ELSEVIER

International Journal of Pharmaceutics 149 (1997) 51–61

international journal of pharmaceutics

Optimization of the operation conditions of an Andersen-Cascade impactor and the relationship to centrifugal adhesion measurements to aid the development of dry powder inhalations

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Received 21 October 1996; accepted 12 December 1996

Abstract

The standardization of the operation conditions of an Andersen-Cascade impactor (Mark II), based on the physical relationship between adhesion and aerodynamic particle behaviour, has been undertaken using the Serevent® Disk-haler. In this case, the drug (Salmeterol xinafoate) is primarily adhered to a carrier material (lactose monohydrate), and thus the standard operation procedure to use this type of impactor had to be modified. For the modified procedure, operating conditions such as air flow rate, thickness of a silicon coat of the impaction plates and drug loading were optimized, bearing in mind the findings reported in the literature about the relationship between adhesion and impaction on plates. For this particular drug, the optimal thickness of the silicon film was found to be 3.75 μm, and the drug loading should not exceed 400 μg. Although any air flow velocity between 20 and 60 l min^{-1} gave a physically correct aerodynamic particle size for this drug and the chosen inhaler device, a maximum amount of drug was released from the device only applying flow rates between 50 and 60 l min^{-1}. Four different industrially manufactured Serevent® batches were studied under these optimized operation conditions. Using centrifugal adhesion force measurements, the median adhesion force between Salmeterol xinafoate particles and lactose monohydrate carrier particles was established and linked to the aerodynamic behaviour of the drug using the Cascade impactor. The median adhesion force (F_{ad}) and the mass median aerodynamic diameter (MMAD) were found to be directly proportional. Statistical analysis, however, indicated that the differences in F_{ad} and MMAD were not significant between the tested batches. The procedure established that the formulation had been produced with high accuracy and reproducibility. The sensitivity of the adhesion force measurements to variability in the manufacturing process would allow an in-process control of the mixture and could help to assure this high standard. © 1997 Elsevier Science B.V.

Keywords: Andersen-Cascade impactor; Centrifugal adhesion measurements; Dry powder inhalations; Serevent®

PII S0378-5173(96)04850-8

1. Introduction

The in vitro test of aerosol products is an essential feature during the development stage and is an in-process and quality control measurement to guarantee a satisfactory in vivo effect after application, for example for the treatment of lung related diseases such as asthma. The size distribution of aerosol particles will strongly influence the therapeutic effect due to its proportional deposition in the respiratory tract. Particles larger than 25 μm are most unlikely to penetrate the airways, whereas those smaller than about 0.3 μm are usually exhaled (Warnke et al., 1994).

In principle, two general ways of in vitro testing exist: (a) the direct determination of the particle size of the aerosol cloud, (b) the fractionated collection of the particles using impactors. The advantage of using impactors is, that the aerodynamic particle size distribution can be found from pure determination of weight (Bürkholz, 1973). However, when compared to direct particle size measurements, the values obtained by impaction will contain considerable errors (Schuch and Löffler, 1975; Bartz et al., 1978; Fißan et al., 1979), which require detailed mathematical analysis. Aiache et al. (1993) studied the performance of different impactors in terms of including them into the European Pharmacopoeia as standard methods. They found, that the use of the Multi-stage Anderson-Cascade Impactor (Mark II) was associated with a larger variability of the results due to the more complex assessment procedure involved. Nevertheless, this method is more often used in the development stage to obtain more detailed information about the aerodynamic particle size distribution of the respirable fraction of the drug, whereas simpler techniques are used for in-process and quality control.

Byron (1986) pointed out that adhesion forces are the main physical factors determining the re-suspension of the micronized drug from the carrier during inhalation. Friction forces are however also largely involved. Hence, it appears useful to link adhesion and friction force measurements, which only require very small quantities of material, to the in vitro testing of dry powder inhalations. Adhesion and friction can be measured between single particles for example using a centrifuge (Podczeck et al., 1995a,b). When measured by the centrifuge method, variables of concern such as surface properties of the carrier particles or carrier particle size (Timsina et al., 1994) can be studied with respect to the adhesion and friction forces between drug and carrier particles.

The aim of this work was to find a general link between the median adhesion force between a drug (Salmeterol xinafoate) and lactose monohydrate carrier particles, as used in the commercially available dry powder inhalation product Serevent®, and the aerodynamic behaviour of the drug using the Andersen-Cascade impactor (Mark II). For this reason, the impactor test procedure had to be modified and optimized conditions had to be found. These optimal operation conditions were sought with respect to the physical laws governing the impaction of airborne particles on plates.

2. Materials and methods

2.1. Materials

Four batches of Serevent® Diskhaler (Allen and Hanburys, Uxbridge, UK), randomly chosen, were tested and are labelled with batch *a–d*.

For the chemical evaluation of the drug by means of an HPLC method, the following materials were used: methanol HPLC grade, analar grades of hexane, sodium dodecyl sulphate, glacial acetic acid, silicon oil (BDH, Poole, UK). The silicon oil is characterised by the following parameters: density 0.976 g cm^{-3}, refractive index 1.403, kinematic viscosity 60 000 m^2 s^{-1} and surface tension 21.5 mNm^{-1}. Salmeterol hydroxynaphthoate (Glaxo Research and Development, Ware, UK) was used as calibration standard (purity 99.7%).

2.2. Methods

The adhesion force between drug and lactose monohydrate carrier particles was determined using the centrifuge technique, which is described in detail by Podczeck and Newton (1995). An Ultracentrifuge (Centrikon T-1080, Kontron Instru-

ments, Milan, Italy) with a vertical rotor (TV-850, DuPont Sorvall, Wilmington, USA) and a set of specially developed adapters (Ventura Scientific, Orpington, UK) was used. The adhesion samples were prepared using aluminium disks as support surfaces, which were covered on one side with a double-sided sticky tape. A paper ring of matching size, which had an inner open area of about 7 mm diameter, was stuck on top of the sticky tape allowing the removal of the disks from the adapters in the experimental stage. Particles of the interactive mixtures (Serevent®) were sprinkled on top of the remaining sticky surface, and the disks were gently tapped to allow an orientation of the lactose monohydrate carrier particles to be attached in their most stable position. The number of drug particles initially adhered to the carrier particles was determined using an image analyzer (Seescan Solitaire 512, Cambridge, UK), attached to a microscope (Olympus BH-2, Tokyo, Japan) in a manual mode. The surfaces were illuminated using a cold light source (High Light 3002, Olympus, Hamburg, Germany), which was attached parallel to the surface, about 1.5 cm from the periphery. The two light beams were placed at an angle of 180° to each other to minimize the formation of shadows. The initial number of drug particles was about 150 per surface, and six surfaces were used parallel in each experiment. A spin-off force was applied, and the number of particles remained adhered was determined. An adhesion force distribution was obtained by successively increasing the spin-off force after each counting. The median adhesion force, which is the force value where 50% of the particles are detached, and the interquartile range, which is the difference of the force values necessary to detach 75% and 25% of the particles initially adhered, was calculated from each individual adhesion force distribution. In this way, a quantitative characterization of the average adhesion force and its variability was possible without making any assumption about the nature of the underlying distribution function. All results are the mean and standard deviation of these characteristic values using six replicates.

The mass median aerodynamic diameter of the drug particles was determined using a cascade impactor (Mark II, Graseby Andersen, Atlanta, GA) attached to a vacuum pump with an adjustable nominal flow rate between 6 and 100 l min^{-1} (Copley, Nottingham, UK). Due to loss impaired by the impactor, an experimental flow rate between 6 and 70 l min^{-1} can be achieved. To reduce the rebouncing effect due to the high elasticity of the drug particles, the stainless steel collection plates were coated with silicon oil. The silicon oil was suspended in hexane (1% w/w for film thickness up to 2.5 μm, 2% w/w for thicker films). An accurately defined volume of the suspension was distributed on each collection plate, and the plates were left to dry under ambient room conditions at least for 1 h. The theoretical film thickness was calculated from the plate surface area and the amount of silicon oil suspended in the defined volume applied. Film thicknesses between 1.25 and 5.00 μm were used. A high-efficiency preseparator (Graseby Andersen, Atlanta, USA) was also used. The glass throat used has been described by the European Community Pharmacopoeia Commission (1993), and is the standard throat required by the European Pharmacopoeia. Prior to each experiment the accuracy of the flow rate selected was tested and adjustments were made when necessary. Afterwards, the preseparator was filled with 5.0 ml methanol and the mouth piece was attached to the throat. The selected number of disks were cleaned with acetone to remove all ink from the aluminium foil. A disk containing four blisters was inserted into the inhaler device, and a blister was opened lifting the inhaler device lid and thus pushing the piercing pin into the blister. The lid was carefully closed avoiding the loss of any powder adhered to the piercing pin. The device was inserted into the mouth piece assuring its correct horizontal position. The pump was switched on for 3 s. The inhaler device was removed from the mouth piece, and the disk was turned so that a further blister could be opened. In this way, the desired number of blisters was used. Empty disks were stored in a Petri dish, and also the inhaler device was stored in a Petri dish, until the analytical assessment of the drug content, which remained, took place.

The amount of drug on each collection plate, in the backup filter, in the throat, preseparator,

device stage (i.e. in the empty disks and the inhaler device) and on the impactor walls (i.e. the stainless steel stages without collecting plates) was determined using an HPLC method. Under standard conditions, i.e. using eight blisters, the drug was washed into volumetric flasks of the following volume using methanol: device, throat, preseparator into 100.00 ml each, collection plates 0 to 7 and backup filter into 25.00 ml each, impactor walls into 50.00 ml. Thus, 13 different samples were collected for each experiment. For experiments using 12 or 16 blisters, the volume of the volumetric flasks was doubled.

A carefully degassed buffered mobile phase, which contained methanol and aqueous buffer (sodium dodecyl sulphate/acetic acid 0.0025 M) in a ratio of 10:1 was used in the HPLC procedure. A short Hypersil column (ODS 5 μm, Shandon HPLC, Runcorn, UK) was placed into a temperature control unit (TC 1900, ICI, Instruments, Dingley, Australia) maintaining a working temperature of 40°C. The flow rate of the mobile phase was set to 1 ml min^{-1} using a standard pump module (Consta Metric 3000, LDC, Milton Roy, UK). The drug content was detected using a fluorescence detector (ABI 980, ABI Instruments, UK). The excitation wave length was 225 nm, and the emission wave length was 345 nm. The light intensity was recorded using a chart recorder (Servoscribe RE 511.20, BBC Goerz, Metrawatt, Vienna, Austria). The area under the peaks was measured using a planimeter (Allbrit, London, UK). Usually, 100 μl were injected via an injection port with an 100.0-μl injection loop. Each sample solution was injected at least twice to guarantee the reproducibility of the results. The sensitivity of the detector was adjusted in accordance with the concentration of the drug. A standard solution of 0.7 μg ml^{-1} salmeterol hydroxynaphthoate was used to calibrate the method. The standard was injected at least twice for each level of sensitivity. A short computer program was written to calculate the amount of drug per sample and blister from the analytical data.

The mass median aerodynamic diameter and its geometric standard deviation were calculated using probit analysis, based on the amount of drug impacted on stages 0 to 7, using the statistical package SPSS V4.0.1 (SPSS, UK).

3. Results and discussion

In standard operation procedures issued for the Andersen-Cascade impactor, usually the amount of drug withheld by the preseparator and deposited on collection plate 0 is analyzed together and quoted as a single drug entity (so-called stage 0). However, in formulations where the drug is delivered as an interactive mixture, i.e. adhered to a carrier material of non-respirable size, the loss of drug in the preseparator can be large, and together with the amount of drug deposited on collection plate 0 more than 50% of the drug is often removed from the air stream. Hence, it is difficult or impossible to derive a mass median aerodynamic diameter (MMAD) for the formulation. Therefore, it appears reasonable to split the stage 0 as defined in the standard operation procedures into its two components and to analyze the amount of drug in the preseparator and on collection plate 0 separately. It is also not known whether the drug found in the preseparator has detached from the carrier particles or not. Presumably, some fine drug particles deposited will still be adhering to the carrier particles, and a further quantity of drug, mainly larger particles above 5 μm, will have been removed at this stage according to their aerodynamic behaviour. Thus, it would be incorrect to use the amount of drug found in the preseparator for the determination of the MMAD, which should be entirely based on the respirable drug, i.e. on the amount of drug detached from the carrier material. In this study, the following definitions were therefore made: first, all drug that was found in the device, throat and preseparator is classified as drug unavailable for respiration. The total amount of drug deposited on the collection plates 0 to 7, lost on the impactor walls and recovered from the filter is regarded as the drug proportion that has been detached from the carrier particles, but the respirable fraction is calculated without the amount of drug lost on the impactor walls. Secondly, the amount of drug found on each collection plate is

calculated as percentage of the drug of the respirable fraction, thus normalized to give a mass aerodynamic particle size distribution spreading between 0 and 100%. Therefore, the MMAD is taken from the cumulative percentage of the respirable amount of drug as a function of the effective cut-off diameter. Both MMAD and the geometric standard deviation were assessed using Probit-Regression analysis.

With respect to the outcome of a cascade impactor analysis, the number of particles adhering to plates placed in an air stream is determined by the particle concentration of the air stream, the properties of the particles and surfaces, the flow velocity and the relative position of the surface to the flow axis. In a cascade impactor the latter is fixed perpendicular to the air stream and hence cannot be regarded as a variable to control particle deposition. However, the properties of the collection surfaces, the air flow velocity and the drug loading are parameters, for which optimization is crucial for the reproducibility of experiments, and which have to be determined for any drug separately to match the physical properties of the drug.

The presence of an oil film on the collection plate surface promotes adhesion and is especially useful, if the impinging particles are very compliant compared to the collection surfaces (Zimon, 1982). The Young's modulus of Salmeterol xinafoate indicates a high elasticity (Podczeck et al., 1995a), which is in contrast to the stiffness of stainless steel collection plates. Hence, a coating with a compliant material such as silicon oil appeared necessary. However, it is essential to control exactly the thickness of such an oil film, because the amount of particles adhering will increase proportionally with the film thickness (Dahneke, 1971). The optimal film thickness to ensure an efficient capture of the particles has been reported to be about half the particle diameter (Zimon, 1982). Therefore, experiments were undertaken to optimize the thickness of the silicon oil film applied to the collection plates in the cascade impactor. An air flow rate of 60 l min^{-1} was chosen as standard condition. The maximum particle size of the drug was 8.3 μm, and hence film thicknesses between 0 and 5 μm were tested. Fig. 1 shows the cumulative aerodynamic particle size distributions, and Table 1 summarizes the results.

The optimum film thickness appears to be 3.75 μm, because the smallest MMAD was found using this film thickness (see Fig. 2). On average, 20 to 30% of the drug initially adhered to the lactose monohydrate carrier particles was freely available regardless of the actual film thickness. Thus, the smaller is the MMAD, the more respirable drug can reach the lower airways. At a film thickness of 5 μm the majority of drug adhered to collection plate 0, indicating that an oil film, which is too thick, causes embedment of the particles. Following Dahneke's results one should in fact optimize the film thickness for each collection plate separately. However, this appears impractical and thus for all further experiments the thickness of the silicon oil film was maintained exactly at 3.75 μm on each plate.

Only initially, when no particles adhere to the collection plate surfaces, will the number of deposited particles be proportional to the number of particles striking the surface. Afterwards, the probability as to whether particles will hit particles already adhering rather than the surface increases, and therefore the probability of particle rebound is increased (Zimon, 1982). However, if too many particles are in the air stream, which then cover the collection plate surface, the adhe-

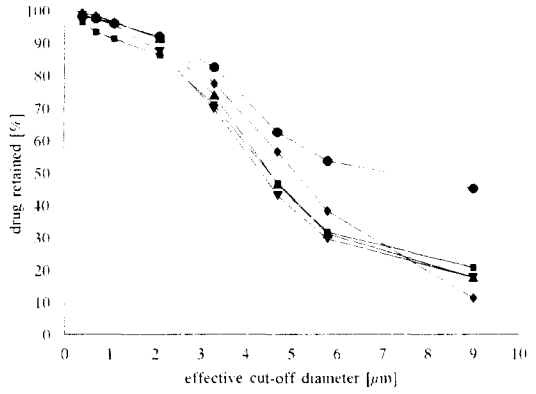

Fig. 1. Cumulative aerodynamic particle size distributions for Salmeterol xinafoate at different silicon oil film thicknesses (air flow rate 60 l min^{-1}, 8 single doses). ■, no film; ◆, 1.25 μm; ▲, 2.50 μm; ▼, 3.75 μm; ●, 5.00 μm.

Table 1
Mass median aerodynamic diameter and loss of drug, tested using different silicon oil film thicknesses at a flow rate of 60 l min^{-1} using eight single doses

	Film thickness (μm)				
	0.00	1.25	2.50	3.75	5.00
1. Drug on carrier					
Device [%]	18.71	10.92	14.69	17.50	9.95
Throat [%]	26.97	24.81	21.04	27.72	19.96
Preseparator [%]	33.54	26.23	36.05	28.66	47.87
Total [%]	78.22	61.96	71.78	74.88	77.78
2. Detached drug					
Total [%]	21.78	38.04	28.22	25.12	22.22
Loss on walls [%]	2.84	12.22	5.59	8.12	3.47
MMAD [μm]	4.42	4.83	4.58	4.31	7.60
GSD [μm]	0.77	0.83	0.83	0.81	0.75

MMAD, mass median aerodynamic diameter; GSD, geometric standard deviation.

sion becomes more and more related to the properties of the particle layer formed. The main feature will be the apparent surface roughness of the particle layer, which if large enough will cause increased particle adhesion. Such phenomenon was previously observed by Graham et al. (1995) and Nasr and Allgire (1995), and can be tested using different numbers of single doses per experiment. Three different loading levels (8, 12 and 16 single doses, equivalent to 400, 600 and 800 μg drug, respectively) were tested. Fig. 3 shows the cumulative aerodynamic particle size distributions for these experiments, and Table 2 summarizes the results.

Overloading is characterized by an increase in MMAD, and therefore the results suggest that 600 μg per experiment already considerably overloads the cascade impactor. To test this assumption, the collection plates were examined microscopically. Multiple layers of drug and fine lactose monohydrate particles were already found for a drug loading of 400 μg, and there was clearly an increase of particle layers with increase

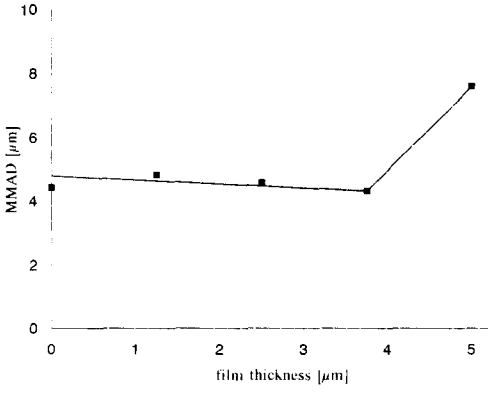

Fig. 2. Mass median aerodynamic diameter (MMAD) as a function of the film thickness of the silicon oil at the collection plates.

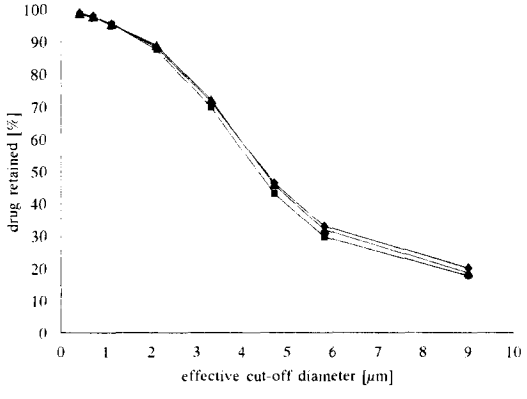

Fig. 3. Cumulative aerodynamic particle size distributions for Salmeterol xinafoate using different drug loadings (air flow rate 60 l min^{-1}, silicon oil film thickness 3.75 μm). ■, 8 single doses (= 400 μg drug); ◆, 12 single doses (= 600 μg drug); ▲, 16 single doses (= 800 μg drug).

Table 2
Mass median aerodynamic diameter and drug loss, tested using different numbers of single doses at a flow rate of 60 l min^{-1} using a film thickness of 3.75 μm

	Number of doses		
	8	12	16
1. Drug on carrier			
Device [%]	17.50	13.72	13.88
Throat [%]	27.72	21.30	20.85
Preseparator [%]	28.66	37.78	36.33
Total [%]	74.88	72.80	71.06
2. Detached drug			
Total [%]	25.12	27.20	28.94
Loss on walls [%]	8.12	6.32	7.18
MMAD [μm]	4.31	4.56	4.50
GSD [μm]	0.81	0.81	0.81

MMAD, mass median aerodynamic diameter; GSD, geometric standard deviation;

in drug loading. Therefore, all further experiments were undertaken using eight single doses, equivalent to 400 μg drug.

One important factor in the use of cascade impactors to determine a MMAD as a characteristic property of a dry powder inhalation is the choice of the correct air flow rate during the experiment. Often, an air flow rate of 60 l min^{-1} is recommended, but the peak inspiratory flow rate equivalent to such an in vitro test flow rate can vary between inhaler devices (Timsina et al., 1993). Hence, the flow rate should be optimized in vitro for both the inhaler device (Timsina et al., 1994), and also for the preparation. Zimon (1982) has shown, that the number of particles adhered to a collection plate changes with air flow rate according to the adhesion properties. Two critical velocities were defined. The first critical velocity determines the possibility of particle rebound from a surface, and depends on particle size, air flow velocity and the elastic properties of the materials in contact. With increasing particle velocity, the force of elastic repulsion increases, and thus particle detachment becomes easier. Thus in a graph of the number of particles adhered to a collection plate as a function of air flow rate, initially the adhesion number will decrease rapidly with increased flow velocity. However, when elastic repulsion and the force of adhesion arrive at an equilibrium, no further decrease in adhesion number will occur, and the first critical air flow velocity has been reached. A subsequent increase in air flow velocity will not change the number of particles adhered until the second critical velocity has been reached. Beyond this point the number of particles adhering will again increase proportionally to the air flow rate. This is due to particles starting to embed into the surface of the collection plate. The degree of particle embedment depends mainly on the particle density and the hardness of the contiguous bodies. Particle embedment increases the true area of contact between particles and surface, which in turn increases the forces of adhesion and friction. Furthermore, the aerodynamic force of the air stream is reduced as a result of the decrease of the drag force acting on the particles. This aerodynamic force becomes zero, if the depth of the embedment is greater than the particle diameter. In this case, no particle detachment can take place.

The following experiments have been undertaken to evaluate the critical velocities for the Diskhaler® if loaded with interactive mixtures of Salmeterol xinafoate and lactose monohydrate (Serevent®). Air velocities between 10 and 70 l min^{-1} have been used. Fig. 4 shows the cumula-

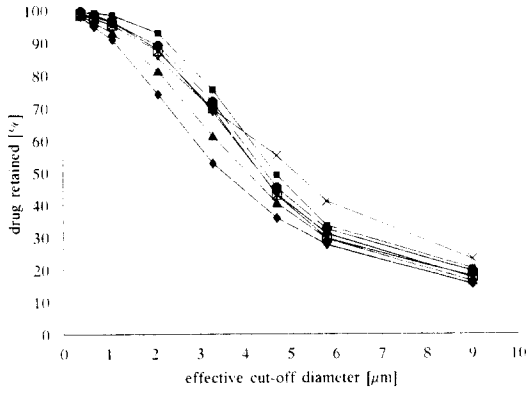

Fig. 4. Cumulative aerodynamic particle size distributions for Salmeterol xinafoate at different air flow rates (8 single doses, silicon oil film thickness 3.75 μm). ×, 10 l min^{-1}; ◆, 20 l min^{-1}; ▲, 30 l min^{-1}; ▼, 40 l min^{-1}; ●, 50 l min^{-1}; ☒, 60 l min^{-1}; ■, 70 l min^{-1}.

Table 3
Mass median aerodynamic diameter and drug loss, tested at different flow rates using a silicon oil film thickness of 3.75 μm and eight single doses

	Flow rate [l min^{-1}]						
	10	20	30	40	50	60	70
1. Drug on carrier							
Device [%]	82.71	51.76	28.28	25.30	16.80	17.50	16.83
Throat [%]	6.30	15.99	18.80	20.06	20.05	27.72	14.29
Preseparator [%]	4.91	14.53	28.04	29.51	37.83	28.66	41.41
Total [%]	93.92	82.28	75.12	74.87	74.68	74.88	72.53
2. Detached drug							
Total [%]	6.08	17.72	24.88	25.13	25.32	25.12	27.47
Loss on walls [%]	3.74	5.45	5.75	6.31	5.11	8.12	4.91
MMAD [μm]	5.03	3.99	3.92	4.36	4.36	4.31	4.86
GSD [μm]	0.83	0.78	0.80	0.82	0.83	0.81	0.84

MMAD, mass median aerodynamic diameter; GSD, geometric standard deviation.

tive aerodynamic particle size distributions for this set of experiments, and Table 3 summarizes the results. Fig. 5 compares the adhesion numbers (i.e. percentage of particles adhered to the plate) for the collection plates 0 and 3 as a function of the air flow rate. Collection plate 0 is the only plate that can be used to apply Zimon's theory, because the amount of particles collected at the following plates depends to a certain degree on the particle number already retained. Collection plate 3 is the plate where, for this particular drug, a large number of particles is expected to impact

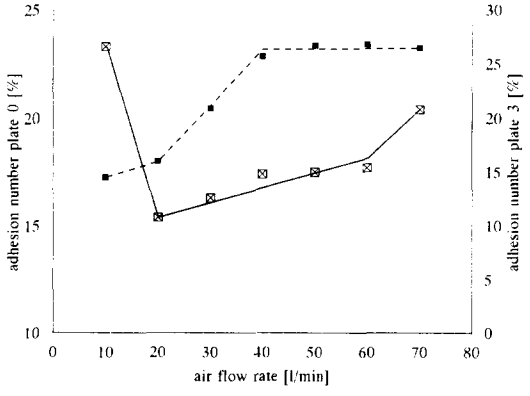

Fig. 5. Relative amount of particles collected at collection plates 0 and 3 as a function of air flow rate (8 single doses, silicon oil film thickness 3.75 μm). ⊠, collection plate 0; ■, collection plate 3.

if the formulation releases the drug in an optimal manner. Hence, here the number of particles collected should increase beyond the first critical velocity, but decrease after the second critical velocity has been reached. From the relative amount of particles collected at plate 0 it can be concluded that the first critical air flow velocity is below 20 l min^{-1}, whereas the second critical air flow velocity appears to be between 60 and 70 l min^{-1}. The relative amount of particles collected at plate 3 confirms the quantity of the first critical velocity (below 20 l min^{-1}), but the second critical velocity might be rather 70 l min^{-1} or more. However, the total aerodynamic behaviour of the formulation, represented by the MMAD (see Table 3) appears to have changed at an air flow velocity of 70 l min^{-1} already, and thus the high air flow velocity of 70 l min^{-1} should be excluded from practical working conditions. In summary, it appears that all in vitro tests involving the Diskhaler® and Serevent® should be undertaken between 20 and 60 l min^{-1} air flow velocity. However, a further criterion to choose the best air flow rate for the in vitro tests using a cascade impactor is the loss of drug in the device and the total amount of drug detached from the carrier material. The results suggest that at least a flow rate of 50 l min^{-1} is necessary to reach the lowest possible loss in the inhaler device (see Table 3).

Table 4
Mass median aerodynamic diameter and drug loss of different Serevent® batches, tested at a flow rate of 60 l min^{-1} using a silicon oil film thickness of 3.75 μm and eight single doses

	Batch code			
	a	*b*	*c*	*d*
1. Drug on carrier				
Device [%]	13.13	11.25	12.27	13.37
Throat [%]	18.26	30.29	28.97	22.20
Preseparator [%]	41.45	38.55	32.83	36.63
Total [%]	72.84	80.09	74.07	72.20
2. Detached drug				
Total [%]	27.16	19.91	25.93	27.80
Loss on walls [%]	6.60	4.61	6.17	5.29
MMAD [μm]	5.14	5.18	4.83	4.71
GSD [μm]	0.83	0.78	0.83	0.83

MMAD, mass median aerodynamic diameter; GSD, geometric standard deviation.

With respect to the amount of respirable drug, i.e. the amount of drug detached from the carrier particles, an air flow velocity between 30 and 60 l min^{-1} appears suitable.

From the results described above, the following standard operating conditions, which are strictly valid only for the Diskhaler® and Serevent® products, were derived: drug loading 400 μg Salmeterol xinafoate, equivalent to eight single doses, a coat of high viscous silicon oil of 3.75 μm thickness for each collection plate, and an air flow rate of 60 l min^{-1}.

Applying these standard operating conditions for the Andersen-Cascade impactor, four Serevent® batches, randomly drawn from industrially manufactured batches, were compared in terms of their aerodynamic properties. Table 4 lists the results obtained.

It can be seen that for batches *c* and *d*, a lower MMAD was obtained than for the batches *a* and *b*. Analysis of Variance, however, failed to detect any significant difference in the MMAD values. Thus, although there appear to be differences in the MMAD values, it cannot be claimed that these are due to differences in the manufacturing process. Therefore, an average aerodynamic drug distribution from Serevent-Diskhalers® in the cascade impactor can be calculated, which is equivalent to a MMAD of 4.97 ± 0.23 μm.

Table 5
Median adhesion force and interquartile range of micronized Salmeterol xinafoate adhering to lactose monohydrate carrier particles in interactive powder mixtures of Serevent® products (arithmetic mean and standard deviation of 6 replicates)

Batch code	F_{ad} [$\times 10^{-12}$ N]	IQR [$\times 10^{-12}$ N]
a	8.07 ± 0.95	16.49 ± 2.18
b	7.97 ± 1.68	18.85 ± 3.93
c	6.50 ± 1.23	15.55 ± 2.32
d	6.70 ± 1.32	17.99 ± 3.23

F_{ad}, median adhesion force; IQR, interquartile range.

If adhesion is one main factor responsible for the aerodynamic behaviour of salmeterol xinafoate when applied using the studied dosage form, there should be a similar trend for the MMAD and the median adhesion force between drug and carrier particles. Hence, experiments were undertaken to establish the median adhesion force between salmeterol xinafoate particles and lactose monohydrate carrier particles. Table 5 lists the median adhesion forces and interquartile ranges obtained for the four batches tested, and Fig. 6 illustrates the adhesion force distributions.

The interquartile range characterizes the spread in adhesion force mainly due to the particle size distribution of the drug, and hence is related to

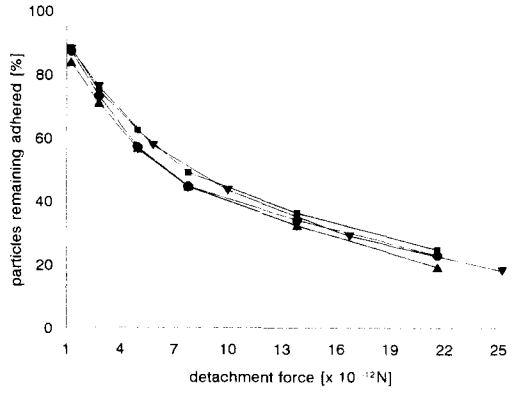

Fig. 6. Adhesion force distributions of micronized Salmeterol xinafoate adhered to lactose monohydrate carrier particles in interactive powder mixtures of Serevent® products. ▼, batch *a*; ■, batch *b*; ▲, batch *c*; ●, batch *d*.

the distribution of the drug over the different stages in the Cascade impactor, whereas the median adhesion force should be related to the mass median aerodynamic particle diameter (MMAD).

Obviously, there is again some variability in the adhesion properties of the Serevent® batches. Therefore, analysis of variance was used to test whether or not an average adhesion performance can be drawn from the results. The batches are statistically not different in their adhesion properties, and average adhesion properties of Serevent® products have been calculated. Typically, the median adhesion force lays between 6.71×10^{-12} N and 7.91×10^{-12} N (95% confidence interval), and the interquartile range of the adhesion force distribution ranges between 15.92×10^{-12} N and 18.52×10^{-12} N (95% confidence interval).

The batches *c* and *d* are characterized by a smaller adhesion force than the other two batches, thus showing a similar trend to the MMAD values. Therefore, the median adhesion force could be regarded as a relevant descriptor of the properties of this dosage form. The measurement is sensitive enough to detect samples, from which an atypical aerodynamic behaviour could be expected, either due to a too low or too high adhesion force between drug and carrier particles. The measurement of adhesion forces could therefore be used as an in-process control parameter.

4. Conclusions

An essential requirement for in vitro testing of dry powder inhalations using the Andersen-Cascade impactor are physically correct standard operating conditions. For inhalation products containing salmeterol xinafoate adhered to lactose monohydrate carrier particles, when applied using the Diskhaler®, the stainless steel collection plates need to be coated with a highly viscous silicon oil to give a film thickness of 3.75 μm. The drug loading of the impactor should not exceed 400 μg, and an air flow rate between 50 and 60 l min^{-1} provides optimum flow conditions. The dry powder inhalation Serevent® (Diskhaler®) is produced under industrial conditions with high reproducibility in its aerodynamic properties. Adhesion force measurements were found to reflect minimal changes in the aerodynamic formulation behaviour, even when these are statistically not significant. Thus, the measurement of adhesion forces could provide a cost effective tool for the in-process control of dry powder inhalations to guarantee a high quality of such products.

Acknowledgements

This work was financed by Glaxo-Wellcome Research and Development, Ware, UK. The author should like to thank Ms. Helen Dicks and Mr. David Prime for providing the Serevent® samples and their support and discussion of the work. The author is also very grateful to Mr. Derek Marley for his technical support with setting up the HPLC equipment.

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