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Characterization of inhalation aerosols: a critical evaluation of cascade impactor analysis and laser diffraction technique

A.H. de Boer *, D. Gjaltema, P. Hagedoorn, H.W. Frijlink

Department of Pharmaceutical Technology and Biopharmacy, Groningen University Institute for Drug Exploration (GUIDE), University of Groningen, Antonius Deusinglaan 1, 9713 AV Groningen, The Netherlands

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Abstract

Cascade impactor analysis is the standard technique for in vitro characterization of aerosol clouds generated by medical aerosol generators. One important reason for using this inertial separation principle is that drug fractions are classified into aerodynamic size ranges that are relevant to the deposition in the respiratory tract. Measurement of these fractions with chemical detection methods enables establishment of the particle size distribution of the drug in the presence of excipients. However, the technique is laborious and time consuming and most of the devices used for inhaler evaluation lack sufficient possibilities for automation. In addition to that, impactors often have to be operated under conditions for which they were not designed and calibrated. Particularly, flow rates through impactors are increased to values at which the flow through the nozzles is highly turbulent. This has an uncontrolled influence on the collection efficiencies and cut-off curves of these nozzles. Moreover, the cut-off value varies with the flow rate through an impactor nozzle. On the other hand, the high air flow resistances of most impactors are rather restricting to the attainable (fixed) inspiratory flow curves through these devices. Especially for breath actuated dry powder inhalers, higher flow rates and flow increase rates may be desirable than can be achieved in combination with a particular type of impactor. In this paper, the applicability of laser diffraction technology is evaluated as a very fast and highly reliable alternative for cascade impactor analysis. With this technique, aerodynamic diameters cannot be measured, but for comparative evaluation and development, comprising most in vitro applications, this is not necessary. Laser diffraction has excellent possibilities for automated recording of data and testing conditions, and the size classes are independent of the flow rate. Practical limitations can be overcome by using a special inhaler adapter which enables control of the inspiratory flow curve through the inhaler, analysis of the emitted fine particle mass fraction and pre-separation of large particles during testing of dry powder inhalers containing adhesive mixtures.

Keywords: Inhalation; Aerosol cloud; Particle size analysis; Aerodynamic diameter; Laser diffraction; Cascade impactor

1. Introduction

One of the standard routines in development and evaluation of drug inhalation systems is to measure the aerodynamic particle size distribution

* Corresponding author. Tel.: +31-50-363-3286; fax: +31-50-363-2500

E-mail address: a.h.de.boer@farm.rug.nl (A.H. de Boer).

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in the generated aerosol cloud as a function of a constant inspiratory flow rate. Thus, to assess the emitted mass fraction of the dose that has the required size range for effective deposition on the site of action in the respiratory tract. The preferred size or size range for inhalation drugs depends on the target area, which is still somewhat controversial for some types of drugs. Many mean particle diameters or size distributions have been recommended as optimal for (deep) lung deposition, most of them are within the range between 0.5 and 7 μm (e.g. Byron, 1986; Morén, 1987; Vidgrén, 1994; Staniforth, 1995).

Different techniques have been recommended for the measurement of aerodynamic particle diameters. Direct measurement in the cloud from an inhaler is possible with apparatus like (cascade) impactors, time of flight 'Aerosizers', sedimentation cells and elutriators, wind sifters, spiral centrifuge aerosol spectrometers and electrical mobility analysers. Some of these techniques require special measures for this rather unusual application, for which none of them was originally designed. Impactors and impingers are currently considered as 'the golden standard' for inhaler testing, because they yield mass fractions of the drug dose (by chemical detection) in aerodynamic size classes that are relevant to particle deposition in the human respiratory tract. Their principle of operation has extensively been described by various authors (e.g. Ranz and Wong, 1952a,b; Hinds, 1982), which gives the impression that inertial impaction is well understood and can well be controlled. Many different types of impactors and impingers have been proposed for inhalation aerosols in the last decades. They vary from simple devices like the modified 'Kirk Lung' (Davies et al., 1976) and the 'twin impinger' (British Pharmacopoeia, 1988) to more complex apparatus, having more collection stages, like the Andersen Mark II (Graseby-Anderson, 1985) or the recently developed new generation impactor (Marple et al., 2000). Inertial impactors have to be designed and constructed according to certain aerodynamic rules, such that they must be operated under strictly defined conditions in order to obtain the desired cut-off efficiencies and to avoid excessive flattening of the cut-off curve. Theoretical cut-off diameters can be calculated as function of nozzle geometry, particle density and air velocity in the nozzle. Recently, John (1999) presented a simple theoretical derivation for the cutpoint of an impactor. However, it is generally recommended that impactors are calibrated with particles of well known aerodynamic size (distribution) obtained from another suitable sizing technique. Asking and Olsson (1997) derived a practical relationship from calibration between the cut-off diameter and the flow rate for the nozzles of a multi-stage liquid impinger, which has been copied by the European Pharmacopoeia, 3rd edition 1997, supplement 2001.

In the last decade, cascade impactor analysis (cia) has been subjected to critical evaluations and some developments in their use. Issues that are of concern for the accuracy of size classification are the incidence of bounce and blow off at the stages. Also wall losses between the stages may occur. Hickey (1990) investigated the deposition characteristics of particles on the individual stages of an inertial impactor and found size fractionation upon a single stage. He observed that larger particles are deposited at the center, and smaller ones at the periphery of an impaction plate. Olsson et al. (1996a) compared the results from different laboratories obtained with the four different impactors described in the European Pharmacopoeia (3rd edition, 1997), for a salbutamol pressurized metered dose inhaler (mdi) and two different dry powder inhalers (dpis). Their study proves that significant differences exist between the results obtained with different devices, as these devices may have different cut-off values for the fine particle fractions and be operated at different flow rates, which reflects on the performance of the dpis. The within-laboratory variation for the fine particle dose in their study was quite large and ranged from 8 to 15.5% for the mdi, respectively from 5.5 to 20% for the dpis. For dpis, only two impactors gave comparable results: they were operated at the same flow rate of 60 l/min and had approximately the same cut-off value (6.4 and 6.8 μm) for the fine particle fraction.

Developments in impactor use include the application of these devices at higher flow rates than those for which they were originally designed,

as well as the re-design of existing impactors for this purpose. However, an increase up to 90 l/min, as for the Marple–Miller Impactor (e.g. Hindle et al., 1996), may not be sufficient, because recent guidelines demand that dpis are tested at a pressure drop of 4 kPa across the device. For low resistance inhalers, like the Spinhaler (Aventis Pharma) and Rotahaler (Glaxo SmithKline), this pressure drop corresponds with much higher flow rates of 125 and 155 l/min, respectively. Another limitation of inertial impactors is that breath controlled dry powder inhalers cannot be tested for the variable flow conditions under which they are operated by the patient. Impactors are intended to sample at fixed flow rates (for which they have been calibrated). Operating at a variable flow rate should be avoided as this yields mass fractions with undefined size distributions. As a possible solution for this problem, inhalation simulators have been developed, such as the Electronic Lung^{TM} (Brindley et al., 1994). With these simulators, well-defined flow curves are drawn through the inhalers that have to be tested. The aerosol clouds generated by the inhalers at these variable flow rates are first discharged in a large spacer before they are conducted through an impactor at a prescribed constant operational flow rate for this impactor. This procedure has the disadvantage that the size distribution of the cloud may change inside the spacer as the result of particle drop out by sedimentation, especially for the larger particles. Furthermore, electrostatic separation and droplet coalescence may occur. Developments in cascade impactor analysis also include their combination with casts of the human throat, serving as the induction port to the first impactor stage, as for instance reported by Niven et al. (1994) and Olsson et al. (1996b). Most of these evaluations and developments arise from the desire to predict airway deposition based on in vitro deposition data, using models like LUng Dose Evaluation Program (LUDEP), developed by the UK National Radiological Protection Board (Moore, 2001).

The use of laser diffraction technique for particle size measurement in the aerosol clouds from nebulizers dates from the eighties of last century. Ho et al. (1986) concluded that laser diffraction technique provides more realistic size distributions for nebulized aqueous drug solutions than inertial impaction techniques. They claimed that droplet bounce and re-entrainment, as well as droplet evaporation during laser diffraction is less relevant. Ranucci (1992) described the use of laser diffraction technique for particle size analysis in the plumes discharged from mdis. He concluded that lda can be a valuable characterization technique for this type of aerosol generator, because it allows real-time plume measurements to be taken as function of distance from the actuator orifice. This is relevant to mdi-testing because the droplets contain volatile components that evaporate in the air stream. As a result, size distribution changes as function of the travelled distance, which is an aspect that can not be studied with impactors and liquid impingers. In the nineties of the past century, several studies of medical nebulizers with laser diffraction technique have been reported (Hurley et al., 1994; McCallion et al., 1995, 1996; Bridges and Taylor, 1998). It has been concluded that the technique is robust and reliable and that it measures size parameters relevant to the clinical situation (Clark, 1995). However, in most of these studies there is no reference to proper air extraction from the measuring zone. Thus, to avoid re-entry of aerosol droplets into the laser beam and also to study the effect of the inspiratory flow rate on the droplet size distribution. The reason is that controlled air suction through the nebulizer requires a connection between the mouthpiece of the nebulizer and the vacuum system, which is technically problematic because of interference with the laser beam.

Previously mentioned references indicate that there is a growing interest in laser diffraction for the characterization of inhalation devices. This includes breath operated dry powder inhalers, for which a well-controlled air flow through the device is particularly relevant. In this paper the applicability of laser diffraction technique is evaluated as an alternative, though not a substitute, for cascade impactor analysis for the in vitro characterization of various types of medical aerosol generators. Specific pros and cons of both techniques from theoretical and practical viewpoint are discussed. Also, some practical and operational limitations of

standard laser diffraction technology for inhaler testing are mentioned.

2. Theoretical background

2.1. Equivalent particle diameters

Inhaled drugs vary not only in size distribution, but also in their physical state (liquid or solid), particle density (ρ_P), shape and velocity. All these parameters are relevant to particle deposition in the respiratory tract, which is the result of a dynamic system of forces acting on airborne particles travelling through this tract. It includes the force of gravity (F_G), the drag (resistance) force of the inspiratory air (F_D) and inertial forces (F_I), which all depend on previously mentioned particle properties. Specifically the ratio of drag force to either of both other forces determines whether the particle is carried on by the air stream into a deeper lung region, or brought in contact with the walls of the airway ducts by sedimentation or a high particle momentum. This, for the situation in which particles are not charged (absence of Coulombic forces) and particle interception in narrow passageways does not occur.

In order to predict or compare the deposition behaviour of particles with different densities and shapes, it is standard practice to make corrections for these parameters (e.g. Hinds, 1982). For instance, the aerodynamic behaviour of particles with different shapes can be compared by expressing them in terms of equivalent volume diameter (D_E), and dynamic shape factor (χ), which is the ratio of the actual resistance force acting on a non-spherical particle to the resistance force on a sphere having the same volume and velocity. For aerosol particles, inertial effects of the air are negligible compared to viscosity effects and thus, Stokes's law applies for the drag force. Accordingly, the dynamic shape factor (χ) can be expressed as:

$$\chi = (F_A)/(3 \cdot \pi \cdot \eta \cdot V \cdot D_E) \quad \text{so,}$$
$$F_A = 3 \cdot \pi \cdot \eta \cdot V \cdot D_E \cdot \chi \tag{1}$$

where F_A = the actual resistance force acting on the particle; η = the dynamic viscosity of the air; V = the particle velocity relative to the air.

Eq. (1) is valid for particles larger than 3 μm. For smaller particles (within the range 0.1–3 μm), the Cunningham correction factor for slip flow (C_C) has to be introduced. Alternatively, the Stokes' diameter (D_S) can be used which is the diameter of a sphere that has the same density and terminal settling velocity (V_{TS}) in still air as the irregular particle.

A correction for both particle shape and particle density can be made by using the aerodynamic diameter (D_A). By definition, the aerodynamic diameter of a particle is the diameter of a sphere with unit density ($\rho = 1$), having the same terminal settling velocity in still air as the particle in consideration. Under the condition of stationary settling, only two forces act on a particle, which are exactly equal in size and opposite in direction. These are the force of gravity (F_G) and the resistance force of the air (F_D). A correlation between previously mentioned diameters (D_E, D_A and D_S) and the dynamic shape factor (χ) can be obtained by expressing these parameters in the terminal settling velocity (V_{TS}), for which F_G equals F_D.

In general terms for the diameter (D),

$$\pi/6 \cdot D^3 \cdot \rho_P \cdot g = 3 \cdot \pi \cdot \eta \cdot V_{TS} \cdot D, \quad \text{so}$$
$$V_{TS} = (\rho_P \cdot D^2 \cdot g)/(18 \cdot \eta)$$

where ρ_p = the particle density; g = the acceleration of gravity.

After substitution of the specific diameters:

$$V_{TS} = (\rho_P \cdot D_E^2 \cdot g)/(18 \cdot \eta \cdot \chi) = (\rho_0 \cdot D_A^2 \cdot g)/(18 \cdot \eta)$$
$$= (\rho_P \cdot D_S^2 \cdot g)/(18 \cdot \eta) \tag{2}$$

Eq. (2), after rearrangement, yields the correlations:

$$D_A = D_E \cdot (\rho_P/\chi)^{0.5} = D_S \cdot \rho_P^{0.5} \tag{3}$$

2.2. Theoretical cutpoint of an impactor

Most apparatus for measuring aerodynamic particle diameters, such as inertial impactors, rely on the counterbalance of two opposing forces, of

which one is the drag force. The nature of the force opposing to this drag force can be different for different principles. Typically, this might be centrifugal forces, the force of gravity, Coulombic forces and particle momentum which can be considered as a force acting over a certain period of time. For inertial impactors, the counter force is a centrifugal force. Cutpoints of such impactors are normally presented as particle diameters with 50% collection efficiency (D_{50}), and because classification is by two competitive forces, as during stationary settling of a particle, it is believed that impactors separate into aerodynamic size fractions. Diameters with 50% collection efficiency (d_{50}) can be derived from the Stokes number (Stk), which governs the collection efficiency of a single jet impactor nozzle. Stokes number is defined as the ratio of particle stopping distance (S) to the nozzle radius (R) of the impactor. Correspondingly, the Stokes number that gives 50% collection efficiency is referred to as Stk_{50}. The particle stopping distance can be written as the product of particle relaxation time (τ) and particle velocity at the nozzle exit (U), whereas the relaxation time is the product of particle mass (m) and particle mobility (B), which is the particle velocity (U) per unit force (F). Because particle velocity at the nozzle exit (U) is established by the action of the drag force ($F_D = 3\cdot\pi\cdot\eta\cdot U\cdot D\cdot C_c^{-1}$), stopping distance S (after rearrangement of terms) may be written as:

$$S = m\cdot B\cdot U = (D^2\cdot C_c\cdot\rho_P\cdot U)/(18\cdot\eta) \quad (4)$$

So,

$$\mathrm{Stk}_{50} = S/R = m\cdot B\cdot U/R = (D_{50}2\cdot C_c\cdot\rho_P\cdot U)/(18\cdot\eta\cdot R) \quad (5)$$

Rearrangement of the terms in Eq. (5) yields the particle diameter with 50% collection efficiency:

$$D_{50}\cdot\sqrt{C_c} = \{(18\cdot\eta\cdot R\cdot\mathrm{Stk}_{50})/(\rho_p\cdot U)\}^{0.5} \quad (6)$$

Stk_{50} is a single number that applies for well designed impactors of the same type that are used for a confined range of flow rates. The Reynolds numbers in the nozzles have to be within 500 and 3000. Design criteria for circular nozzles include that the separation distance between the nozzle and the impaction plate is larger than the nozzle diameter. Under these conditions, Stk_{50} equals 0.22. It may be clear that D_{50} obtained with Eq. (6) is a geometric diameter, since its value varies with the particle density (ρ_P).

3. Critical evaluation of cia and lda

3.1. Cascade impactor analysis (cia)

In contrast with the force of gravity during terminal settling, the centrifugal force acting on a particle travelling between the nozzle and the collection plate of an impactor is not constant. Neither is the drag force. In the region beyond the impactor jet, both forces continuously increase from zero as a particle travels from the jet to the collection plate, whereas they also change their direction (Fig. 1). In this dynamic force system, irregular particles may exhibit a behaviour which is quite different from that during stationary settling. They may start to rotate, in which case their (average) dynamic shape factor (χ) is different from the one during stationary settling. Consequently, the aerodynamic diameter measured with an inertial impactor, is not necessarily the same as its equivalent obtained from a sedimentiation experiment, which is the only technique yielding the aerodynamic diameter by definition. So, different proportionality constants $(\rho_P/\chi)^{0.5}$ between D_A and D_E for the same particle may apply for different situations and different techniques. This is a neglected aspect when in vitro deposition data from cia are used to predict lung deposition.

The conditions under which inertial impactors should be operated include laminar flow through their nozzles ($500 < \mathrm{Re} < 3000$). Most impactors do not meet this criterion during inhaler testing, as shown in Table 1 for the four stage ASTRA impactor. Actual Reynolds numbers during measurement may be even higher than the numbers presented in Table 1. This is a consequence of the design of the nozzles, which are relatively short. As a result of that, the contribution of inlet and outlet geometry to the flow regime inside the nozzle is quite large. Especially for the flow through the

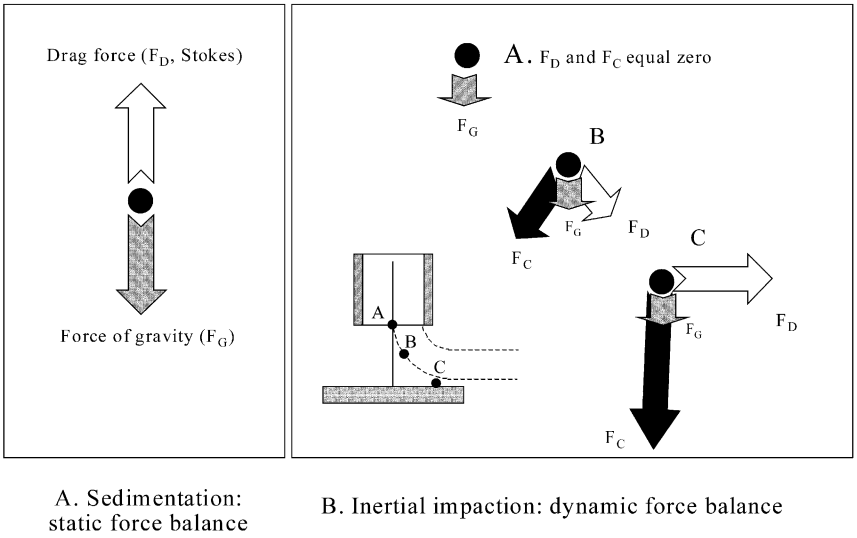

Fig. 1. Schematic presentation of the forces acting on a particle (A) during stationary settling and (B) after leaving the nozzle of an inertial impactor. In (B), F_C is the centrifugal force.

Table 1
Calculated Reynolds numbers in the nozzles of the four stage ASTRA impactor

Stage:	1	2	3	4
Nozzle diameter (mm)	25	14	8	2
20 l/min	1132	2018	**3552**	1485
30 l/min	1701	3030	**5334**	2250
40 l/min	2267	**4043**	**7115**	3002
50 l/min	2835	**5049**	**8886**	**3749**
60 l/min	**3401**	**6061**	**10667**	**4500**
70 l/min	**3962**	**7073**	**12449**	**5252**

Stage 4 is a multi-jet with seven identical orifices. Values printed in bold are outside the recommended range ($500 < Re < 3000$).

Table 2
Some relevant differences between in vitro (cia) and in vivo deposition

In vitro:
1. Deposition is by inertial impaction only
2. Particle collection is (near-)complete
3. The throat (inlet tube) is often a dry bent tube (in which powder disintegration is continued)
4. The inspiratory flow curve is well controlled and monitored
5. The conducting tubes (between the impactor stages) have fixed dimensions

In vivo:
1. Deposition is by inertial impaction, sedimentation and diffusion
2. Collection efficiency varies with particle size
3. The throat is wet (particles stick on impact)
4. The inspiratory flow curve is highly variable and often not known
5. Air passageways differ from person to person and from moment to moment

nozzles of the second and third stages, which are relevant to the fine particle fractions, the Reynolds number is much higher than 3000 at flow rates that are still below the range of interest for dpi testing. This, in contrast with the in vivo situation, where the target area (for the fine particle fractions) is beyond the segmental bronchi, in which (at 60 l/min) the Reynolds number is only approximately 1200, and further decreasing with increasing generation. Other relevant differences between in vitro (impactor) and in vivo (lung) deposition have been listed in Table 2. Most important is the difference in deposition mechanisms and their efficiencies. In the impactor, deposition is by inertial impaction only, whereas in the respiratory tract, particle collection is also by sedimentation and diffusion, especially in the deeper lung regions for the smallest particles. Deposition efficiencies in all regions of the lungs decrease with decreasing

Table 3
Review of some practical drawbacks and limitations of inertial impaction

✓ Classification is into only a small number of size classes
✓ The cut-off diameters of nozzles vary with the flow rate through the impactor
✓ High impactor resistance limits the adjustable range of flow rates and reduces the flow increase rate
✓ Fine particle adhesion occurs onto inner impactor walls
✓ Electrostatic charge may disturb particle collection
✓ Collection efficiency of the final stage may be less than 100%
✓ Droplet evaporation can not be studied
✓ Cascade impactor analysis lacks sufficient possibilities for automation
✓ Cascade impactor analysis is time consuming and laborious
✓ A large inter-device and inter-laboratory variation has been reported

particle diameter to a minimum of approximately 20% for total lung deposition corresponding with a particle diameter of 0.5 μm (e.g. Martonen and Katz, 1993). In vitro, particle collection may be complete for all size classes, providing that the final stage (impinger or filter) performs adequately. Therefore, lung deposition can not be predicted from cascade impactor results, unless these differences in collection efficiences are taken into account. And even then, predicted values based on cascade impactor results will not reflect actual lung deposition, unless the inspiratory flow curves during both the in vitro and in vivo experiments are kept exactly the same. This requires not only good flow control during cia, but also adequate monitoring of the inhalation manoeuvre during clinical studies.

The most relevant practical drawbacks and limitations of cascade impactor analysis have been summarized in Table 3. The flow rate dependent cut-off diameters of the nozzle stages make it difficult to compare the fine particle fractions from different types of dpis obtained at the same pressure drop of 4 kPa through these devices. The flow rates for a number of marketed dpis at this pressure drop are given in Table 4. This table also shows the theoretical cut-off values with 50% collection efficiency for the second stage of the Erweka impactor at these flow rates, which differ by a factor of 2 for the extremes. Especially for impactors with a relatively low number of stages (<5) for the fine fractions, calculation of cumulative size distribution curves is rather arguable. Deriving comparable size fractions with the same span from such curves or mass median aerodynamic diameters by intra- or extrapolation is therefore nearly impossible, as shown in Fig. 2 for a practicle result from a four stage Fisons type of impactor at 60 l/min. From an inhalation experiment with a dpi, 4.57 μg of drug deposition was obtained on the second, 8.79 μg on the third and 26.58 μg on the fourth stage from a 100 μg dose. The theoretical cut-off diameters (D_{50}) of this impactor at 60 l/min for this type of drug are 17.06 μm for the first, 8.74 μm for the second and 4.31 μm for the third stage. These data yield the following cumulative size distribution curve: 68.08% $<$ 4.31 μm; 88.29% $<$ 8.74 μm and 100% $<$ 17.06 μm for the total 'fine particle' mass

Table 4
Flow rates through some marketed dpis corresponding with 4 kPa and cut-off diameters for the second stage of the Erweka impactor at these flow rates

Inhaler	Resistance	Flow rate		U_{NOZZLE}[1]	D_{50}[2]
	($kPa^{0.5}$·min/l)	(l/min)	(10^{-3} m^3/s)	(m/s)	(μm)
Inhalator Ingelheim	0.057	35	0.58	3.77	9.38
Astra/Zeneca Turbuhaler	0.043	46	0.77	5.00	8.15
Glaxo Diskus	0.034	59	0.98	6.37	7.22
Sofotec Novolizer	0.028	71	1.19	7.73	6.55
ISF inhaler	0.019	105	1.75	11.37	5.40

[1] U_{NOZZLE} is air velocity in the nozzle of the second stage corresponding with the flow rate.
[2] D_{50} is the theoretical cut-off diameter for the second stage nozzle with 50% collection efficiency (for particles with $\rho_P = 1.5$ g/cm^3).

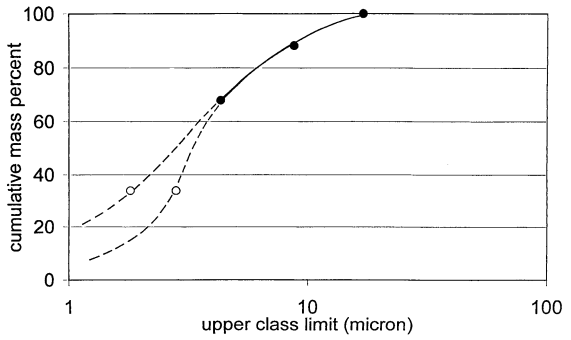

Fig. 2. Typical cumulative mass distribution curve(s) as function of theoretical cut-off point, expressed as upper class limit, for budesonide fractions retained from a four stage Fisons type of impactor at 60 l/min. The sum of the mass fractions on stages 2–4 (39.04% of the nominal dose) has been put to 100%. The results are for a special test inhaler with an adhesive mixture.

fraction of 39.04 μg (including the third and second stage deposition for some extra datapoints). In fact, only the fraction on the fourth impactor stage (<4.31 μm) is relevant to the target area. But in spite of these extra datapoints, extrapolation of the size distribution curve towards the range <4.31 μm is nearly impossible. Particularly if the distribution is not log-normal, which is mostly the case for the mixture of primary particles and small agglomerates in a narrow size fraction from a dpi. The mass median aerodynamic diameter of the fine fraction <4.31 μm, could as well be 2.8 as 1.8 μm, as can be concluded from graphic presentation of the cumulative size distribution curve. However, total deposition efficiency for a 2.8 μm particle is approx. 55%, whereas that for a 1.8 μm particle is only 35%. This uncertainty makes prediction of lung deposition from such a cascade impactor result nearly impossible and it is not very helpful for comparative evaluation of inhalation systems either. With laser diffraction analysis, 13 size classes are obtained (with a 50 mm lens) within the fraction smaller than 4.50 μm. At least for comparative evaluation, this enables a much better discrimination between devices and conditions, in spite of the fact that not so much aerodynamic diameters are obtained.

The high Reynolds numbers in the nozzle throats are not the only limitation to the flow rates through dpis connected to an impactor. The high air flow resistance of most impactors, is another, more practical, constraint in this respect. This resistance, in combination with the relatively large volume of the impactor, also reduces the flow increase rate which is particularly relevant to the performance of dpis like the AstraZeneca Turbuhaler (e.g. de Boer et al., 1997). Another practical problem of relevance is the fact that uncontrolled and undesired deposition mechanisms may occur in an impactor, such as particle collection by adhesion and electrostatic forces. Electrostatic particle collection onto inner impactor walls can be reduced by using metal devices that are connected to the earth during inhaler testing. However, contact charging occurs mainly inside the plastic inhalers connected to the impactors, and because electrostatic forces can be much higher than drag or inertial forces, charged particles may influence each other's trajectories also in the nozzle regions of uncharged impactors. Particle adhesion onto impactor walls of earthed devices is mainly the result of Van der Waals forces. Fine particles may stick to the surfaces with which they make contact during passage. Unless they are entrained again, they contribute to the drug fractions on the stages for coarser size classes. The extent of adhesion highly depends on the design of the impactor. Extreme circulation of the aerosol stream on upper stages of some devices increases the number of contacts between particles and the inner walls of these stages. However, none of the previously mentioned drawbacks of cascade impaction analysis is as problematic as the fact that the technique is laborious and time consuming. Moreover, automation of the test is nearly impossible for most devices.

3.2. Laser diffraction analysis (lda)

Laser diffraction apparatus measure random geometrical particle dimensions. The method has the potential to solve some of the major problems related to cascade impactor analysis. The measuring is fast and not dependent on the flow rate. However, micronized inhalation powders, in most cases, approach the spherical shape, with a few exceptions like salbutamol sulfate. Therefore,

calculated laser diffraction diameters (D_{LD}) do not differ much from the particles' equivalent volume diameters (D_E). A strong indication for this was found in our work with carefully prepared sieve fractions of crystalline alpha lactose monohydrate, having a well-defined wedge shape. The mean ratio of crystal length to base width for such particles is 1.66 (from optical observation). For wedge shaped particles, the crystal base is the characteristic particle diameter upon which fractionation takes place. Therefore, it is not surprising that this ratio of crystal length to base width is confirmed by laser diffraction analysis, yielding X_{100}-values being (on average) 1,68 times higher than the upper class limits of the prepared sieve fractions. The calculated median volume diameters (D_{LD}) from laser diffraction analysis of these fractions are (also on average) 1.21 times the mean fraction diameter, which is the arithmetic mean of lower and upper sieve diameter. It can also be calculated that the equivalent volume diameter (D_E) of such particles with unit base width is 1.17. These values for D_{LD} and D_E are in good agreement with each other (the difference is only 3%). Especially considering the fact that not all fractions exhibit a natural size distribution: for narrow size fractions that are taken from the tails of the size distribution of the starting material, the arithmetic mean of upper and lower class limit may not equal the median diameter of the fraction.

From the small difference between D_{LD} and D_E for typically wedge shaped particles, it may be expected that this difference for most solid micronized inhalation drug particles is of the same order of magnitude, or even smaller. This, if desired, enables the estimation of median aerodynamic diameters from median laser diffraction diameters using Eq. (3), since particle density (ρ_P) and dynamic shape factor (χ), in many cases, can be obtained from other techniques. For nebulizers, aerosolizing aqueous drug solutions, calculations are not even necessary, because droplets are spherical and drug concentrations are quite low. Thus, dynamic shape factor and droplet density both equal unity, and so does the proportionality constant $(\rho_P/\chi)^{0.5}$ between D_A and D_E. For mdis, mostly only particle density differs slightly from unity. For dpis, calculation and correct understanding of laser diffraction data may be simple if particles in the aerosol cloud are single entities. For such particles, density and shape factor (both being >1) normally widely compensate each other in the proportionality constant between D_E and D_A. For instance, the dynamic shape factor of typical airborne particles with various shapes ranges from approximately 1.0 (for spheres) to 1.3 (e.g. cylinder with $L/D=4$). The density of solid inhalation drugs ranges from approximately 1.2 to 1.5 g/cm^3, unless porous particles are being used (e.g. Edwards et al., 1997). So, the proportionality constant $(\rho_P/\chi)^{0.5}$ for these particles is between 0.96 and 1.22 for the extremes mentioned. However, particles discharged from dpis are not only primary entities, but also small clusters of single particles, and assessments to be made for such small agglomerates are more complex. Table 6 shows the data for some chain-type of clusters, containing up to four primary spherical particles of the same size, which is the most unfavourable type of agglomerate for laser diffraction measurement. The difference between the expected mean laser diffraction diameter (D_{LD}) and estimated aerodynamic diameter (D_A) increases to 68% (as percent of D_A) for a 4-chain cluster. The difference is not so much caused by the proportionality constant between D_E and D_A, but rather by the difference between calculated D_E and estimated D_{LD}, which is quite large for chain-like agglomerates. Four-chain agglomerates are highly unlikely to exist in the turbulent air stream from a dpi however, compact-types of agglomerates stand a much better chance. Even much more important for dpi development is the fact that differences between D_{LD} and D_A (or D_E) are not at all relevant, because such a development (with lda) is based upon reference measurements. Starting point for any dpi development is the drug in the correct aerodynamic particle size distribution for the desired therapeutic effect (obtained from an appropriate sizing technique). Depending on drug density and particle shape, laser diffraction analysis of this drug (using a dispersion apparatus like RODOS, Sympatec GmbH, Goslar Germany) may yield a different primary particle size distribution (in terms of D_{LD}). This however, is of no concern, because the objective of powder formula-

Table 5
Some theoretical limitations and drawbacks of laser diffraction technique that are relevant to the characterization of inhalation aerosols

✓ Measurement of geometrical instead of aerodynamic particle size (at random measurement of numerous planes of symmetry)
✓ Volume distribution curves are calculated on the assumption that particles are spherical
✓ Apparent particle density and dynamic shape factor of drug agglomerates are not known
✓ A choice between Mie and Fraunhofer has to be made
✓ Over- and underestimation of fractions in bimodal mixtures may occur

tion (with this drug) and device development is to get as close as possible to this RODOS result during inhalation experiments whereby the same measuring principle is used. Therefore, errors inherent in the measuring principle are basically the same for all measurements undertaken during development. Inadequate de-agglomeration of the powder, resulting in the release of small agglomerates (or a mixture of primary particles and small agglomerates) from the dpi, reflects on the size distribution of the aerosol cloud which will be different from the result obtained with RODOS dispersion. This does not imply that data interpretation is simple. It requires good understanding of the working principle of a dpi and the properties of powder formulations for inhalation to draw the correct conclusions. This could limit the application of lda for dpi development.

The choice between the Mie and Fraunhofer theory for aerosol measurement is another item needing careful consideration. This choice has been subject of many discussions and is particularly related to the final aspect mentioned in Table 5. This has been shown by Annapragada and Adjei (1996) who investigated the nature and magnitude of errors in determining size distributions with the Fraunhofer technique. They concluded that the Fraunhofer diffraction pattern analysis method works well for unimodal systems, but for sharp-peaked multimodal systems, there is a tendency to skew the distribution towards the mode that produces the strongest peak in the diffraction pattern. It should be recognized that the choice between Mie and Fraunhofer is not so much a choice between diffraction theories however, but rather one between deconvolution and smoothing techniques. Therefore, a rational choice between Mie and Fraunhofer cannot always be made, unless the algorithms used to solve the complex diffraction integral are known, as well as the effect of the smoothing techniques on the distribution curve. There is no doubt that Mie is the better theory, but practically, Fraunhofer results may give better correlations with cascade impactor data. One important reason for this is, that the optical parameters (refractive index and absorption coefficient) needed for a correct use of the Mie theory are often not exactly known. They depend not only on the chemical nature of the particles to be measured, but (partly) also on physical particle properties, such as size (Boeck, 1983), shape, concentration and temperature, which all may vary during inhalation (e.g. by droplet evaporation). Small variations in these optical parameters may result in dramatic changes in the calculated size distribution curve, as has been shown in several studies (e.g. Müller and Schuhmann,

Table 6
Clusters of spheres with unit diameter and density $\rho_P = 1.3$ g/cm^3

Cluster	Mean D_{LD}	V_{TOT}	D_E	χ	ρ_S	$(\rho_P/\chi)^{0.5}$	$D_{A(E)}$	Ratio
Single sphere	1	0.52	1	1	1.30	1.140	1.14	0.88
2 chain	1.5	1.31	1.36	1.12	1.04	0.964	1.31	1.15
3 chain	2	2.10	1.59	1.27	0.98	0.876	1.39	1.44
4 chain	2.5	2.88	1.77	1.32	0.95	0.846	1.49	1.68

D_{LD} = mean of the extreme particle dimensions; V_{TOT} = total cluster volume; ρ_S = apparent particle density; $D_{A(E)}$ = aerodynamic diameter calculated from D_E (using Eq. (3)); Ratio = ratio of measured laser diffraction diameter (D_{LD}) to calculated aerodynamic diameter ($D_{A(E)}$); χ = dynamic shape factor, derived from Hinds (1982).

1996). It must therefore be concluded, that if the correct optical parameters are not known, or if they change during the measurement, then the Mie theory is no option.

More confining for laser diffraction testing of inhalation aerosols than the previously discussed theoretical drawbacks and limitations, are certain practical and operational limitations. Most frequently mentioned problems have been summarized in Table 7. Up to date, these problems have excluded widespread testing of breath controlled dpis. Not only flow control through the device is impossible with the present standard laser diffraction apparatus; neither can emitted mass fractions (of fines) be measured. Furthermore, measurement of drug–drug or drug–excipient mixtures may be difficult, if the size distributions of such binary mixtures cover each other largely. Finally, the presence of larger carrier crystals makes accurate fine drug particle measurement impossible. de Boer et al. (2000) recently presented the development of a special inhaler adapter to take away most of these operational shortcomings of lda. The system can be used for all types of inhalers; the configuration for dpis comprises an airtight in-line arrangement of a pre-separator, a central housing and a fine particle collector in between the inhaler and the vacuum system, through which the inspiratory flow curve can be adjusted.

Limits to the fine particle concentration in the aerosol cloud or the total mass of fines to be measured are not typically a problem related to laser diffraction technique. Limits also exist for cascade impactors, for which overload of the impaction plate may result in particle bounce or re-entrainment. With laser diffraction technique, very high particle concentrations in the cloud can be measured quite well, whilst high total mass amounts do not accumulate in the measuring zone, but are extracted with the air. Analytical problems with low particle concentrations in the aerosol cloud (as from very low drug doses) can be solved for impactor technique by inhaling a large number of doses for one single analysis. With laser diffraction technique, single drug doses of 2–4 μg can still be measured accurately, as a result of recent improvements in detector sensitivity. The wide measuring range with laser diffraction technique should not be considered as a problem. Lenses with focal lengths of 50 (range 0.45–87.5 μm) or 100 mm (range 0.9–175 μm) have to be used because of the required measuring volume within the aerosol cloud. This volume should not become too small, so as to measure a representative cross section of the cloud. But this overrange is compensated by the large number of size classes within the total measuring range (e.g. 31 for Sympatec HELOS) and the logarithmic increase in class width with increasing mean diameter. As a consequence, there are still ten size classes within the range from 0.9 to 5.0 μm for the 100 mm lens (R3), against even 14 classes within the range from 0.45 to 5.25 μm for the 50 mm lens (R2) for the Sympatec HELOS/BF-Magic (Sympatec GmbH, Goslar Germany). Most impactors, in comparison, have only one (e.g. twin impinger) to seven (e.g. Next Generation Impactor) classes within total size ranges that are even slightly larger. A clear advantage of laser diffraction is the fact that the size classes are independent of the inspiratory flow rate. A further unique characteristic of laser diffraction technology is the possibility to carry out so-called time-sliced measurements, that enable one to follow the size distribution in the cloud as function of the inhalation time. With this technique, the total measuring time is cut into very short periods within which single measurements are performed. Optical concentration expresses particle concentration in the cloud, and this parameter can be used to measure total

Table 7
Some frequently mentioned practical limitations and drawbacks of laser diffraction technique that are relevant to the characterization of inhalation aerosols

✓ The flow curve through the inhalation device can not be controlled
✓ Fine particle mass fractions are not obtained
✓ Measurement of mixtures (drug–drug or drug–excipient) may be problematic
✓ Low particle concentrations may not be measurable (dose weights < 4 μg)
✓ The measuring range is much wider than the size distribution of the drug particles
a. for a 100 mm lens, the measuring range is 0.9–175 μm
b. for a 50 mm lens, the range is 0.45–87.5 μm

emission time of an inhaler or to estimate at what moment, from the start of the inhalation, the bulk of the dose is released. But by far the most profitable features of laser diffraction are time saving, reproducibility and automatic data recording and processing.

4. Conclusions

The principle of inertial impaction with so-called multi stage impactors or liquid impingers is widely used for performance testing of medical aerosol generators. But the relevance of this technique is practically confined to comparative in vitro evaluation of these devices within rather narrow spans for (a constant) peak flow rate and inhalation time. At the highest possible flow rates through impactors, within the range between 60 and 90 l/min, the cut-off diameters and collected mass fractions are of lower confidence, because the flow regime inside the nozzles is highly turbulent. Fine particle outputs at flow rates being higher than 90 l/min, as can easily be attained by most patients during prescribed use of low resistance dry powder inhalers, cannot be measured because of the high impactor resistance. Experiments at high flow increase rates, which is the decisive flow parameter for inhalers like the Turbuhaler (Astra Zeneca), are not possible. High inter and intra laboratory variations have been described with impactors of the same design. Comparison of results from different types of impactors with different upper class limits for the fine particle fractions at the same flow rate are even more problematic. Especially, when the number of size classes is low and composition of a cumulative mass distribution curve as function of particle diameter is impossible. As a consequence of all these limitations, impactor data are a poor source of information for the prediction of (bio-)equivalence or therapeutic efficacy.With laser diffraction analysis, geometric particle diameters are measured instead of their aerodynamic equivalents, unless the particles are spherical and have unit density, such as droplets generated by nebulizers from aqueous drug solutions. Standard laser diffraction apparatus offer insufficient control of the inspiratory flow curve through the inhaler. Next to that, the mass fraction of the dose that is emitted as fine particles cannot be measured. Therefore, special means are necessary which minimally comprise a closed system through which the aerosol cloud can be drawn from the inhaler, a flow control unit and a fine particle collector for the determination of the emitted fine particle mass fraction. Additionally, for the measurement of dpis containing adhesive mixtures, a pre-separator for the larger carrier crystals is a requisite. With such means, designed as a modular concept, laser diffraction can become a highly valuable aerosol sizing technique for testing of all types of inhalers. This includes application for the development of dpis, for which the primary drug particle size distribution (from RODOS dispersion) can serve as a reference. Whether lda will also become acceptable as a standard for regulatory authorities for certain applications, depends on further development of the technique and its estimation based on validation programs and comparative error analyses (with cascade impaction). Advantages of laser diffraction technique over cascade impactor analysis are the many size classes within the relevant drug fraction for lung deposition, the short measuring time, size distribution measurement as function of inhalation time and automatic data recording.

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