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Research Paper

Characterisation of a Carrier-Free Dry Powder Aerosol Formulation Using Inertial Impaction and Laser Diffraction

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Purpose. The purpose of the study was to examine the suitability of using laser diffraction to measure the fine particle fraction (FPF) of drugs emitted from carrier-free dry powder aerosol formulations.
Materials and Methods. Particle size distribution of terbutaline sulphate from Bricanyl™ Turbohaler™, which contained loose agglomerates of drug particles only, was measured separately by laser diffraction apparatus equipped with a metal throat and a twin-stage, multi-stage liquid impingers, or Andersen cascade impactor at flow rates ranging from 28.3 to 100 l min^{-1}. In-line measurements were then conducted which allowed the same aerosolised particles to be measured first by laser diffraction then captured by an impactor or impinger for subsequent chemical analysis.
Results. A significant linear correlation ($p < 0.001$, $R^2 = 0.96$, ANOVA) existed between the results obtained from two techniques when measurements were conducted independently. There was little difference in FPFs measured by inertial impaction and laser diffraction at the same flow rate. When in-line measurements were conducted, the FPFs measured by inertial impaction were approximately 0.7–0.9 times the aerosol FPFs measured by laser diffraction. This linear relationship was statistically significant and had a statistically insignificant *y*-intercept, regardless of inhaler batches, impinger types and measuring position of the laser beam.
Conclusion. Laser diffraction could prove to be a reliable technique for development, evaluation and quality control of carrier-free, dry powder aerosol formulations.

KEY WORDS: Bricanyl™ Turbohaler™; dry powder aerosols; formulations; inertial impaction; laser diffraction; particle size distribution.

INTRODUCTION

Laser diffraction has been successfully employed to examine aerosols for inhalation from nebulisers (1) and pressurised metered dose inhalers (pMDI) (2), but its application to dry powder aerosols has been little studied (3,4). This is because the spray from a pMDI or mist from a nebuliser is primarily composed of the drug particles or droplets. Particle size results measured by laser diffraction can be related to the aerodynamic properties of the drug particles. Indeed, the results obtained from laser diffraction were found to correlate well with those obtained from an Andersen cascade impactor for nebulisers (5) and pMDI (6). However, the validity of laser diffraction to measure the drug particles from dry powder aerosol formulations remains unconfirmed largely due to the fact that most dry powder inhaler formulations are binary interactive mixtures composed of micronized drug blended with a coarse carrier which almost always contains fine carrier particles of similar size to the micronized drug. In the absence of chemical analysis, laser diffraction is incapable of differentiating between the drug and fine carrier particles. However, our previous studies demonstrated that for a typical binary interactive mixture, examining only the particles with diameter <60 μm obtained by laser diffraction highlighted the fine fraction (<5 μm) and enabled the aerosolisation of different blends to be compared at a range of different flow rates (7). There was a significant linear relationship between aerosol fine fractions measured by laser diffraction and drug fine fractions determined by inertial impaction. Such correlation was also found for formulations containing added fine lactose that would have interfered with the measurement of micronized drug. Yet, particle size measured by laser diffraction under well-controlled conditions could reflect the aerodynamic characteristics of the drug in formulations containing added fine carrier particles.

There is an increasing interest in formulations that do not need the coarse carrier to aid the flowability of micronized drug, namely, the carrier-free formulations. Some commercially available dry powder inhalers have already employed carrier-free formulations, such as those contained in the Turbohaler™ (8) and the Twisthaler™ (9). In these inhalers, micronized drug particles are loosely agglomerated into larger particles to improve flowability such that the

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agglomerates can flow from drug reservoirs into metering chambers of the devices. On inhalation, the loose agglomerates would be broken up into primary particles that can penetrate into the lung. A different type of carrier-free formulation is exemplified by Exubera®, which contains spray dried insulin particles (10). Instead of jet milling which could denature proteins, spray drying has been employed to manufacture respirable particles containing the active ingredient and stabilizing agents. Particles made by spray drying are spherical and may flow sufficiently well to be filled accurately into blisters or capsules without the need for coarse carriers. Besides, lactose is a reducing sugar and it is not compatible with drugs that have primary amine moieties such as proteins/peptides (11). Therefore, formulations similar to that in Exubera® are expected to play an important role in delivery of proteins/peptides to the lungs.

To achieve maximal delivery of drugs to the lung from these formulations, extensive investigations have to be conducted to optimize both the formulation and manufacturing processes. Current compendial techniques for testing dry powder inhalers are all based on inertial impaction principles (7). However such techniques are unable to generate information that is sufficiently detailed for detecting any subtle differences that might be vital to formulation and process optimisation. Besides, techniques based on inertial impaction are laborious, time-consuming and difficult to set up as in-line control techniques. Therefore, there is an urgent need for a robust technique that is capable of conducting on-, in- and/or at-line measurement of particle size distribution. It is therefore the purpose of this study to establish whether a laser diffraction method has the potential to characterize the aerodynamic properties of a carrier-free formulation.

MATERIALS AND METHODS

Materials

Bricanyl™ Turbohalers™ (AstraZeneca, King's Langley, Herts, UK, BNs: VF 565 and VC 552) each containing 100 doses (500 μg terbutaline sulphate per dose) were purchased and used as supplied. Standard solutions of terbutaline were prepared from terbutaline sulphate (Sigma Chemicals Ltd., Poole, Dorset, UK: lot 33H0964). Chemicals and solvents used were *p*-hydroxybenzoic acid ethyl ester (ethyl paraben) (Sigma, Poole, UK), methanol (HPLC grade) (Rathburn Chemicals Ltd, Walkerburn, Scotland), ammonium acetate (HPLC grade) (BDH Lab Supplies, Loughborough, UK) and distilled water (MilliQ grade) (Millipore, Watford, UK).

Analysis of Terbutaline Quantities in Samples from Inertial Impaction Measurements

A UV or an HPLC method was validated to measure terbutaline quantities in samples from measurements by inertial impaction. The UV method was employed to measure terbutaline in samples collected from independent measurements by inertial impaction since without the interference from any bulking agent, sufficient number of doses (e.g., ten doses) could be fired into the impinger/impactor in order for the UV method to measure terbutaline deposited in each stage of the impingers accurately. However, the number of doses needed to be kept to a minimum when sizing by inertial impaction was used in line with laser diffraction so as to avoid powder build-up within the laser diffraction apparatus that could cause errors to measurements by both techniques. Five doses were fired into the system and drug deposition in each stage of the impinger/stage was then measured using the HPLC method.

For UV assay, a series of standard solutions with varying terbutaline concentrations was prepared to construct a calibration curve for terbutaline. For each series, 100 ml of stock solution of terbutaline (100.0 μg ml^{-1}) in methanol:water (70:30) mixture was prepared. From this stock solution, the range of solutions containing 10.0, 20.0 and 50.0 μg ml^{-1} terbutaline was obtained. Three replicate series of the three standard solutions were made, each from an independently prepared stock solution, and the absorbance of each standard solution was determined at 280 nm. Plotting each absorbance as a function of concentration produced a straight calibration curve.

The HPLC assay employed a mixture of methanol and 0.0013 M ammonium acetate (pH 4.5) (55:45, v/v) as the mobile phase running at a flow rate of 0.8 ml min^{-1}, *p*-hydroxybenzoic acid ethyl ester (2 μg ml^{-1}) as an internal standard and UV detection at 276 nm. The HPLC system consisted of a pump (CM 4000 Multiple Solvent Delivery System, LDC Analytical, FL, USA), a multiple wavelength UV detector (SpectroMonitor 3100, LDC Analytical, FL, USA) and a 15 cm S5ODS2 C_{18} column (Anachem). The retention times for terbutaline and ethyl paraben (IS) were found to be 2.3 and 5.8 min, respectively. The HPLC method was found to be linear in the range of 0.8–20 μg ml^{-1} terbutaline, with a recovery of 99–101% and relative standard deviations of less than 1% in the intra- and inter-day variability of the results.

Particle Size Characterisation by Inertial Impaction

The aerodynamic particle size distribution of the aerosols generated by the Turbohaler™ was measured by either an Andersen cascade impactor (ACI) at 28.3 l min^{-1}, or a twin stage liquid impinger (TSLI) at 60 l min^{-1} or a multistage liquid impinger (MSLI) at 60 and 100 l min^{-1}. The impingers and impactor were operated under pharmacopoeial conditions (12). Five or ten doses were discharged from the Turbohaler™ (approximately 2.5 mg terbutaline sulphate) per experiment and each dose was withdrawn by 4 l of air through the inhaler at the predetermined flow rate. Each stage of the impinger or impactor was then washed individually using the mobile phase containing the internal standard (MPIS) or the methanol:water (70:30) mixture and the washing solution was then made up to a fixed volume (50 ml) with the same solvent for analysis of terbutaline content using the HPLC or UV method.

The MSLI (Astra Draco, Lund, Sweden) was fitted with a TSLI glass throat. The ACI (Andersen Samplers Inc., Atlanta, Georgia, USA) was assembled with a glass adaptor (14/23 male Quickfit™ socket) on top of the preseparator to accept the TSLI glass throat. For each impinger or impactor, a silicone rubber insert was attached to the entrance of the glass throat to accept the mouthpiece of the Turbohaler™. A

backup filter (Qualitative 1, Whatman Labs. Div., Maidstone, Kent) was introduced to the last stage of the ACI or MSLI. To minimise particle bounce on the plates of the ACI during testing, the preseparator and collection plates were coated in hexane containing 1% (w/v) silicone fluid and left to air dry in a fume hood.

Particle Size Characterisation by Laser Diffraction

A metal throat was designed and made in-house to receive the aerosol cloud generated by the inhaler and permit particle sizing to be made by a Malvern 2600 laser diffraction sizer (7). The Turbohaler™ mouthpiece was connected with the teflon entrance seal at the mouthpiece of the metal throat. A metal throat exit seal with a brass fitting was used to connect a variable pump via a solvent trap that allowed constant flow rates of 28.3 to 60 l min^{-1} to be generated through the metal throat with the Turbohaler™ in line.

The Malvern 2600 sizer was fitted with a 100 mm lens and positioned with the laser beam passing through the top window of the metal throat. Background particle size measurements were taken over 1,000 sweeps of the detector elements with the pump activated but without the primed Turbohaler™ in place. A sweep is the number of times a reading is taken of light falling on each of the concentric photodiode detectors. Particle size distribution measurements of each aerosol dose were made over 200 sweeps of the detector elements. Since the aerosol emerged from the Turbohaler™ as soon as the pump was switched on, the measurement cycle of 200 sweeps was initiated immediately before the pump was started. This ensured that the total aerosol cloud was representatively viewed by the laser beam. The complete dose passed the beam within the 200 sweep cycle.

Five doses were discharged and measured from each Turbohaler™ batch at each flow rate. The aerosol particle size distribution was presented according to an independent model based on the diffracted light from all detector rings ('virgin' kil (0,0) distribution). The independent model calculated particle size distribution from the light scattering data recorded without making an *a priori* assumption about the nature of the size distribution. Since the formulation consisted of pure terbutaline, all particles should be included in the calculation of fine fraction of the drug. This was in contrast to the particle size measurement of carrier-based formulations, where the light scattering data recorded in the first inner ring of the detector had to be removed in order to eliminate the interference from the larger (>60 μm) carrier particles with the assessment of the finer drug particles (<10 μm) (7). The size measurement of the carrier-free formulation as carried out in this study was based on all light scattering data, including that recorded in the first inner ring of the detector, in order to represent the entire particle size distribution of the drug.

Simultaneous Characterisation by Inertial Impaction and Laser Diffraction

In the above studies described, sizing by laser diffraction and inertial impaction was carried out independently of each other, i.e., carried out on dry powder aerosols from the same Turbohaler™ device but on different aerosol doses in separate experiments. However the metal throat was also combined in line with the impactor in order to allow the same aerosol, generated under inspiratory air flow, to pass through both the laser beam crossing the metal throat window and the stages of the inertial impactor (Fig. 1). Sizing could therefore be carried out by the two techniques 'simultaneously.' The Teflon™ seal designed to connect the metal throat with the entrance port of the Andersen cascade impactor preseparator, was not used in this study. This was because the seal protruded into the interior of the metal throat and trapped a proportion of the aerosol powder before it could enter the impactor. The Teflon™ seal was therefore replaced by a glass funnel with cylindrical sides, the neck of which fitted tightly within the preseparator entrance port (Fig. 1). The metal throat was held in place centrally over the neck by a moulded silicone rubber seal. The silicone rubber did not interfere with the HPLC assay for terbutaline. When sizing at 60 and 100 l min^{-1} was required, an alternative glass funnel with a silicone rubber mould insert was used to seal the metal throat via the glass neck to the multistage liquid impinger entrance port which had an internal diameter larger than that of the preseparator.

Since the sizing by inertial impaction was carried out simultaneously with that by laser diffraction, five Bricanyl™ Turbohaler™ doses were discharged through the metal throat at any one flow rate per experiment. After five doses had been discharged and the particle size distribution of each separate dose determined by laser diffraction, the complete throat and impactor apparatus were washed to prepare analysis samples for one experiment. Five impaction experiments were carried out at each flow rate. Hence the generated aerosols were sized by laser diffraction over 25 measurements and by inertial impaction over five measurements.

Aerosol Characterisation Before and After the 90° Bend in Metal Throat

The presence of two sets of viewing windows on the metal throat, one before and one after the 90° bend, enabled

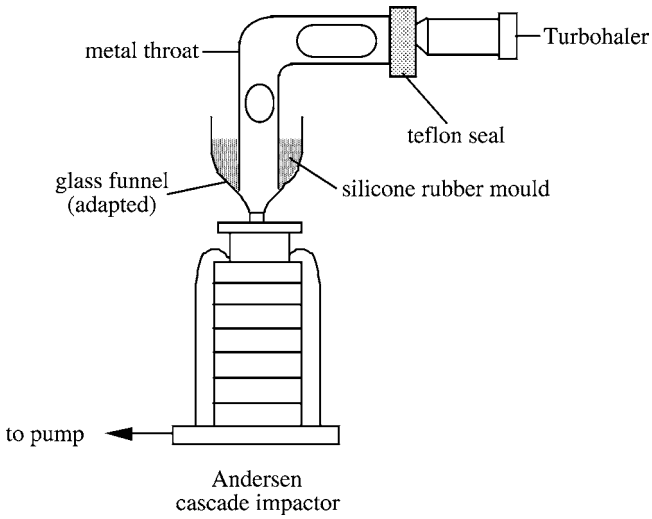

Fig. 1. Schematic diagram of combined apparatus of metal throat and inertial impactor (represented as an Andersen cascade impactor) to permit simultaneous particle sizing of Bricanyl™ Turbohaler™ dry powder aerosols by laser diffraction and inertial impaction.

the generated aerosol to be viewed at one of two different points in its flight path to the inertial impactor. The aerosol was viewed through the bottom window to ascertain the effect on aerosol particle size distribution of any impaction or deposition in the upper part of the throat. For this purpose it was important to ensure that any aerosol particles which impacted in the throat remained deposited there, and did not bounce off to rejoin the aerosol passing on to the bottom window. The internal metal surfaces of the throat were therefore coated with pressure sensitive double-sided adhesive tape (3M Loughborough, UK) to increase their adhesiveness and to resemble the tackiness of the throat walls found *in vivo*. One strip of adhesive tape was applied to the upper/rear internal surface and one to the lower/forward surface of the throat, as depicted in Fig. 2. The internal sides of the throat, housing the glass windows, were not coated due to the practical difficulties of cutting and positioning the tape to cover the walls without touching the windows.

On completion of each experiment with a coated throat, the pieces of adhesive tape were carefully removed and sonicated in 25 ml washing solution (MPIS) for 5–10 min. This solution was then added to those washings from the rest of the throat apparatus for HPLC analysis. In summary, generated aerosols were sized by laser diffraction through the top window of both a coated and an uncoated metal throat, and through the bottom window of a coated throat.

Deposition within the Metal Throat

To examine this throat deposition further, inertial impaction experiments were repeated at the three flow rates, 28.3, 60 and 100 l min^{-1} using a metal throat coated internally as described before. On the completion of each experiment, however, the washings from the throat were prepared from three areas (Fig. 2): 1) the Teflon™ seal housing the Turbohaler™; 2) the "upper" part of the throat containing the top windows, and 3) the "lower" part of the throat containing the 90° bend and the bottom windows.

To help maintain a consistent position of the arbitrarily selected line of division between the upper and lower throat parts, the coating tape was cut into four measured lengths for each experiment before positioning them within the throat (Fig. 2). The break between tapes A and B and between tapes C and D marked the division between the upper and lower throat parts.

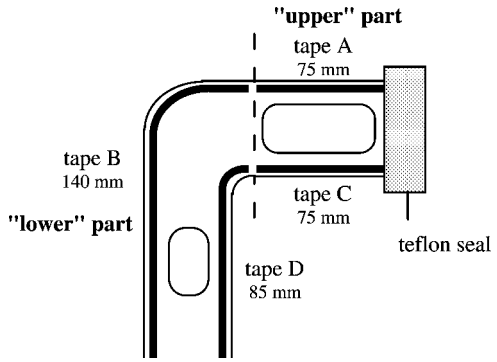

Fig. 2. Schematic diagram of internally coated metal throat displaying the arbitrarily selected line dividing the "upper" from the "lower" part of the throat for examination of deposited terbutaline.

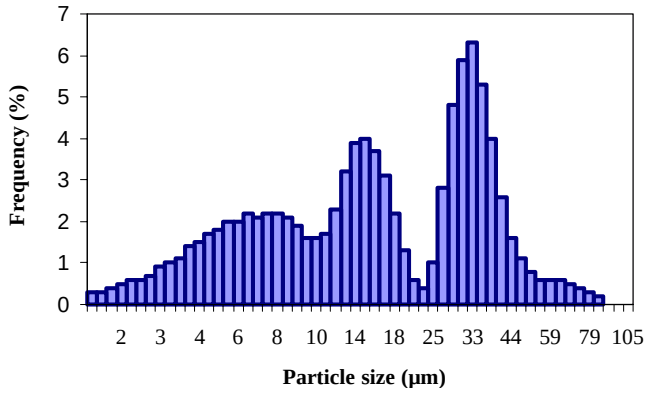

Fig. 3. Particle size distribution of the aerosol dose generated from a Bricanyl™ Turbohaler™ at 28.3 l min^{-1} sized through the top window of the metal throat by laser diffraction.

On the completion of one additional experiment at each flow rate, the throat was not washed for analysis but instead, the coating tapes were removed carefully and examined, by scanning electron microscopy, to assess the particle size, position and relative quantity of deposited material with aerosol generating flow rate.

RESULTS

Particle Size Distribution by Laser Diffraction

Figure 3 shows a representative Malvern particle size distribution of a Turbohaler™ aerosol dose generated at 28.3 l min^{-1} whilst Fig. 4 displays that part of the average distribution which highlights the aerosol fine fractions up to 10.3 μm for each flow rate. No significant differences were apparent between the aerosol distributions generated from Turbohalers™ derived from the two different batches (p = 0.948, ANOVA). There were differences, however, between the distributions measured from aerosols generated at both 28.3 and 60 l min^{-1} (Fig. 4).

Correlation between Inertial Impaction and Laser Diffraction

The aerosol fine fractions by laser diffraction and terbutaline fine fractions by inertial impaction at 28.3 and 60 l min^{-1} are summarised in Table I. As found with the laser diffraction data, the aerosol particle size distributions from the two different batches were not different (p = 0.849, ANOVA). The mean aerosol fine fraction (<5.5 μm) measured at 60 l min^{-1} was reduced from a mean value of 34.7 to 13.1% at 28.3 l min^{-1} (<5.8 μm). The mean emitted dose was also found to be flow dependent, being reduced significantly from 80.9% at 60 l min^{-1} to 58.4% at 28.3 l min^{-1} ($p < 0.001$, Student's t-test).

A significant linear correlation ($p < 0.001$, $R^2 = 0.96$, ANOVA) existed between the distribution results from the two techniques (Fig. 5). There was little difference in fine particle fractions measured by different impactors and laser diffraction.

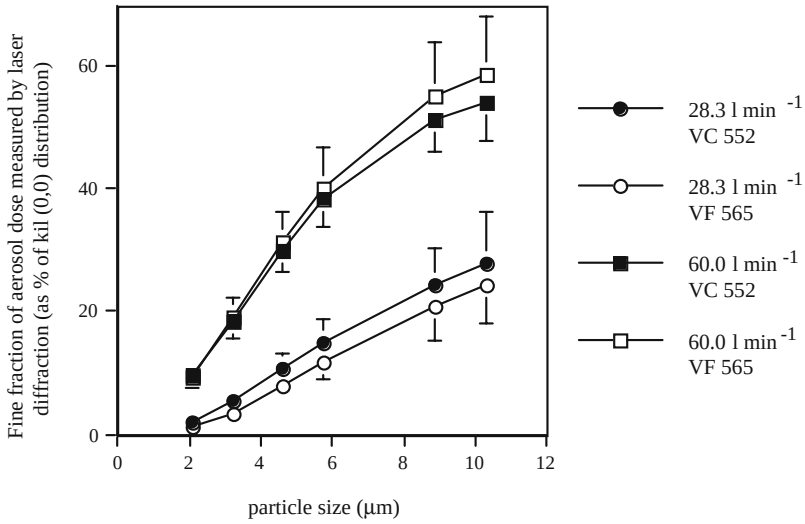

Fig. 4. Partial particle size distributions of aerosol doses generated from Bricanyl™ Turbohalers™ (BNs: VC 552 and VF 565) at 28.3 and 60 l min^{-1} measured by laser diffraction (mean (sd), $n = 5$).

Particle Size Characterisation by Inertial Impaction In-Line with Laser Diffraction

Internal Coating of Metal Throat

The aerosol particle size distribution data determined by inertial impaction and carried out using a coated or uncoated metal throat on top of the impaction apparatus at 28.3, 60 and 100 l min^{-1} are detailed in Tables II, III and IV, respectively. The data are expressed both as percent of emitted dose deposited on each stage and cumulative percent undersize of emitted dose for each size fraction. The data in Tables II to IV show that there was little difference between the distributions of aerosols deposited in apparatus which included either a coated or uncoated throat. This suggested that the increased adhesiveness was not affecting the deposition of fine particles which would normally pass through an uncoated throat to the impactor.

Deposition within the Metal Throat

Table V details the mean terbutaline deposited (per emitted aerosol dose) analysed by HPLC within the three areas of the throat and seal apparatus at flow rates of 28.3, 60 and 100 l min^{-1}. Although the Turbohaler™ mouthpiece fitted into the Teflon™ seal so that the end of the mouthpiece was virtually flush with the inner edge of the seal, some terbutaline did deposit in this area. The spiral channels within the Turbohaler mouthpiece are known to impart centrifugal forces on the aerosol particles in the air flow (13). These forces are likely to have promoted the particles' impaction on the walls of the seal (and throat) as they left the mouthpiece in an outward spiralling trajectory. The deposition within the seal was found to increase with increasing flow rate. A degree of electrostatic attraction may also have contributed to this deposition. It was also clear that at each flow rate, the amount of terbutaline deposited in the upper

Table I. Aerosol Particle Size Distributions Measured by Laser Diffraction (LD) (mean(sd), $n = 5$) and Inertial Impaction (II) (mean(sd), $n = 3$) from Two Batches of Bricanyl™ Turbohaler™ at 28.3 and 60 l min^{-1}

	Particle size (μm)		Batch VC 552		Batch VF 565	
Flow rate (l min^{-1})	LD	II	LD (percent of aerosol volume)	II (percent of recovered terbutaline)	LD (percent of aerosol volume)	II (percent of recovered terbutaline)
28.3	<10.3	<10.0	22.40 (1.4)	27.8 (8.6)	17.28 (2.6)	24.4 (6.2)
	<8.9	<9.0	15.26 (0.9)	24.5 (5.8)	14.89 (2.2)	20.9 (5.6)
	<5.8	<5.8	13.03 (1.3)	14.8 (3.8)	13.07 (1.6)	11.8 (2.9)
	<4.6	<4.7	11.27 (1.1)	10.6 (2.7)	11.61 (1.1)	7.9 (2.0)
	<3.3	<3.3	7.48 (1.7)	5.3 (1.1)	5.71 (0.2)	3.4 (0.9)
	<2.1	<2.1	3.93 (2.0)	1.9 (0.5)	2.03 (0.6)	1.1 (0.3)
60	<10.3	<10.5*	38.63 (2.0)	54.1 (6.3)	47.33 (1.0)	58.6 (9.4)
	<5.4	<5.5*	30.53 (4.0)	38.2 (4.4)	38.86 (5.0)	40.1 (6.6)
	<6.6	<6.4**	36.3 (9.0)	43.2 (4.6)	40.20 (4.0)	46.8 (9.4)

*Multi-stage liquid impinger.
**Twin-stage liquid impinger.

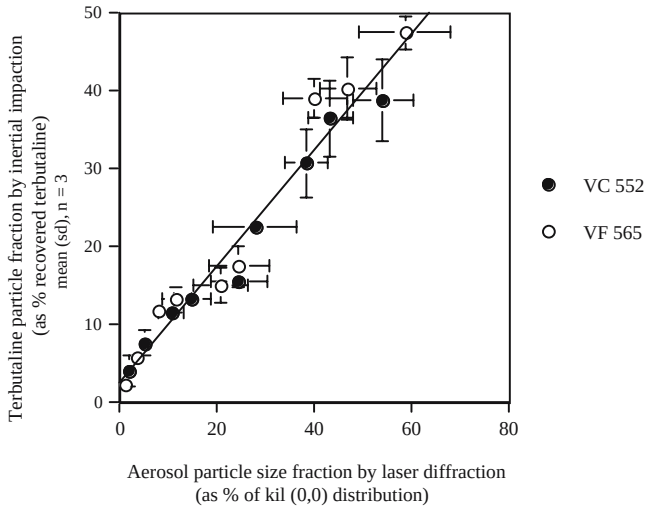

Fig. 5. Terbutaline particle size fractions measured by inertial impaction from Bricanyl™ Turbohaler™ aerosols generated at 28.3 and 60 l min^{-1} plotted as a function of aerosol particle size fractions measured by laser diffraction (mean (sd), $n = 5$).

part of the metal throat was much greater than that deposited in the lower part ($p < 0.001$, Student's t-test). The ratio of deposited material in the upper throat:lower throat was lowest at 60 l min^{-1}.

At 28.3 l min^{-1} the bulk of the deposited material in the upper throat was found on the lower (tape C, Fig. 2) rather than on the upper surface (tape A, Fig. 2) of the throat (SE micrographs not shown). At this low flow rate the deposited material was present as large aggregates, some up to 70 μm in diameter. Some smaller aggregates created at 28.3 l min^{-1} did follow the air flow streamlines through this part of the throat only to impact at the back of the throat at the 90° bend (tape B in Fig. 2). The bulk of the fine particles were able to follow the air flow streamlines through the throat to the inertial impactor below. Overall, the evident loss of large aggregates in the upper throat and back of the 90° bend would account for the significant increase in aerosol fine fraction measured at the bottom window by laser diffraction.

The least amount of terbutaline was deposited at 60 l min^{-1} relative to that at the other flow rates but when throat deposition was expressed as a percent of the emitted dose at each flow rate, very little difference was observed. At 60 l min^{-1}, the ratio of deposited terbutaline in the upper part of the throat to that in the lower part of the throat was markedly lower than at the other flow rates. This suggested that, at this flow rate, more of the emitted dose was able to follow the air flow streamlines through the upper throat but then impacted in the lower throat before it reached the impactor. At 60 l min^{-1}, the comparatively large quantity of impacted material on the back of the throat (tape B in Fig. 2) was found to exist in particle size ranges from 1–10 μm to up to 60 μm (SE micrographs not shown).

At 100 l min^{-1} very little terbutaline was deposited in the lower throat as there were few aerosol particles of a size with sufficient inertia at this flow rate to impact. What was evident, however, was the deposition of fine particles (<10 μm) on the roof (tape A in Fig. 2) and on the floor (tape C in Fig. 2) of the upper throat (SE micrographs not shown). Particles of this size, at this flow rate, would normally follow the air flow streamlines through the throat. However, as discussed previously, the flight of the aerosol within the internal spirals of the Turbohaler™ mouthpiece is such that the trajectory of the emerging aerosol could impact with the walls of the upper metal throat, particularly at such high flow rates. A high degree of air turbulence in the upper throat would also be anticipated.

Correlation between Laser Diffraction and Inertial Impaction After In-Line Measurement

Top Window of the Throat

Characterisation of Bricanyl™ Turbohaler™ dry powder doses (BN: VM 589) by the two techniques, laser diffraction and inertial impaction were found to give comparable results of size distribution for aerosols generated at 28.3, 60 and 100 l min^{-1} (Table VI). This was true for laser diffraction measurements of the aerosols through the top throat window. When the results (Table VI) by laser diffraction were plotted against those by inertial impaction, a correlation of significant linearity was revealed ($p < 0.001$, $R^2 = 92.6\%$, ANOVA, Fig. 6). If this linear correlation is compared with that found for aerosols from the other Turbohaler™ batches (VC 552 and VF 565) examined previously, the equation of linear regression was not found to be dependent upon the batch of Turbohaler™ used ($p = 0.661$, ANOVA).

Table II. Terbutaline Size Distribution from Turbohalers™ at 28.3 l min^{-1} through a Coated and Uncoated Metal Throat Fitted on Top of an Andersen Cascade Impactor (mean(sd), $n = 5$)

		Uncoated throat		Coated throat	
Andersen stage	Particle size cut-off (μm)	Percent of emitted dose	Cumulative percent undersize	Percent of emitted dose	Cumulative percent undersize
Metal throat		63.01 (2.9)		66.03 (4.2)	
Preseparator	>10.0	21.33 (2.6)	36.98 (2.8)	18.53 (3.2)	34.94 (3.6)
0 and 1	10.0	3.50 (1.2)	15.65 (2.6)	4.22 (2.6)	16.41 (3.0)
2	5.8	1.50 (0.5)	12.15 (2.0)	1.56 (0.7)	12.19 (4.1)
3	4.7	4.88 (1.3)	10.65 (1.7)	4.52 (1.3)	10.63 (2.9)
4	3.3	3.39 (0.6)	5.77 (1.1)	3.22 (1.2)	6.11 (2.2)
5 to filter	2.1	2.37 (0.9)	2.37 (0.9)	2.90 (1.1)	2.90 (1.1)

Table III. Terbutaline Size Distribution from Turbohalers™ at 60 l min^{-1} through a Coated and Uncoated Metal Throat Fitted on Top of a Multi-stage Liquid Impinger (mean(sd), $n = 5$)

		Uncoated throat		Coated throat	
MSLI stage	Particle size cut-off (μm)	Percent of emitted dose	Cumulative pecent undersize	Percent of emitted dose	Cumulative percent undersize
Metal throat		50.67 (4.4)		55.42 (3.7)	
1	>13.0	8.12 (2.1)	49.34 (4.4)	6.00 (2.0)	44.58 (3.7)
2	13.0	5.93 (2.0)	41.21 (6.0)	4.54 (1.1)	38.12 (3.3)
3	6.8	26.48 (2.8)	35.30 (4.5)	25.97 (2.3)	33.58 (3.1)
4	3.1	8.80 (1.9)	8.80 (1.9)	7.61 (3.5)	7.61 (3.5)

Bottom Window of the Throat

In order to compare the laser diffraction results obtained through the bottom window with those produced by inertial impaction on the same aerosol, the inertial impaction results were adjusted to account for the deposited terbutaline lost from the aerosol prior to its arrival at the bottom window. The impaction results for a coated throat (Tables II, III and IV) presented the quantity of deposited terbutaline on each impactor stage as a fraction of the emitted dose recovered (D_1) from the complete apparatus. In adjusting these figures, to allow for their comparison with laser diffraction data through the bottom window, the terbutaline deposited in the seal and upper part of the throat (Table V) was subtracted from D_1 to calculate the dose recovered from the lower throat and impactor (D_2). Like D_1, D_2 differed at each flow rate. This quantity (D_2) is a closer representation than D_1 of the aerosol viewed by laser diffraction through the bottom window. The amount of terbutaline deposited on each stage was then presented as a fraction (%) of D_2, as shown in Table VI. These adjusted inertial impaction results were then compared with the particle size results by laser diffraction through the bottom window (Table VII) and the plot of the correlation (Fig. 7) represented in a similar manner to that carried out for comparative measurements through the top window (Fig. 6).

A good linear correlation was found between the particle size results by inertial impaction (as percent of D_2) and those by laser diffraction through the bottom window ($p < 0.001$, $R^2 = 97.2\%$, ANOVA). It was further found that when the correlation between measurements through the top window was compared with the correlation between measurements through the bottom window, no dependency on throat window position was found ($p = 0.094$, ANOVA). It could be concluded therefore that measurements by inertial impaction could be predicted from the corresponding measurements made by laser diffraction on the same Bricanyl™ Turbohaler™ aerosol through either throat window.

DISCUSSION

Both sizing techniques, laser diffraction and inertial impaction, detected differences in the aerosol particle size distributions at flow rates of 28.3 and 60 l min^{-1}. Both techniques also revealed evidence of a significant degree of aggregated material in the aerosols generated at these flow rates. Indeed, even at 60 l min^{-1} less than 35% of the emitted dose appeared to be of such a fine nature which would be deemed to be "respirable." These changes in fine fraction and emitted dose noted with an increase in flow rate from 28.3 to 60 l min^{-1} are similar to those reported in the literature (13–16).

Laser diffraction has been used as a sizing technique for particles and droplets for many years (17). The technique is suitable for use with liquid-borne and gas-borne suspensions, and is therefore well suited for use in the characterisation of aerosol dosage forms. In recent years, much interest has been directed towards diffraction analysis in the assessment of nebuliser clouds (5). The nebulised cloud can be sized by this technique as soon as it emerges from the mouthpiece before any appreciable evaporation can take place, and the technique is much faster than conventional sizing by impaction. The robustness of laser diffraction and generation of a sizing parameter relevant to the clinical situation for nebulisers is largely due to the sphericity of the particles and the narrow distribution and homogeneous nature of the nebulised cloud. The same may also be true for dry powder aerosol formulations containing loose agglomerates of micronized drug particles or spray dried particles containing the drug and excipients.

Table IV. Terbutaline Size Distribution from Turbohalers™ at 100 l min^{-1} through a Coated and Uncoated Metal Throat Fitted on Top of a Multi-stage Liquid Impinger (mean(sd), $n = 5$)

		Uncoated throat		Coated throat	
MSLI stage	Particle size cut-off (μm)	Percent of emitted dose	Cumulative percent undersize	Percent of emitted dose	Cumulative percent undersize
Metal throat		49.34 (3.0)		52.55 (4.4)	
1	>10.07	8.14 (4.1)	50 67 (2.9)	7.08 (2.4)	47.44 (4.4)
2	10.07	6.14 (1.0)	42.27 (6.7)	7.26 (0.8)	40.37 (4.7)
3	5.27	26.00 (6.5)	36.19 (6.0)	25.96 (3.9)	33.11 (4.1)
4	2.40	10.19 (2.2)	10.19 (2.2)	7.15 (1.6)	7.15 (1.6)

Table V. Terbutaline Deposition (μg) within Different Parts of the Coated Metal Throat after Aerosolising a Dose from the Turbohaler™ at 28.3, 60 and 100 l min^{-1} (mean (%RSD), $n = 3$)

Apparatus part	28.3 l min^{-1}	60 l min^{-1}	100 l min^{-1}
Teflon™ seal	14.0 (4.0)	36.4 (2.9)	62.0 (1.4)
Upper part of coated throat	191.1 (2.1)	119.0 (1.3)	164.2 (0.8)
Lower part of coated throat	15.7 (2.0)	23.4 (1.8)	6.7 (0.7)
Mean total	220.8 (8.1)	178.8 (6.0)	232.9 (2.9)

According to Lorenz-Mie theory, the scattering of light by a particle is a function of the equivalent sphere diameter of the particle, the wavelength of the incident light, the difference in refractive indices including light absorption coefficient between the particles and the medium in which they disperse (18). Particles in a carrier-free formulation are sufficiently homogeneous in terms of chemical composition such that the component particles in each formulation have virtually the same refractive index and light absorption coefficient, both of which can be determined (19). In addition, both the micronized and spray dried particles can be treated as spherical during laser diffraction measurement (20). It is therefore possible to use instruments based upon Lorenz-Mie theory such as the Malvern Mastersizer series (Malvern Instruments, Malvern, U.K.) to measure accurately the particle size distribution of a carrier-free dry powder aerosol formulation.

If the particles are opaque and do not transmit any light, greater than 40 times the wavelength of the laser light (i.e., 25 μm when a He-Ne laser is used) and those of different size scatter light with the same efficiency, the Lorenz-Mie theory can be reduced to the simpler Fraunhofer diffraction theory, also referred to as static light scattering or low-angle forward light scattering. In the present study, particle size measurement was performed using the Malvern 2600 Particle sizer, which is based on the Fraunhofer approximation (21). It has been reported that the Fraunhofer approximation works well for unimodal size distributions, but may skew the reported distribution towards the mode that produces the strongest peak in the diffraction pattern for multimodal systems (22). The particles released from the Turbohaler™ exhibited a unimodal size distribution generally\ whilst the particles within a binary mixture (i.e., micronized drug blended with coarse carrier particles) followed a bimodal or even multi-modal distribution. Therefore, laser diffraction is better suited for characterising the formulations of unimodal size distributions than formulations of bi- or multimodal distributions. For unimodal size distribution, fine fractions may be accurately calculated from the entire size distribution without the need to exclude the large fraction (i.e., > 60 μm) from the calculation as described for the bimodal size distribution (7). Consequently, the fine fraction results measured by laser diffraction are similar to the results by inertial impaction. In a binary blend, the particle size distribution by laser diffraction is likely to exaggerate the fraction of large carrier particles at the expense of the aerosol fines (<5 μm), leading to an underestimate of the fine fractions. Errors such as these may have contributed to the levels of aerosol fines measured by laser diffraction being consistently lower than those by inertial impaction (7).

From the data obtained (Fig. 6) in these studies, the following equation of linear regression (Eq. 1) with an insignificant y-intercept ($p = 0.125$, ANOVA) existed between the terbutaline fractions (II) measured by inertial impaction and the aerosol fractions (LD_{top}) measured by laser diffraction through the top window:

$$\mathrm{II} = 0.747(LD_{top}) \quad (1)$$

A second linear regression (Eq. 2) equation was obtained (Fig. 7), also with an insignificant y-intercept ($p = 0.311$, ANOVA) showing that a correlation existed between the terbutaline fractions (II_{D2}) measured by inertial impaction and the aerosol fractions (LD_{bot}) measured by laser diffraction through the bottom window:

$$\mathrm{II}_{D2} = 0.948(LD_{bot}) \quad (2)$$

When the correlation between measurements through the top window (Eq. 1) was compared with the correlation

Table VI. Particle Size Distributions of Bricanyl™ Turbohaler™ Aerosol Doses, Measured by Inertial Impaction (II) and Laser Diffraction (LD), as they Pass the Top Window of the Metal Throat

	Size range of aerosol		Aerosol fraction (*mean % (sd))	
Flow rate (l min^{-1})	By II (μm)	By LD (μm)	By II (as percent of emitted dose)	By LD through top window (as percent of aerosol volume)
28.3	<10.0	<10.3	15.65 (2.6)	22.2 (10.1)
	<5.8	<5.8	12.15 (2.0)	10.6 (5.0)
	<4.7	<4.6	10.65 (1.7)	8.0 (3.7)
	<3.3	<3.3	5.77 (1.1)	3.3 (1.6)
60	<13.0	<12.3	41.21 (6.0)	56.6 (11.7)
	<6.8	<6.7	35.30 (4.5)	38.1 (8.4)
	<3.1	<3.2	8.00 (1.9)	11.1 (3.6)
100	<10.07	<10.3	42.77 (6.7)	50.8 (21.6)
	<5.27	<5.4	36.19 (6.0)	31.4 (14.7)
	<2.4	<2.4	10.19 (2.2)	8.3 (6.0)

*Mean (sd) where $n = 5$ for inertial impaction and $n = 25$ for laser diffraction.

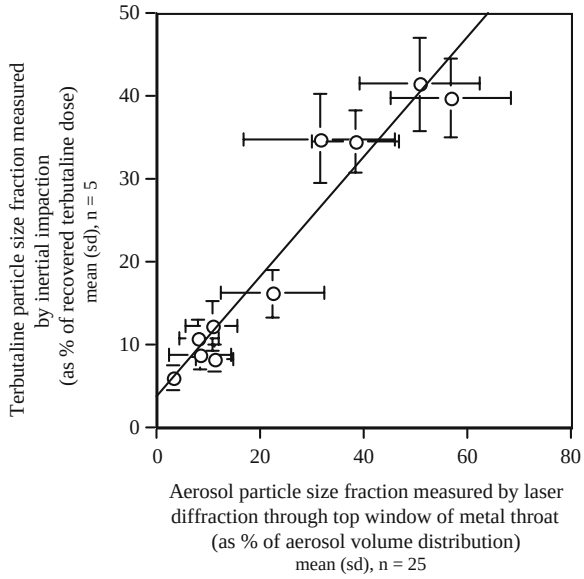

Fig. 6. Plot of Bricanyl™ Turbohaler™ (BN: VM 589) aerosol particle size distributions measured by laser diffraction (*through top window of metal throat*) against the distributions measured by inertial impaction ($p < 0.001$, $R^2 = 92.6\%$, ANOVA).

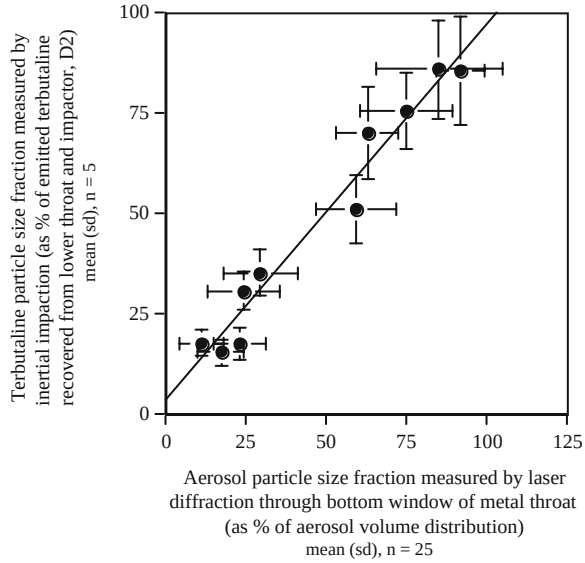

Fig. 7. Plot of Bricanyl™ Turbohaler™ (BN: VM 589) aerosol particle size distributions measured by laser diffraction (*through bottom window of metal throat*) against the distributions measured by inertial impaction ($p < 0.001$, $R^2 = 97.2\%$, ANOVA).

between measurements through the bottom window (Eq. 2), no dependency on throat window position was found ($p = 0.094$, ANOVA). The two equations of linear regression (Eqs. 1 and 2) were not found to be significantly different ($p = 0.912$, ANOVA). According to Stokes' law, the aerodynamic fine fraction (measured by inertial impaction) should be the geometric fine fraction (measured by laser diffraction) divided by the square root of density. The density of solid inhalation drugs ranges from approximately 1.2 to 1.5 g cm^{-3}, unless porous particles are being used (23). Therefore, the fine fractions measured by inertial impaction should theoretically be 0.8–0.9 times the fine fractions measured by laser diffraction at two extremes of the density. This theoretical calculation correlates very well with the experimental data obtained in the present study. Particle density can be easily measured and then used to calculate aerodynamic particle size distribution from the distribution measured by laser diffraction (20). The linear correlations between the aerosol measurements by two techniques demonstrated how the more rapidly determined laser diffraction measurements could be used to predict the aerodynamic particle size distribution results normally obtained laboriously by inertial impaction. Indeed, the absence of a statistically significant *y*-intercept in the linear regression equations suggests that there is a strong correlation between the two techniques when dry powder aerosols containing solely drug as opposed to drug and excipient blends.

Table VII. Particle Size Distributions of Bricanyl™ Turbohaler™ Aerosol Doses Measured by Inertial Impaction (II) and Laser Diffraction (LD) as they Pass the Bottom Window of a Coated Metal Throat

	Size range of aerosol		Aerosol fraction (*mean % (sd))	
Flow rate (l min^{-1})	By II (μm)	By LD (μm)	By II (as percent of estimated terbutaline dose D_2)	By LD through bottom window of coated throat (as percent of aerosol volume)
28.3	<10.0	<10.3	50.97 (8.5)	59.16 (12.3)
	<5.8	<5.8	34.89 (5.7)	29.43 (11.6)
	<4.7	<4.6	30.42 (4.9)	24.28 (11.0)
	<3.3	<3.3	17.49 (3.3)	10.94 (6.9)
60	<13.0	<12.3	85.51 (12.4)	85.07 (19.9)
	<6.8	<6.7	75.33 (9.6)	74.76 (14.4)
	<3.1	<3.2	17.07 (4.0)	23.10 (8.1)
100	<10.07	<10.3	85.10 (13.5)	91.81 (7.7)
	<5.27	<5.4	69.79 (11.6)	62.69 (6.9)
	<2.4	<2.4	15.07 (3.2)	17.11 (7.0)

*Mean where $n = 5$ for inertial impaction and $n = 25$ for laser diffraction.

CONCLUSIONS

The aerosol fine fractions from Bricanyl™ Turbohalers™ measured by a laser diffraction technique were found to correlate linearly with data gathered by inertial impaction. Therefore, laser diffraction offers a reliable alternative to inertial impaction in the characterisation of dry powder aerosol formulations. This technique may be particularly useful for systems filled with agglomerates of micronized drugs, biodegradable microspheres, and spray-dried protein powders. As demonstrated in the present study, the higher degree of sphericity and more homogeneous nature of these particles enabled them to be sized by laser diffraction with greater accuracy than fractured crystalline powders. Sizing aerosols by laser diffraction can be conducted at predetermined times and under varying air flow rates simulating patients' inhalation profiles since the need to capture deposited aerosol for analytical determination is obviated. Such studies would expand the formulator's understanding of the influence of different rates and modes of inhalation on fine particle fraction. It may be possible to construct inhalation devices with glass windows, akin to the metal throat, to enable sizing of aerosols by laser diffraction in the early stages of development.

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