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The Effect of Dose on the Characterization of Aerodynamic Particle-size Distributions of Beclomethasone Dipropionate Metered-dose Inhalers

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Abstract

The aerodynamic particle-size distribution for two doses of a Becloforte metered-dose inhaler (MDI) was measured by use of a twin-stage impinger (TSI), the new multi-stage (five-stage) liquid impinger (MSLI) and the Andersen cascade impactor (ACI) (n = 5 for each apparatus). The mean (s.d.) fine-particle doses measured by the three techniques for the Becloforte MDI were 40·3 (1·2), 45·7 (0·5) and 41·8 (0·4)% w/w, respectively; the median mass aerodynamic diameters (MMAD) measured using the MSLI and the ACI were 3·50 and 3·73 μm, respectively. The MSLI fine particle (< 6·8 μm) doses for 2, 5, 10, 20, 30 and 40 doses from Becloforte MDIs (n = 5 for each dose) were 49·7 (0·7), 52·9 (1·2), 45·3 (0·6), 45·5 (0·71), 45·9 (0·7) and 46·4 (0·7)% w/w, respectively. Values obtained using the ACI (< 5·8 μm) were 40·8 (1·0), 41·0 (0·8), 44·4 (0·5), 43·1 (0·4), 42·8 (0·5) and 40·4 (0·4)% w/w (n = 4). MMAD values measured with the MSLI were 3·39, 3·46, 3·75, 3·91, 4·15 and 4·45 μm, respectively; using the ACI they were 3·46, 3·54, 3·61, 3·66, 3·73 and 3·85 μm.

The results indicate that the measured aerodynamic particle-size distributions of beclomethasone dipropionate MDIs are affected by the dose dispensed and by the apparatus used for measurement.

The Pharmacopoeias of Europe and the USA have recently begun a process of consultation leading to harmonization of test methodologies for in-vitro analysis of the aerodynamic particle-size of inhaled products (Aiche et al 1993; Byron et al 1994; Ganderton & Byron 1996). The European approach advocates the measurement of the aerodynamic particle-size distribution using one of four methods: the glass and metal twin-stage impingers (TSI), the newly modified multi-stage liquid impinger (MSLI, otherwise referred to as the Astra–Draco Impinger) and the Andersen cascade impactor (ACI). In the USA the ACI is widely used and the Marple Miller impactor has recently been introduced. Previous studies have shown that the shape and size of the sample introduction port of the impinger can influence the measured particle-size distribution of a dose from a metered-dose inhaler (Van Oort et al 1994) and thus standardization of this is required. Standardization of the number of doses used in the measurement of aerodynamic particle-size distribution is also necessary. In the past, workers have used many doses because of the low analytical sensitivity of their method. Cyr et al (1991) first revealed the importance of using individual doses from a salbutamol metered-dose inhaler (MDI). Further work by Nasr & Allgire (1995), using the old-style multi-stage liquid impinger, showed that the aerodynamic particle-size distribution measured for ten doses from a salbutamol MDI were larger than when only one dose was used.

We have developed a high-performance liquid chromatography (HPLC) assay of sufficient sensitivity to enable the aerodynamic particle-size distribution of therapeutic doses from beclamethasone dipropionate metered-dose inhalers (BDP MDIs) to be measured. This in turn has enabled us to compare the distributions obtained from the three most commonly used impingers (glass TSI,

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MSLI and the ACI). We have also investigated the effect of dose on the measured aerodynamic particle-size distribution of BDP MDIs using the recently modified MSLI and also the ACI.

Materials and Methods

Comparison of TSI, MSLI and ACI particle-size distributions using therapeutic doses from BDP MDIs

The TSI was the glass impinger (Apparatus A British Pharmacopoeia 1993, Appendix XVII) and the MSLI was the new five-stage impinger. Both have the same glass throat for the introduction port. The introduction port for the ACI was a metal throat.

Initially the test MDI canister was removed from its original actuator (mouthpiece) and placed into another actuator. The original actuator was washed, rinsed with distilled water and dried in a stream of nitrogen. Each MDI canister, fitted into the temporary actuator, was shaken for 5 s and a dose was discarded to waste. This was repeated at 25-s intervals until five doses had been discarded. The test MDI canister was then taken out of the temporary actuator and put back into the original (washed and dried) actuator for each determination of particle-size distribution.

Before introduction of the first dose into the TSI, MSLI or ACI the flow rate of each apparatus was adjusted accordingly (60 L min^{-1} for the TSI and MSLI, 28·3 L min^{-1} for the ACI). The pump was then switched on for 30 s before introduction of the first dose and remained operational until 30 s after the last dose. For each dose the MDI was shaken for 5 s before the release of each single dose. The MDI was tightly fitted into the introduction port and after firing it was left in-situ for 20 s. The MDI was removed from the introduction port and the whole procedure, starting with shaking for 5 s, was repeated. The time interval between doses was 30 s. This dosing procedure was repeated until the required number of doses had been delivered into the appropriate impactor. Measurement of the aerodynamic particle-size distribution for the required number of doses was repeated five times for each method of impaction.

Each of the four stages of the MSLI was charged with 20 mL of the mobile phase used for HPLC (acetonitrile–potassium dihydrogen phosphate (0·05 M; pH 7; 60:40 (v/v)). The mobile phase was also placed in the upper (stage 1, 5 mL) and lower (stage 2, 20 mL) impinger chambers of the TSI. The aerodynamic particle-size distribution was measured for two doses from a Becloforte (Allen & Hanbury, UK) MDI, which delivers 250 μg BDP per dose. After the vacuum pump had been switched off each apparatus was disassembled. The solutions from stages 1 and 2 of the TSI were transferred into separate 100-mL volumetric flasks. Each stage was rinsed with mobile phase (3 × 25 mL), to recover all the BDP and the washings were added to the appropriate flasks. Each solution was then diluted to volume with mobile phase. The actuator of the MDI was rinsed with mobile phase (3 × 25 mL) and the washings collected in a volumetric flask and diluted to the appropriate volume. This procedure was repeated for the throat of the TSI. Stages 1–4 of the MSLI, and the actuator and the throat, were handled as for the TSI. The filter paper of the MSLI (Whatman GF/A filter) in mobile phase (25 mL) in a beaker was placed in an ultrasonic bath for 5 min and the solution was then poured into a 100-mL volumetric flask. This was repeated twice and the combined extracts were then diluted to the appropriate volume. The eight stages of the ACI were transferred to individual dishes containing mobile phase (25 mL) and placed in an ultrasonic bath for 5 min. Each solution was transferred to separate 100-mL volumetric flasks. This was repeated twice to recover all the BDP from each stage of the ACI. All solutions were made up to the required volume with mobile phase. The filter paper, MDI actuator and throat of the ACI were handled using the same procedure as the MSLI. The content of BDP in each solution was determined by HPLC.

The effect of BDP dose on the measured aerodynamic particle-size distribution

The procedures for the MSLI and ACI (detailed above) were repeated using 2, 5, 10, 20, 30 and 40 doses from a Becloforte MDI. A modification of the MSLI method was necessary for 20, 30 and 40 doses because of significant evaporation of acetonitrile, which could have affected the solubility of BDP. For these doses the pump was switched off every 10 doses and the acetonitrile that had evaporated was replaced. Measurement of the aerodynamic particle-size distribution for the required number of doses was repeated five times for the MSLI and four times for the ACI.

Data analysis

The amount of BDP on each stage of the apparatus, deposited in the throat, left in the actuator and trapped in the final filter of the TSI, MSLI and ACI (as appropriate) were determined. Aerodynamic particle-size distributions were generated from the MSLI and ACI depositions by use of probability log plots (Nasr 1993; Hindle et al 1996). The BDP left in the actuator and deposited on the throat (and

for the MSLI the amount on stage 1 was also included) was subtracted from the total recovered. This value was used to calculate the cumulative percentage (undersize) on stages 3 and 4 and on the final filter of the MSLI. On the probability scale this was plotted against the logarithm of the effective cut-off diameter (ECD) of each stage. Linear regression analysis was applied to the data and the MMAD (mass median aerodynamic diameter) was determined as the value of the effective cut-off diameter at a cumulative percentage $< 50\%$. Similar aerodynamic particle-size distribution analysis was used for the ACI data (using stages 1–7 and the final filter) with the exception that linear regression analysis was applied only to the middle four points (ECDs 2·1, 3·3, 4·7 and 5·8 μm) because of the unreliability of the data if all points are used (Nasr & Allgire 1995). The geometric standard deviation (GSD) for each was calculated by dividing the MMAD by the ECD value for the cumulative percentage undersize at 15·87%.

Results

The mean (s.d.) total recoveries of BDP from a Becloforte MDI measured by use of the TSI, MSLI and ACI were 102·7 (1·0), 104·0 (0·5) and 104·0 (0·7)% w/w (of the labelled dose), respectively. The mean (s.d.) fine-particle doses measured by use of the TSI (particles $< 6{\cdot}4$ μm), MSLI ($< 6{\cdot}8$ μm) and ACI ($< 5{\cdot}8$ μm) for the Becloforte MDI were 40·3 (1·2), 45·7 (0·5) and 41·9 (0·4)% w/w. From the probability log plots the MMAD obtained by use of the MSLI was 3·50 μm and that by use of the ACI was 3·73 μm. The GSD values obtained by use of the MSLI and ACI were 1·67 and 1·41, respectively. Only one Becloforte was used for these determinations.

Nine Becloforte MDIs were used to determine the effect of dose on particle-size distribution. Five MDIs (one set of doses from each) were used to generate the particle-size distributions measured by the ACI and one set of doses from each of the other four MDIs were used to generate those measured by MSLI. The order of doses into each apparatus was randomized. The total recovery of BDP from the MSLI after 2, 5, 10, 20, 30 and 40 doses was 104·8 (1·7), 99·6 (2·2), 97·9 (2·7), 99·0 (2·0), 97·4 (1·8) and 96·4 (2·8)% w/w (of the labelled dose), respectively. Similar values for the ACI were 103·3 (4·43), 101·4 (2·7), 101·6 (5·6), 100·1 (5·0), 94·6 (2·3) and 99·1 (5·4)% w/w. Tables 1 and 2 list the percentage distribution obtained from the MSLI and ACI, respectively, for the different numbers of doses from the Becloforte MDIs. From these, Table 3 shows the fine-particle doses measured by use of the MSLI ($< 6{\cdot}8$ μm) and the ACI ($< 5{\cdot}8$ μm). The MMAD and GSD are also shown in Table 3.

Discussion

It was not possible to determine the particle-size distribution for one dose of beclomethasone because the limits of quantification were reached at this assay level. Each apparatus used for the measurement of the aerodynamic particle-size characteristics has a different design and thus the definition of the fine-particle dose is dependent on the cut-off diameters of their stages. Although it has been shown that the MMAD and GSD for 1, 4 and 10 doses from a salbutamol MDI were similar when using uncoated plates from the Andersen and Marple Miller cascade impactors (Nasr et al 1997), differences between the two impactor results were noted when the plates were coated with silicone or glycerine to prevent the phenomena of particle bounce and the loading effect (Graham et al 1995; Nasr & Allgire 1995). It is unclear why the coating caused the differences between the results from the two impactors but it could be because of an effect on the cut-off diameters of the plates. Holzner &

Table 1. Mean distribution (% of emitted dose), for different numbers of doses, from the Becloforte metered-dose inhaler (MDI) measured using the multi-stage liquid impinger.

Deposition site	Effective cut-off diameter (μm)	Dose					
		2	5	10	20	30	40
MDI actuator		11·9 (0·9)	8·9 (0·6)	5·6 (0·8)	4·4 (0·5)	4·6 (0·5)	4·5 (0·2)
Throat		37·9 (1·5)	37·6 (1·7)	48·2 (2·7)	48·3 (2·3)	47·5 (2·5)	46·0 (2·9)
Stage 1	> 13	0·5 (0·2)	0·7 (0·1)	1·0 (0·1)	1·7 (0·2)	2·1 (0·3)	3·2 (0·3)
Stage 2	13·0	4·5 (0·4)	4·9 (0·5)	5·1 (0·5)	6·0 (0·8)	7·3 (0·7)	7·8 (0·7)
Stage 3	6·8	23·2 (1·0)	25·6 (1·2)	22·6 (0·6)	22·8 (0·6)	23·1 (0·9)	23·2 (0·8)
Stage 4	3·1	17·1 (0·6)	16·7 (1·6)	12·9 (0·8)	12·1 (1·0)	11·7 (1·2)	12·4 (1·2)
Final filter	1·7	4·9 (0·4)	5·7 (0·7)	4·7 (0·4)	4·6 (0·7)	3·8 (0·3)	3·0 (0·4)

The figures in parentheses are standard deviations (n = 5).

Table 2. Mean distribution (% of emitted dose), for different numbers of doses, from the Becloforte metered-dose inhaler (MDI) measured using the Andersen cascade impactor.

Deposition site	Effective cut-off diameter (μm)	Dose					
		2	5	10	20	30	40
MDI actuator		16·4 (0·9)	11·7 (1·8)	9·5 (1·8)	8·4 (1·3)	8·4 (0·8)	8·5 (0·5)
Throat		42·0 (4·5)	46·1 (5·3)	44·6 (2·9)	46·9 (4·8)	46·9 (2·5)	48·5 (4·7)
Stage 0	10·0	0·8 (0·3)	1·2 (0·2)	1·4 (0·2)	1·7 (0·2)	1·9 (0·2)	2·5 (0·2)
Stage 1	9·0	2·3 (0·6)	2·2 (0·2)	2·5 (0·3)	2·9 (0·2)	3·0 (0·2)	3·3 (0·3)
Stage 2	5·8	5·4 (0·2)	5·8 (0·3)	6·7 (0·6)	6·6 (0·4)	6·6 (0·3)	7·6 (0·7)
Stage 3	4·7	14·7 (2·5)	16·4 (1·5)	18·4 (1·6)	17·9 (1·2)	17·6 (1·2)	16·4 (0·8)
Stage 4	3·3	12·0 (2·4)	12·1 (2·3)	13·3 (0·8)	12·5 (0·6)	12·6 (1·1)	10·6 (1·0)
Stage 5	2·1	4·0 (0·9)	3·1 (0·6)	2·8 (0·3)	2·6 (0·2)	2·6 (0·3)	2·1 (0·2)
Stage 6	1·1	1·0 (0·1)	0·6 (0·2)	0·4 (0·1)	0·3 (0·1)	0·2 (0·1)	0·2 (0·1)
Stage 7	0·7	0·4 (0·3)	0·2 (0·2)	0·1 (0·1)	0·1 (0·1)	0·1 (0·1)	0·1 (0·1)
Final filter	0·4	1·0 (0·3)	0·6 (0·2)	0·2 (0·1)	0·2 (0·1)	0·1 (0·1)	0·1 (0·1)

The figures in parentheses are standard deviations (n = 4).

Table 3. Aerodynamic particle-size characteristics.

Multi-stage liquid impinger	Number of doses					
	2	5	10	20	30	40
Mean fine particle dose (%)	49·7 (0·7)	52·9 (1·2)	45·3 (0·6)	45·5 (0·8)	45·9 (0·8)	46·4 (0·8)
Median mass aerodynamic diameter (μm)	3·39	3·46	3·75	3·91	4·15	4·45
Geometric standard deviation	1·80	1·82	1·83	1·85	1·90	1·93
Andersen cascade impactor						
Mean fine particle dose (%)	40·8 (1·0)	41·0 (0·8)	44·4 (0·5)	43·1 (0·4)	42·8 (0·5)	40·4 (0·4)
Median mass aerodynamic diameter (μm)	3·46	3·54	3·61	3·66	3·73	3·85
Geometric standard deviation	1·48	1·48	1·44	1·45	1·44	1·46

The mean fine-particle dose is expressed as a percentage of the emitted dose.

Muller (1995) have reported excellent agreement for the fine-particle dose between Appendix A (British Pharmacopoeia 1993) and the four-stage liquid impinger and between Apparatus B (British Pharmacopoeia 1993) and the Andersen cascade impactor. The three impactors used in our study gave different results. In general the fine-particle dose from the beclomethasone MDIs determined by use of the Andersen cascade impactor was smaller than that determined by use of the multi-stage liquid impinger. The effect on the aerodynamic particle-size characteristics of increasing the dose is more pronounced for the multi-stage liquid impinger.

For salbutamol MDIs the MMAD of one dose (Miller & Schulz 1992; Nasr 1993) has been reported to be lower than for 5–40 doses (Kim et al 1985; Fults et al 1991). Nasr & Allgire (1995) have shown that this was because of particle bounce and loading. The effect on the aerodynamic particle-size distribution measured by the Andersen system with increasing beclomethasone dose clearly demonstrates the effect of particle bounce and loading. These are highlighted in Table 2 by the different deposition on each stage of the Andersen cascade impactor with increasing dose. Whether this phenomena occurs with the multi-stage liquid impinger is not yet proven but the result of increasing the number of doses is similar to that for the Andersen cascade impactor. The different deposition on stages 2 and 4 and the final filter of the multi-stage liquid impinger (Table 1) do suggest this effect. The decrease in deposition on the actuators, expressed as a percentage of the dose emitted, of the Andersen cascade impactor and of the multi-stage liquid impinger when increasing numbers of doses are used implies overloading in that the actuators can retain only a certain amount of drug. The effect of this is increased deposition in the throat which could also be caused by the

loading effect. The results could explain why Barnes & Nash (1996), who measured the aerodynamic particle-size distribution of 10 beclomethasone doses in their multi-stage liquid impinger, reported the fine-particle dose to be different from that measured by Kenyon et al (1995) who used 40 doses.

If particle bounce does occur in the multi-stage liquid impinger it could result either from the liquid surface or from the glass wall of each stage. The effect of dose on the result from the multi-stage liquid impinger could also be a result of electrostatic forces. Similarly, the effect of loading is difficult to explain. Solubility problems might have affected the results because beclomethasone is not very water-soluble, and thus a mixture of water and acetonitrile was used on each plate. After introduction of each tenth dose the pump was switched off and acetonitrile added to each stage to replace the amount lost by evaporation (as a result of the continuous air stream sucked through the impinger). Stopping the pump every 10 doses could explain why the fine-particle dose does not change as the number of doses is increased. However, the particle-size distribution within this parameter does change, as shown in Table 1.

It is proposed that there is a need for some standardization consensus in the choice of procedure. In this respect the Andersen cascade impactor provides more detailed information on particle-size distribution because of the greater number of plates. At present the Anderson cascade impactor is the most commonly used procedure for measuring particle size. It has recently been proposed that immersion of the plates in 1% silicone (in hexane) to coat the stages will prevent particle bounce. The drawback is that this might enhance the loading effect if many doses are used. If this coating procedure is to be adopted to minimize particle bounce (and the loading effect), then recalibration of the cut-off diameters might be necessary. Also the usefulness of the prevention of particle bounce should be considered, because this phenomena will occur in the respiratory tract. Similarly there should be some examination of the number of doses used. This is an easier decision to make because on no occasion should the number of doses be more than the number used in clinical practice. Furthermore, the lower the number of doses the smaller the loading effect.

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References

Aiche, J. M., Bull, H., Ganderton, D., Olsson, B., Wright, P. (1993) Inhalations: collaborative study on the measurement of the fine-particle dose using inertial impactor. Pharmeuropa 5: 386–389

Barnes, A. R., Nash, S. (1996) Comparisons of aerodynamic particle size from 250 μg per dose beclomethasone dipropionate metered-dose inhalers. Int. J. Pharm. 139: 255–258

Byron, P. R., Kelly, E. L., Kontny, M. J., Lovering, E. G., Puochikian, G. K., Sethi, S., Thiel, G. G., Vadus, E. B. (1994) Recommendations of the USP advisory panel on aerosols on the USP general chapters on aerosols [601] and uniformity of dosage units [905]. Pharm. Forum. 20: 7477–7503

Cyr, T. D., Graham, S. J., Li, K. Y. R., Lovering, E. G. (1991) Low first spray drug content in albuterol metered-dose inhalers. Pharm. Res. 8: 658–680

Fults, K., Cyr, T. D., Hickey, A. J. (1991) The influence of sampling chamber dimensions on aerosol particle size measurements by cascade impactor and twin impinger. J. Pharm. Pharmacol. 43: 726–728

Ganderton, D., Byron, P. R. (1996) Harmonising Inhaler Testing Across the Pharmocopeias, Repiratory Drug Delivery V. Interpharm Press, Buffalo Grove, II. 283–292

Graham, S. J., Lawrence, R. C., Ormsby, E. D., Piker, R. K. (1995) Particle-size distribution of single and multiple sprays of salbutamol metered-dose inhalers. Pharm. Res. 12: 1380–1384

Hindle, M., Byron, P. R., Miller, N. C. (1996) Cascade impaction methods for dry powder inhaler using the high flow rate Marple Miller impactor. Int. J. Pharm. 134: 137–146

Holzner, P. M., Muller, B. W. (1995) Particle size determination of metered-dose inhalers with inertial separation methods. Apparatus A and apparatus B (BP), 4-stage impinger and Andersen Mark-II cascade impactor. Int. J. Pharm. 116: 11–18

Kenyon, G. J., Dewsbury, N. J., Newman, S. P. (1995) Differences in aerodynamic particle-size distributions of innovator and generic beclomethasone dipropionate aerosols used with and without a large volume spacer. Thorax 50: 846–850

Kim, C. S., Trujillo, D., Sackner, M. (1985) Size aspects of metered-dose inhalers. Am. Rev. Respir. Dis. 132: 137–142

Miller, N. C., Schulz, R. K. (1992) Size analysis of single shots of metered-dose inhaler (MDI) sprays by cascade impactor. Pharm. Res. 9 (Suppl.): S-142

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

Nasr, M. M., Allgire, J. F. (1995) Loading effect on particle size measurements by inertial sampling of albuterol metered-dose inhalers. Pharm. Res. 12: 1677–1681

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 using the Andersen and Marple Miller cascade impactors. Pharm. Res. 14: 1437–1443

Van Oort, M., Gullmar, R. U., Bohirski, R. J. (1994) Effects of sampling chamber volume and geometry on aerodynamic size distributions of metered dose inhalation aerosols measured with the Andersen cascade impactor. Pharm. Res. 11: 604–607