. After releasing 10 puffs of aerosol into the apparatus, the impinger was carefully shaken to ensure that all of the drug particles would dissolve. Then five solutions were prepared: The actuator was rinsed with solvent and diluted to volume (50.0 ml). The throat and the first jet were rinsed with solvent, the washing solution combined with the solution in stage 1 and diluted to volume (50.0 ml). The other jets were also rinsed with the solvent of the appropriate stage below before the drug solutions were taken out. The filter of stage four was placed into 50.0 ml of solvent and put into an ultrasonic bath for 1 min. Jet number four was then rinsed with this solution.

2.2.4. Andersen Mark II Cascade Impactor

The Andersen Mark II Cascade Impactor was used at an air flow of 28.3 l/min. The modified 50 ml round flask that served as inlet port for the Apparatus A was also used here. The metal impaction plates were coated with a thin film of Span 85 to ensure that the deposited particles would remain on the impaction plates and not bounce off again. After releasing 10 puffs of the aerosol into the impactor, all drug in the apparatus was rinsed out yielding 10 fractions: fraction 1 was obtained by rinsing the actuator with 50.0 ml of demineralized water. For fraction 2, the adapter, inlet port, and preseparator were rinsed with altogether 40.0 ml of water. The other eight fractions were obtained by placing the impaction plates of the single stages (no. 0–7) in 20.0 ml of water each. To ensure that all particles dissolve the plates were then put into an ultrasonic bath for 1 min.

2.3. Determination of drug content

Prior to analysis all the solutions were filtered through a 0.45 μm cellulose acetate filter. The cromolyn sodium solutions of Apparatus A, B and the Four Stage Impinger were determined by UV absorption at 238.4 nm. The cromolyn sodium solutions of the Andersen Impactor had to be analysed by HPLC because of their high content of Span 85 that would have interfered with UV determination. Beclomethasone 17,21-dipropionate solutions were analysed by HPLC at 237 nm. The quantities of drug recovered were calculated as percentages (% w/w) of the total amount of drug found in each experiment. Confidence intervals for the mean value on a 95% level were calculated to check for statistically significant differences.

2.3.1. UV determination

Cromolyn sodium in Apparatus A, B and the Four Stage Impinger was determined by a Uvikon 930 Spektrophotometer (Kontron Instruments, Milano, Italy) set at 238.4 nm. The washing solutions had to be diluted up to 10-fold depending on their concentration. The diluted solutions were measured in quartz cuvettes of 1.00 cm length against demineralized water.

2.3.2. HPLC methods

The HPLC system consisted of a Gynkotec High Precision Pump Model 300CS (Gynkotec, München, Germany), a Kontron Autosampler HPLC 360 (Kontron Instruments, Milano, Italy), a Hypersil ODS 5μm column (4.8 × 250 mm), a Shimadzu UV spectrophotometric detector and a Shimadzu Chromatopac C-R3A Integrator (Shimadzu, Kyoto, Japan). Samples of 100 μl were injected.

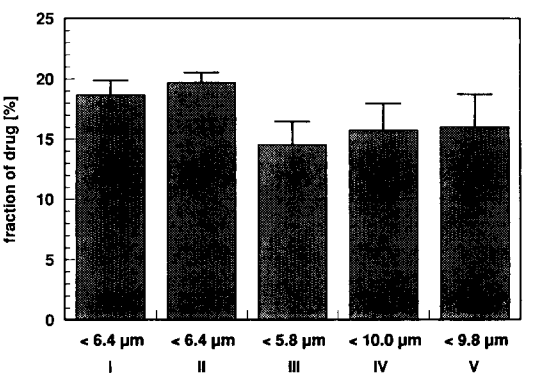

Fig. 2. The fine particle fraction of the cromolyn sodium aerosol A as determined with Apparatus A (I), Four Stage Impinger (II), Andersen Impactor (III, IV) and Apparatus B (V). Error bars represent the confidence intervals of the mean values ($a = 0.05$, $n = 3$, except Apparatus A where $n = 6$).

For cromolyn sodium the mobile phase consisted of 10% component A and 90% component B, with component A being 20% methanol in water and component B consisting of 5 mM KH_2PO_4 and 10 mM tetrabutylammonium hydrogen sulfate in 50% methanol at pH 6.50 (adapted from Thoma and Marschall, 1992). Flow rate was 1.5 ml/min yielding a pressure of 190 bar. Retention time was 4.8 min.

For beclomethasone 17,21-dipropionate, the mobile phase consisted of 65% acetonitrile in water. The flow rate was 2.0 ml/min, yielding a pressure of 133 bar. Before analysis, the samples had to be diluted with 25% of water in order to achieve a satisfactory baseline. Retention time was 4.3 min.

3. Results and discussion

3.1. Comparison of Apparatus A, B, Four Stage Impinger and Andersen Impactor with Aerosol A

In the first part of this study the standard aerosol formulation A was compared with the four different impactors. The results are summarised below in Table 1 for Apparatus A, Table 2 for Apparatus B, Table 3 for the Four Stage Impinger and Table 4 for the Andersen Cascade Impactor.

In Apparatus A 18.7% (w/w) of the particles of Aerosol A were found to be smaller than 6.4 μm in diameter. This is very close to the 19.7% found in stages 3 and 4 of the Four Stage Impinger together (Fig. 2). With the Andersen Impactor 14.5% of the particles were found to be below 5.8 μm (stages 2–7) or 15.8% below 10.0 μm (stages 0–7). Almost the same is seen with Apparatus B where 16% of the particles are found to be smaller than 9.8 μm. The differences between Apparatus A and the Four Stage Impinger on the one hand and between Apparatus B and the Andersen Impactor are not significant ($a = 0.05$). The difference between Andersen Impactor/Apparatus B and Apparatus A/Four Stage Impinger is, however, statistically sigificant.

In Apparatus B there was a visible deposition of drug particles on the walls of the impaction chamber, mainly around the chamber exit towards the filter. This wall loss accounted for 2.3% of the drug. The same amount of drug deposited on the impaction plate.

The fraction of aerosol remaining in the actuator was identical for Apparatus A and the Four Stage Impinger (21.5% vs 20.4%). Using the Andersen Impactor a greater amount (38.3%) remained in the actuator. This may be explained by the lower flow rate and the higher resulting repulsion. For Apparatus B more than half of the aerosol remained in the actuator. Additionally, 22% remained in the inlet tube so that only around 20% of the drug reached the impaction plate where the separation should occur.

Table 1
Deposition of aerosol A in Apparatus A

Fraction	Particle size	Average (%)	SD
Actuator	not defined	21.46	1.49
Upper stage	> 6.4	59.84	2.18
Lower stage	< 6.4	18.70	1.46

The amount of drug in the different fractions is calculated as a percentage of the total amount of drug recovered ($n = 6$). SD, standard deviation.

Table 2
Distribution of the test aerosol in the Apparatus B

Fraction	Particle size (μm)	Average (%)	SD
Actuator	not defined	57.10	1.23
Inlet tube	not defined	22.16	2.62
Impaction plate	> 9.8	2.37	0.25
Impaction chamber	not defined	2.32	0.28
Filter	< 9.8	16.04	2.39

The amount of drug in the different fractions is calculated as a percentage of the total amount of drug recovered ($n = 3$).

Table 3
Deposition of aerosol A in the Four Stage Impinger

Fraction	Particle size (μm)	Average (%)	SD
Actuator	not defined	20.43	0.92
Stage 1	> 15.3	53.43	0.31
Stage 2	15.3–6.4	6.42	0.49
Stage 3	6.4–2.8	16.15	0.46
Stage 4	< 2.8	3.56	0.27

The amount of drug in the different fractions is calculated as a percentage of the total amount of drug recovered ($n = 3$).

Table 4
Deposition of aerosol A in the Andersen Mark II Cascade Impactor

Fraction	Particle size (μm)	Average (%)	SD
Actuator	not defined	38.34	3.19
Preseparator	> 10.0	45.90	3.86
Stage 0	10.0–9.0	0.23	0.03
Stage 1	9.0–5.8	0.98	0.29
Stage 2	5.8–4.7	3.69	0.26
Stage 3	4.7–3.3	2.39	0.54
Stage 4	3.3–2.1	4.78	0.53
Stage 5	2.1–1.1	3.27	0.31
Stage 6	1.1–0.7	0.31	0.25
Stage 7	0.7–0.4	0.11	0.10
Filter	< 0.4	0.00	0.01

The amount of drug in the different fractions is calculated as a percentage of the total amount of drug recovered ($n = 3$).

Table 5
Deposition of aerosols B–F in actuator, upper stage and lower stage of the twin impinger, calculated as a percentage of the total amount of drug recovered

Aerosol	Deposition (%) in					
	Actuator	SD	Upper stage	SD stage	Lower	SD
B	15.0	2.38	55.7	3.53	29.3	2.69
C	16.9	0.92	65.9	0.82	17.2	2.38
D	15.4	1.65	71.6	1.43	13.0	0.85
E	20.4	0.20	61.1	1.38	18.5	0.94
F	10.2	0.70	42.7	2.81	47.1	2.82

The standard deviations (SD) are indicated ($n = 3$).

The correlation and the differences seen here can be explained by the separation conditions in the four devices. These are not exactly identical. Apart from a different flow rate in the Andersen Impactor there are two slightly different separation mechanisms. In Apparatus A and the Four Stage Impinger the particles impinge upon a liquid surface. The inlet ports of Apparatus A, the Four Stage Impinger and the Andersen Impactor are geometrically identical round flasks. These identical conditions explain the good correlation seen with Apparatus A and the Four Stage Impinger. In Apparatus B and the Andersen Impactor, the particles impact upon solid plates. Apparatus B has a very narrow inlet tube (inner diameter 19 mm) with a 90° bend. This inlet tube geometry is responsible for the fact that most of the aerosol particles do not reach the actual separation stage.

Each of the four devices is useful for determining the particle size of an aerosol. Particles between 0.8 and 5 μm are theoretically able to reach the lower regions of the human respiratory tract and stay there (Heyder et al., 1986; Aerosol Consensus Statement, 1991). While Apparatus A and B allow for an estimation of the respirable dose, the 50% cut-off diameter of Apparatus B is too high for this. There are also differences regarding the amount of information given on the particle size distribution and regarding the analytical effort necessary to obtain the results. Apparatus A and B yield information on only two size fractions, but the analytical effort is small, too. The Four Stage Impinger already shows a simple particle size distribution with roughly the same amount of labour necessary. The most time-consuming method, the Andersen Cascade Impactor

Table 6
Deposition of aerosols B–F in the actuator and the different stages of the Four Stage Impinger, calculated as a percentage of the total amount of drug recovered

Aerosol	Deposition (%) in									
	Actuator	SD	Stage 1	SD	Stage 2	SD	Stage 3	SD	Filter	SD
B	14.4	1.59	52.8	0.91	5.7	0.89	20.1	0.97	7.0	0.97
C	10.0	3.22	63.5	5.07	7.5	0.66	14.9	1.06	4.1	0.31
D	10.2	3.80	69.9	3.04	6.3	0.36	11.0	0.49	2.6	0.18
E	13.2	4.58	63.4	11.4	6.5	2.28	14.0	4.62	2.9	0.61
F	10.0	0.33	33.3	1.54	9.9	1.77	39.4	1.90	7.4	1.14

The standard deviations (SD) are indicated ($n = 3$).

gives the most complete information on the particle size distribution. Considering all these aspects, Apparatus A and the Four Stage Impinger were chosen for a more detailed comparison in the second part of the study.

3.2. Comparison of Apparatus A and the Four Stage Impinger with Aerosols B–F

In the second part of this study five more metered dose inhalers (B–F) were compared using Apparatus A and the Four Stage Impinger. The fractions of drug depositing in the different stages of the impingers are shown in Table 5 for the Twin Impinger and Table 6 for the Four Stage Impinger.

The theoretically respirable fraction of fine particles below 6.4 μm is deposited in the lower stage of Apparatus A. With the Four Stage Impinger it is split further into two fractions that are found in stage 3 and 4 (filter). For all the aerosol formulations tested the percentage of drug that is found in these two stages together is very well in agreement with the lower stage of Apparatus A (Fig. 3). There are no statistically significant differences ($a = 0.05$) between the two methods to be found.

Looking at the aerosol formulations the data of Apparatus A show that there is a significant difference between the cromolyn sodium formulations A to E and the beclomethasone aerosol F.

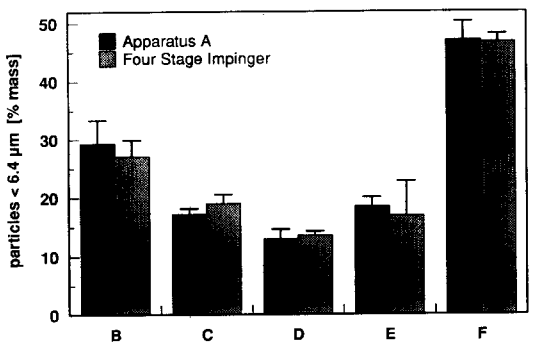

Fig. 3. The fraction of particles smaller than 6.4 μm of aerosols B–F as determined with Apparatus A and the Four Stage Impinger. Error bars represent the confidence intervals of the mean values ($a = 0.05$, $n = 3$).

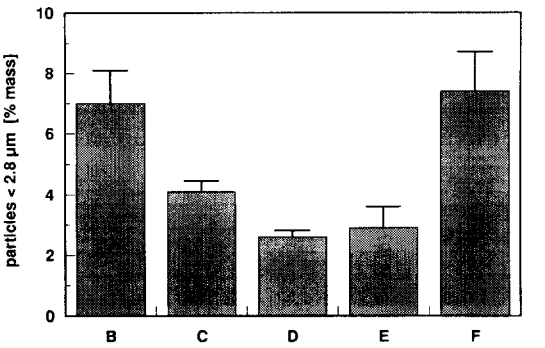

Fig. 4. The fraction of particles smaller than 2.8 μm of aerosols A–F as determined with the Four Stage Impinger. Error bars represent the confidence intervals of the mean values ($a = 0.05$, $n = 3$).

The cromolyn sodium aerosols differ in their propellant composition. Therefore different particle size distributions of the formulations should be expected. Significant differences between Aerosol B, C, D and E can be seen. It is, however, not possible to distinguish between the formulations C and E by the Twin Impinger data. The same picture is seen with the Four Stage Impinger when the particle fractions in stage 3 and stage 4 are taken together ($d < 6.4$ μm). If, however, the fractions of particles in stage 4 ($d < 2.8$ μm) are taken into account separately, then the fractions of all the cromolyn sodium formulations show statistically ($a = 0.05$) significant differences (Fig. 4). Therefore, the Four Stage Impinger allows for a better distinction of these aerosol formulations by splitting the fine particle fraction of Apparatus A into two separate fractions.

All in all, the results and especially the broad agreement in the fine particle fraction ($d < 6.4$ μm) suggest that both Apparatus A and the Four Stage Impinger are useful devices for assessing the particle size of pharmaceutical aerosols. By splitting up the two fractions of Apparatus A into four, the Four Stage Impinger gives more information on the actual particle size distribution and allows for a better distinction of the formulations with an analytical effort that is just slightly higher. So when examining similar aerosol formulations, the Four Stage Impinger may be superior to Apparatus A.

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