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Research Paper

Influence of Realistic Inspiratory Flow Profiles on Fine Particle Fractions of Dry Powder Aerosol Formulations

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Purpose. The purpose of the study was to determine how air flow profiles affect fine particle fractions (FPF) (<5 μm) from dry powder aerosol formulations and whether laser diffraction (LD) could be used to measure FPF of aerosols generated by variable flows.
Materials and Methods. Carrier-based formulations containing 1.5% w/w micronized salbutamol base blended with the 63–90 μm fraction of alpha-lactose monohydrate or sorbitol or maltose were aerosolised from a model glass device using either a constant flow rate or a predetermined flow profile. The FPFs of the same aerosolised particles were first measured by LD and then by a liquid impinger. Volunteer inhalation airflow profiles and 3-phase (acceleration, constant flow rate and deceleration) square wave airflow profiles were generated using the Electronic Lung™ and an Inhalation Profile Recorder. Similar experiments were conducted for a carrier-free formulation from the Bricanyl Turbohaler™.
Results. Salbutamol FPFs of all carrier-based formulations were found to increase by increasing the initial flow increase rate (FIR) from 200 to 600 l min^{-1} s^{-1} although they could be placed in an increasing order of maltose blend < sorbitol blend < lactose blend. A significant linear correlation was found between FPFs measured by LD and by inertial impaction (R^2=0.95, p<0.01, ANOVA). For the Bricanyl Turbohaler™, increasing FIR from 120 to 600 l min^{-1} s^{-1} for a constant peak flow rate (PFR) of 60 l min^{-1} increased the mean Terbutaline FPF from 18.2% to 45.5%. For the volunteer inhalation profiles, a higher FIR tended to be associated with higher PFR, leading to a marked increase in drug FPF due to the combined effect of FIR and PFR.
Conclusion. Drug FPF from either carrier-free or carrier-based formulations is determined by both FIR and PFR. LD is a viable technique to measure the performance of dry powder aerosol formulations at realistic inspiratory flow profiles.

KEY WORDS: dry powder inhaler; fine particle fraction; flow increase rate; inertial impaction; laser diffraction; peak flow rate.

INTRODUCTION

The majority of currently marketed dry powder inhalers (DPIs) utilise the energy of the patient's inspiration to generate an effective dry powder aerosol for delivery to the lungs (1). For many of these inhalers the aerosol particle size distribution is influenced by the inhaled flow rate, inspiration time and inhaled volume (2,3), and initial rate of inspiration (4–7). These parameters can in turn affect lung deposition (8) and clinical efficacy (9).

Current *in vitro* testing methods to characterise such aerosols use inertial impactors which operate at calibrated constant flow rates. Although the impactors may be used at several different constant flow rates through laborious recalibration of the apparatus, they are not able to operate under conditions of changing flow rate, typical of a patient's inhalation profile. The use of a constant flow rate to measure aerodynamic particle size distribution of medicinal aerosols has been seen as a major drawback of inertial impaction techniques (10) since the actual inhalation flow rate achieved by patients through DPIs clearly varies during the time of inhalation. As the vital capacity of a patient is substantially larger than the internal volume of a DPI, the metered dose is drawn out of the device before the peak flow rate (PFR) has been achieved. Therefore, the flow rate that has released the aerosol dose from the device is expected to be lower than the PFR. An inhalation carried out using a higher flow increase rate (FIR) will reach the PFR sooner thereby subjecting the initially static dose to higher flow rates at an earlier stage in the inspiration even if ultimately the same PFR is achieved. Thus, drug delivery from DPIs is determined by both FIR and PFR. Inertial sizing methods with constant flow rates do not account for the effect of FIR on drug delivery, leading to questions over the clinical relevance of such techniques (11).

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A number of techniques have been proposed to overcome the potential problems associated with measurements at fixed flow rates and to simulate patient use of DPIs (10,12,13). However, these techniques can be complicated and may not be suitable for routine testing of DPIs.

Laser diffraction (LD) techniques have been found to correlate well with inertial impaction when used to measure aerosol fine particle fraction at constant flow rates (14,15). If such a correlation can be shown to exist when variable flow rates are employed, LD could be used to measure aerosol particle size distribution generated by patients' inspiratory flow profiles. In addition, no studies have been reported that deal with the effects of inhalation flow profiles on the performance of different dry powder aerosol formulations containing the same drug. If such effects can be identified *in vitro* then important performance variables intrinsic to the formulations might be identified that could have been overlooked as a consequence of testing under constant flow rates. It is therefore the aim of this study to use both LD and inertial impaction techniques to characterise the aerosols generated from different dry powder aerosol formulations at airflow conditions closely mimicking those created by patients inhaling through dry powder inhalers. To this end, it was planned to employ an inhalation simulator termed the Electronic Lung™ (16), with a view to assessing the influence of various profile parameters, e.g., FIR and PFR, on aerosol generation *in vitro*.

MATERIALS AND METHODS

Materials

Micronized salbutamol base (Allchem International, Maidenhead, UK), regular grade α-lactose monohydrate (Borculo Whey Products, Saltney, Cheshire UK), sorbitol (Fisons Laboratory Supplies, Loughborough, UK) and maltose (Sigma, Poole, UK) were obtained from the suppliers indicated. *p*-Hydroxybenzoic acid ethyl ester (ethyl paraben) (Sigma, Poole, UK), methanol (HPLC grade) (Rathburn Chemicals Ltd, Walkerburn, Scotland), ammonium acetate (HPLC grade) (BDH Laboratory Supplies, Loughborough, UK) and distilled water (MilliQ grade) (Millipore, Watford, UK) were obtained from the indicated suppliers.

Formulation Development and Analysis of Salbutamol Base

Both carrier-based and carrier-free dry powder formulations were investigated in the study. The carrier-based formulations were prepared by blending micronized salbutamol base (volume mean diameter (d_v): 2.42 μm; span: 1.01) (3.0 g) with 202.5 g of a coarse (63–90 μm) sugar carrier to obtain a target concentration of 1.45% w/w. The sugar carrier, comprising regular lactose, sorbitol, or maltose, was obtained by sieving 2 kg of the sugar particles sequentially through test sieves with an aperture width of 90 and 63 μm using an air-jet sieve (Alpine, Augsburg, Germany) for 15 min. The blending of the drug with the carrier was conducted according to the following protocol. The drug was added to approximately 50% of the coarse excipient, in a 'sandwich' arrangement to limit the adherence of micronized drug to the glass blending jar, and the powder blended in a Turbula mixer (model T2C, Willy A Bachofen, Basel, Switzerland) at 42 rpm for 20 min. The remaining 50% of the excipient was then added and mixing continued for a further 10 min. The blend was then passed through a 212 μm sieve to break up any loose agglomerates before being mixed for a further 10 min. The blending procedure was carried out under controlled conditions of 22–23°C and 45% RH.

Investigations on carrier-free formulations utilised a commercial Bricanyl Turbohaler™ since its formulation was composed solely of the agglomerates of micronized terbutaline sulphate.

Salbutamol base was analysed by HPLC employing 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) and a 15 cm S5ODS2 C_{18} column (Anachem). The retention times for salbutamol base and the internal standard were 2.6 and 5.7 min, respectively. The HPLC method was found to give a recovery between 99–101% at salbutamol concentrations between 0.25 and 1.25 mg% with relative standard deviations of ca.1% being obtained in intra-day and inter-day variability of the results.

The Electronic Lung™ and Inhalation Profile Recorder

The Electronic Lung™ (EL) (The Technology Partnership, Melbourn, Herts, UK) is essentially a computer-controlled inhalation simulator. This equipment is schematically represented in Fig. 1. It comprises a large vertical piston and cylinder which draws air in through the inhaler device via an inlet port and then exhales the aerosol cloud out through a standard inertial impactor sizing apparatus at a constant calibrated flow rate via an outlet port. The piston is driven by a computer-controlled servo-motor to generate the desired inhalation profile. This profile may be arbitrarily defined through a PC user interface or may be a real inhalation profile reprogrammed directly from the Inhalation Profile Recorder (IPR) (see next section). The movement of the piston determines the inhalation air flow profile which generates the aerosol cloud, but on exhalation a constant

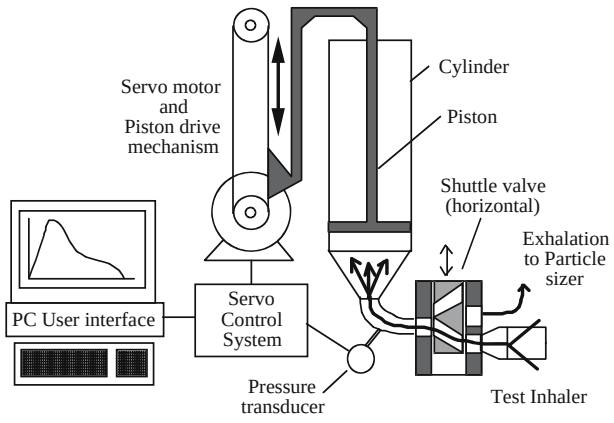

Fig. 1. Schematic diagram of the Electronic Lung™ (16).

flow rate is determined by a conventional pump. The piston simply follows the particle cloud down the cylinder to promote maximum clearance of the aerosol.

The Inhalation Profile Recorder (IPR), schematically represented in Fig. 2, consists of the inhaler of interest fitted with a miniature pressure transducer (3 mm in diameter) and small-bore sensing tube leading into the inhaler mouthpiece. The transducer is connected via a cable to the signal-conditioning electronics unit, and the output is processed using the appropriate PC software. The IPR thus measures the pressure drop in the mouthpiece with time throughout the inhalation profile. This pressure drop profile can then be defined in terms of a flow rate *versus* time profile using the device specific resistance obtained by an independent calibration.

The inhalation profile can be viewed on the PC screen in real time and/or stored on disk for later recall and reprogramming of the EL. As far as the volunteer is concerned, the adaptation of the inhaler is sufficiently non-intrusive to make inhalation behaviour though the device as near normal as possible.

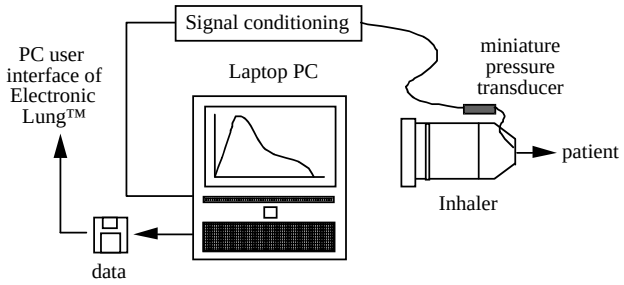

Fig. 2. Schematic diagram of the Inhalation Profile Recorder (*IPR*) (16).

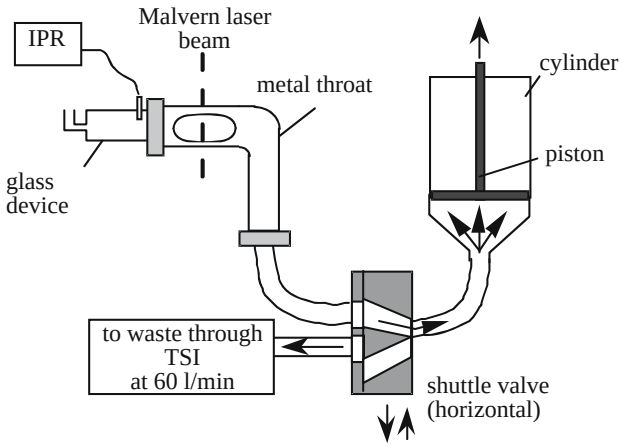

Fig. 3. Schematic of apparatus comprising a glass device (as a representative inhaler), metal throat through which the dry powder aerosols were sized by laser diffraction, and the Electronic Lung™ (*IPR*—Inhalation Profile Recorder, *TSI*—Twin-stage liquid impinger).

Aerosol Characterisation by Laser Diffraction

Dry powder aerosols generated by the inhalation profiles drawn by the EL were characterised using laser diffraction (Malvern 2600, Malvern Instruments, Malvern, Worcs., UK). In order to make this measurement, a metal throat containing optically plain windows was placed in-line with the inhaler and the EL inlet port (14). A schematic diagram of the apparatus is shown in Fig. 3. The profile recorded by the IPR from the transducer in the dry powder device was compared with the profile recorded by an internal pressure transducer within the EL to ensure that there was no influence of the metal throat and additional tubing on the device specific resistance, programmed into the software.

The LD sizer was positioned so that the laser beam passed through the windows of the metal throat, and the recording lens was as close as possible to the windows. The LD sizer was either fitted with a 63 mm lens or a 100 mm lens during the characterisation of aerosols from either salbutamol blends or the Turbohaler™ formulation, respectively.

Each size measurement involved a pre-programmed 200 sweeps of the detector elements in the absence of any aerosol, followed by the air flow profile drawn by the EL. Passage of the aerosol dose through the laser beam, was completed within a further 200 sweep cycles. The number of sweeps was the number of times a reading was taken of light falling on each of the concentric photodiode detectors of the LD sizer. The exhalation of the aerosol was carried out at 60 l min^{-1} through a twin stage impinger simply as a means of clearing the apparatus and drawing the aerosol to waste rather than for aerodynamic sizing of the aerosol. Measurements were made on 10–12 aerosol doses at each air flow profile, with the apparatus including the metal throat being dismantled and cleaned after the passage of every three doses.

Doses of salbutamol blend (approximately 25 mg) were dispensed into the dosing port of an in-house designed glass inhaler device (14) and were aerosolised and discharged upon initiation of the inhalation profile. In a previous study, the dosing port was occluded prior to discharge, to allow time to achieve the required steady constant flow rate before the dose was released (14). However in this work, it was important to subject the powder dose to the complete air flow profile drawn and not only the steady constant flow. The port was therefore not occluded prior to discharge.

When the Bricanyl Turbohaler™ was examined, the inhaler doses were metered with the inhaler in a vertical position and it was then placed horizontally into the throat inlet seal which was of appropriate geometry for its mouthpiece. The Turbohaler™ doses were discharged upon initiation of the profile as described for the blend doses.

The complete particle size data obtained from each salbutamol blend aerosol were saved but the aerosol fine particle fraction (% <5 μm) was presented as a fraction of the aerosol volume from diffracted light on the detector elements omitting the light on the detector's first inner ring i.e., the designated "kil (1,0) distribution" (14). For a lens of focal length 63 mm, this meant that only particles less than approximately 60 μm would be included in the distribution. Since the "kil (1,0) distribution" eliminated the interference from larger carrier particles, any small difference in the therapeutically important finer fractions (e.g., <10 μm) of the drug could now be detected by the technique. In contrast, the aerosol fine particle fraction measured from each Turbohaler™ was presented as a fraction of the aerosol volume from the diffracted light from all detector rings ('virgin' kil (0,0) distribution) (15). Since the Turbohaler™ formulation consisted of loose agglom-

erates of pure drug, all particles should be included in the calculation of drug fine particle fractions.

Aerosol Characterisation by Inertial Impaction

The EL was employed in a more conventional fashion to measure the aerosol fine particle mass of salbutamol blend doses by inertial impaction. A schematic diagram of this apparatus is shown in Fig. 4.

The glass device was inserted directly into the EL inlet port and each dose was drawn into the vertical piston/cylinder by the imposed individualised air flow profile, before being drawn out via the outlet port at a constant flow rate of 60 l min^{-1} over 4 s through a twin stage liquid impinger (TSI) (BP 2002). Stages 1 and 2 of the TSI were filled, respectively, with 7 and 30 ml of mobile phase containing internal standard (MPIS) for subsequent analysis of salbutamol by HPLC. Five aerosol doses were discharged per experiment in this way before the complete apparatus was dismantled and washed with MPIS. Washings from the glass device were made up to 50 ml with MPIS, while those from the EL inlet port, shuttle valve, main piston/cylinder and associated pipework were combined and made up to 200 ml with MPIS. Washings from stages 1 and 2 of the TSI were each made up to 100 ml with further MPIS. The salbutamol aerosol fine particle mass (<6.4 μm) was obtained from the washing solution derived from stage 2 of the TSI and this amount was expressed as a fraction of the total emitted dose. Each experiment was carried out in triplicate.

Generation of Variable Flow Profiles

Square Wave Air Flow Profiles

Prior to the effects of "real" patient inhalation profiles on aerosol performance being assessed, various parameters of the profile were investigated using square wave air flow profiles. The parameters investigated were PFR and the FIR to a pre-determined PFR.

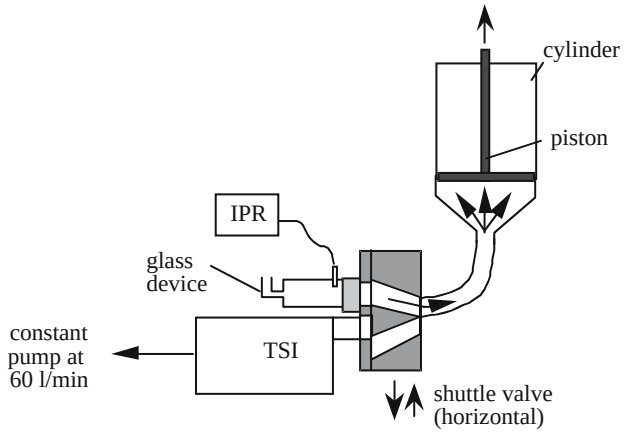

Fig. 4. Schematic of apparatus comprising a model glass inhaler device, the Electronic Lung™ and twin-stage impinger (BP 2002) through which the dry powder aerosols are sized by inertial impaction at 60 l min^{-1} (*IPR*—Inhalation Profile Recorder, *TSI*—Twin-stage liquid impinger).

A series of square wave air flow profiles were programmed into the EL based on a profile with three phases as depicted in Fig. 5a:

- An initial phase of constant acceleration to PFR, which was complete in 0.1 to 0.5 s
- A second phase of constant PFR (60 l min^{-1} for 2.5 s, 100 l min^{-1} for 2 s, or 120 l min^{-1} for 1.5 s)
- A third phase of constant deceleration to zero flow rate, which was completed in 0.2 s

Each square wave air flow profile drew an air volume of approximately 3 to 3.5 l through the dry powder inhaler.

Volunteer Inhalation Profiles from the Glass Inhaler

"Real" inhalation profiles were taken from a selection of profiles stored on an IPR which had been generated by human volunteers through different dry powder inhalers. Since the glass device had been custom made for this research, no volunteer profiles using this particular device were saved on disk. Instead, profiles recorded through the Diskhaler™ (Allen & Hanburys, Uxbridge, UK) were selected since this inhaler had the specific resistance (0.06 mbar l^{-1} min) closest in value to that of the glass device (0.05 mbar l^{-1} min). Even so, the Diskhaler™ profiles were adjusted to account for the higher flow rates which would be expected from the same inspiratory effort through the glass device of lower resistance. The adjusted profiles (B1, B2 and B3) employed in the study are shown in Fig. 5b. These three profiles were chosen to reflect the range of FIRs and PFRs achievable through such a device by healthy human volunteers.

Volunteer Inhalation Profiles from the Turbohaler™

Three stored volunteer inhalation profiles obtained previously through a Turbohaler™ were similarly selected to cover a range of conditions achievable through this inhaler. These stored profiles (Fig. 5c) were used without alteration to generate and size aerosols from the same dry powder inhaler.

RESULTS

Salbutamol Blend Aerosol Characterisation by Laser Diffraction

Constant Air Flow Rates Produced by a Conventional Vacuum Pump

The fine particle fraction of each formulation was determined at constant flow rates of 60 and 80 l min^{-1} by the laser diffraction technique described previously (14). The results are listed in Table I.

From the use of the IPR connected to the glass device in this experimental set-up, it was possible to measure the air flow profiles actually generated in the device by the constant air flow vacuum. At each flow rate, as soon as the sample port was unoccluded the air flow rate rose sharply to the required peak level but not without a small degree of initial

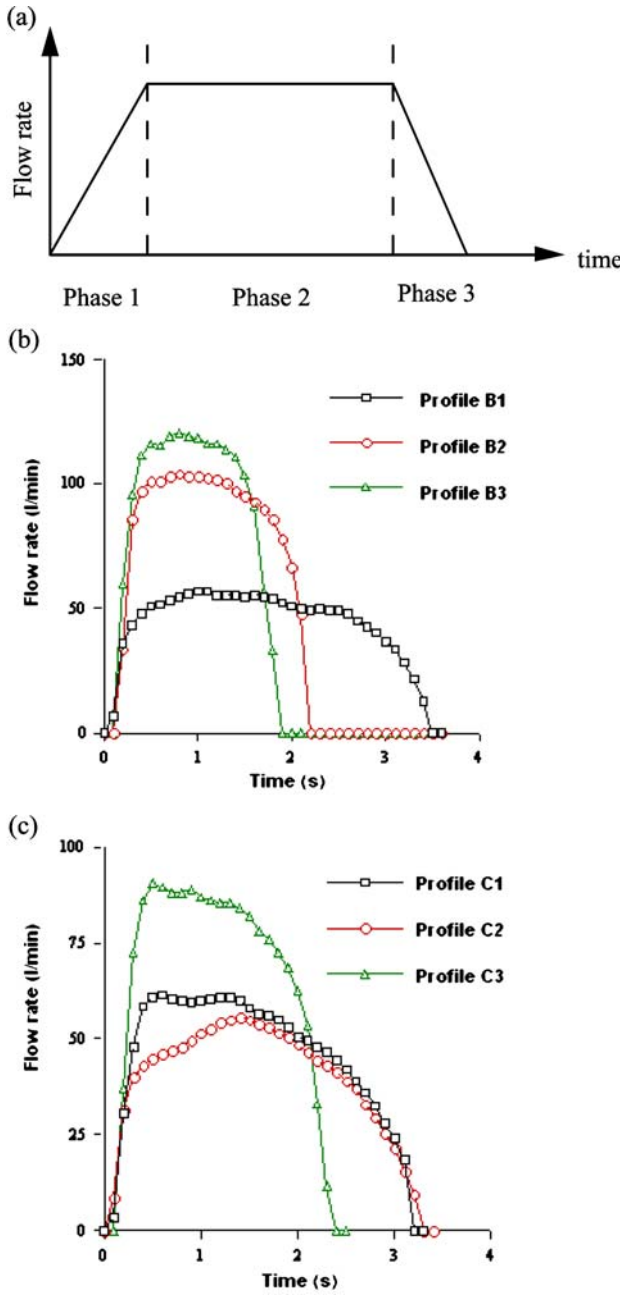

Fig. 5. Schematic of various variable flow profiles. (a) a square wave air flow profile programmed by the Electronic Lung™; (b) volunteer inhalation profiles, B1, B2 and B3 used by the Electronic Lung™ to generate salbutamol blend dry powder aerosols from the glass inhaler device; and (c) volunteer inhalation profiles, *C1*, *C2* and *C3* used by the Electronic Lung™ to generate dry powder aerosol doses from the Bricanyl Turbohaler™.

"overpeaking" in flow rate of approximately 5 l min^{-1}. This degree of "overpeaking", however, was observed to be reproducible over the repeated discharge of 10 to 15 doses.

If, however, the sample port containing the dispensed dose was left unoccluded and the vacuum switched on from rest, the peak air flow level was overshot by up to 15 l min^{-1}, and a change in flow rate occurred which was erratic and variable with repeated doses.

Square Wave Air Flow Profiles by EL

The aerosol blend fine fractions using various square wave air flow profiles were determined by laser diffraction and are summarised in Table II.

When the carrier excipients (lactose, sorbitol and maltose) with a size range of 63–90 μm were discharged alone and measured as controls by this technique, no fines (<5 μm) were detected in any of the air flow profiles.

The aerosol fine fractions from the lactose blend using profiles A1, A2 and A3 were found to be significantly different although a constant PFR of 60 l min^{-1} was reached in each profile. The time to reach that peak differed, however, for each profile. An increase in the acceleration phase was accompanied by an increase in the fine fraction of the aerosol as it emerged from the device. Increasing both the FIR and the PFR of profile A3 to those conditions found in profile A5 increased the lactose blend fine fraction significantly ($p<0.05$, ANOVA). But when only the PFR was raised, the FIR remaining constant at 600 l min^{-1} s^{-1} (as in profile A4), the blend fine fraction was not altered ($p>0.80$, ANOVA). It appeared that for this blend, the FIR of the profile played an important role in the dispersion of the aerosol emerging from the device.

The fine particle fraction from the sorbitol blend measured using profiles A1, A2 and A3 were found to be similarly affected by an FIR to 60 l min^{-1}. In profile A1 no fines were measured but for profile A2, when the FIR was increased to 300 l min^{-1} s^{-1}, fines were detected and the level of these was increased further by imposition of profile A3.

The levels of fine particle fraction measured in the maltose blend aerosols were lower still for all three profiles, but similar trends were observed.

With any of the air flow profiles A1 to A5, the degree of aerosol dispersion measured was found to be dependent on the particular carrier in the blend, the aerosol fine fraction decreasing in the order: lactose > sorbitol > maltose. This is the same trend found in aerosols generated from these blends at constant air flow rates (Table I).

It was noted, however, that for each blend the aerosol fine particle fractions measured with the square wave profiles (A1, A2 and A3) with a PFR of 60 l min^{-1} were lower than those measured at a constant flow rate of 60 l min^{-1} pulled by a conventional vacuum pump (Table I). This could have been due to flow rate "overpeaking" by the vacuum pump which would have generated a greater FIR. This "overpeaking" effect was evident from the pressure drop profile recorded by the IPR, but further fluctuations in air flow rate may have been obscured by limitations of the

Table I. Fine Particle Fractions (% <5 μm) of Salbutamol Base Blends Drawn Through the Metal Throat at Constant Flow Rates of 60 and 80 l min^{-1} Measured by Laser Diffraction (mean(sd), $n = 10 - 15$)

	Flow rates	
Excipient in Salbutamol base blend	60 l min^{-1}	80 l min^{-1}
Lactose	15.3 (2.7)	23.8 (3.4)
Sorbitol	7.3 (1.5)	16.2 (3.0)
Maltose	5.4 (1.3)	7.6 (2.1)

Table II. Fine Particle Fractions (% <5 μm) from Salbutamol Base Blends Aerosolised in Square Wave Air Flow Profiles, and Measured by Laser Diffraction (mean(sd), $n = 10 - 15$)

	Square wave air flow profile conditions						FPF (%)
		Phase 1		Phase 2		Phase 3	
Carrier in blends	Profile	Time to Peak Flow (s)	FIR (l min^{-1} s^{-1})	Time at Constant Flow (s)	PFR (l min^{-1})	Deceleration Rate (l min^{-1}s^{-1})	
Lactose	A1	0.3	200	2.5	60	300	1.8(0.5)
	A2	0.2	300	2.5	60	300	3.8(1.8)
	A3	0.1	600	2.5	60	300	8.2(1.4)
	A4	0.2	600	1.5	120	600	8.3(1.3)
	A5	0.1	1000	2.0	100	500	11.3(0.7)
Sorbitol	A1	0.3	200	2.5	60	300	0
	A2	0.2	300	2.5	60	300	2.8(0.9)
	A3	0.1	600	2.5	60	300	4.5(1.8)
	A5	0.1	1000	2.0	100	500	5.8(2.3)
Maltose	A1	0.3	200	2.5	60	300	0
	A2	0.2	300	2.5	60	300	0.5(0.5)
	A3	0.1	600	2.5	60	300	1.5(0.7)
	A5	0.1	1000	2.0	100	500	2.8(0.5)

computer software which enables the measurement of pressure drop only to be made every 0.1 s. The precise flow profile up until the initial 0.1 s or during the subsequent 0.1 s intervals may not therefore be fully defined.

Volunteer Inhalation Air Flow Profiles

The salbutamol blend aerosol fine particle fractions measured using volunteer inhalation profiles B1, B2 and B3 by LD are shown in Table III.

Since these profiles were generated *in vivo*, they did not exhibit the clear delineation of the three phases that were generated by the square waves, and the FIR was, as expected, variable throughout phase 1 (Fig. 5b). However, to enable some means of comparison with the square wave profiles, the approximate FIR over the initial 0.2 s of phase 1 was determined for each of the profiles B1, B2 and B3 (Table III).

Profiles B2 and B3, with similarly high PFR, generated aerosols of each blend with fine particle fractions which were not significantly different (p=0.95, ANOVA). Their initial FIRs differed but this did not seem to affect significantly the aerosol fine fraction generated. The similarity in fine particle fractions was probably due to the impact on aerosolization of both the high PFRs and the similar average FIRs during phase 1 overall, which were 250 and 270 l min^{-1} s^{-1} for profiles B2 and B3, respectively. These lower average FIRs in comparison to those shown in Table III were calculated for profile B2 over 0.4 s and for profile B3 over 0.45 s (Fig. 5b). The high PFRs at both profiles would therefore be expected to mask the potential difference in FPFs caused by the minor change in FIRs between profiles B2 and B3.

For each salbutamol blend, the fine particle fraction generated in profile B1 was smaller than that generated in either profile B2 or B3. In profile B1 both the FIR and PFR were lower than in B2 or B3. It is interesting to note that volunteer profile B1 and the square wave profile A1, which have the same FIR and PFR, generated aerosols from each blend with similar fine fractions (p=0.89, ANOVA). The same is true for the salbutamol/lactose blend aerosols generated by volunteer profile B3 and square wave profile A4 (p=0.96, ANOVA).

Salbutamol Blend Aerosol Characterisation by Inertial Impaction

Square Wave Air Flow Profiles

Employing the experimental set-up represented in Fig. 4, the salbutamol fine fraction (<6.4 μm) from the salbutamol/lactose blend was assessed by inertial impaction when the aerosols were generated by various square wave air flow profiles A1, A2 and A3. The resulting fine particle fractions

Table III. Fine Particle Fractions (% <5 μm) from Salbutamol Base Blends Aerosolised by Volunteer Inhalation Flow Profiles, and Measured by Laser Diffraction (mean(sd), $n = 10 - 15$)

Volunteer inhalation flow profiles			FPF (%)		
Profile	FIR over initial 0.2 s (l min^{-1} s^{-1})	PFR (l min^{-1})	Lactose blend	Sorbitol blend	Maltose blend
B1	200	60	2.8 (1.0)	0	0
B2	400	100	7.3 (1.0)	2.5 (0.8)	1.0 (0.7)
B3	600	120	8.8 (1.4)	3.3 (1.8)	1.5 (0.7)

Table IV. Salbutamol Fine Particle Fraction (% <6.4 μm) from Salbutamol/Lactose Blend Aerosolised by Square Wave Air Flow Profiles, and Measured by Inertial Impaction at 60 l min^{-1} (mean(sd), n=3)

Profiles	FIR to Reach a PFR of 60 l min^{-1} in the Square Wave Air Flow Profiles (l min^{-1} s^{-1})	FPF (% of emitted dose)
A1	200	4.79 (0.5)
A2	300	8.48 (0.5)
A3	600	13.75 (0.7)

are shown in Table IV. The three profiles were programmed to draw air at constant FIRs of 200, 300 and 600 l min^{-1} s^{-1} during phase 1 of the profile, all reaching the same PFR of 60 l min^{-1}. It was found that although the PFR remained at 60 l min^{-1} in each profile the salbutamol fine particle fraction measured in the aerosol emerging from the EL was highly dependent upon FIR to reach that peak (Table IV). These results were supported by the findings made by the measurements made by LD (Table II).

Volunteer Inhalation Air Flow Profiles

The salbutamol fine particle fractions measured for lactose blend aerosols generated by volunteer inhalation profiles B1, B2 and B3 are summarised in Table V. The fine particle fractions generated by imposition of profiles B3 or B2 were significantly different from that generated by profile B1 (p<0.05, ANOVA).

Comparison of the Results by Laser Diffraction and Inertial Impaction

When a comparison was made of the data obtained by LD and by inertial impaction, a similar trend in differences of fine particle fraction as a function of inspiratory profile was detected by each method (Tables II, III, IV, and V). The pressure transducer and IPR connected to the device mouthpiece were able to confirm that the pressure drop profiles generated during comparable aerosol measurements by laser diffraction and inertial impaction were consistent. Although the same numerical data were not found by each method, a strong correlation of significant linearity was found between the two sets of data (R^2=0.95, p<0.01, ANOVA).

Table V. Salbutamol Fine Particle Fraction (% <6.4 μm) from Salbutamol/Lactose Blend Aerosolised by Volunteer Inhalation Flow Profiles, and Measured by Inertial Impaction at 60 l min^{-1} (mean(sd), n=3)

Profile	FIR over initial 0.2 s (l min^{-1} s^{-1})	PFR (l min^{-1})	FPF (% of emitted dose)
B1	200	60	7.86 (0.2)
B2	400	100	14.94 (1.1)
B3	600	120	17.36 (1.2)

Bricanyl Turbohaler™ Aerosol Characterisation by Laser Diffraction

Square Wave Air Flow Profiles

The terbutaline fine particle fractions (% <5 μm) measured in aerosols generated by the imposition of different square wave profiles are summarised in Table VI. All the profiles had a phase of deceleration to rest over 0.2 s.

The effect of varying inspiratory FIR to reach a constant PFR of 60 l min^{-1} can be seen in Table VI. The fraction of terbutaline fines measured in the aerosol dose was clearly dependent upon FIR, particularly when the FIR was above 200 l min^{-1} s^{-1}. The relative standard deviation of the mean fine particle fraction was found to increase with lowering FIRs. Despite reaching a PFR of 60 l min^{-1} profiles A6 and A7 generated low fine particle aerosol fractions (18%). The change in flow rate over the first 0.3 and 0.5 s, respectively, in these profiles probably resulted in aerosol emission before the PFR was reached, and aerosol characterisation at flow rates < 60 l min^{-1}.

From the evidence provided by the fine particle fractions generated by profiles A8 and A10, where the FIR remains constant, the importance of peak flow is also clear. The fine particle fraction measured in profile A10 [9.2 (2.6)] was found to increase three-fold to 29.8 (3.6) for a two-fold increase in PFR as imposed in profile A8.

Previous fine particle fraction data reported on the aerosolization of Bricanyl Turbohaler™ doses at constant flow rates of 28.3 and 60 l min^{-1} drawn by a conventional vacuum pump (15) were not dissimilar from data generated here using air flow profiles A9 and A10 which reached the constant PFR in 0.1 s. The attainment of 30 l min^{-1} constant flow through a Bricanyl Turbohaler™ by a conventional pump did closely resemble that of profile A10 with an FIR of 300 l min^{-1} s^{-1}. However reaching 60 and 100 l min^{-1} through this device, as in profiles A9 and A11, was observed to produce a marked degree of "overpeaking." The IPR recorded a brief "overpeaking" to 75 l min^{-1} when attempting to reach 60 l min^{-1} in 0.1 s, while almost 150 l min^{-1} was achieved at the peak in reaching a constant flow of 100 l min^{-1} through this inhaler in 0.1 s.

Table VI. Terbutaline Fine Particle Fractions (% <5 μm) Obtained from Bricanyl Turbohaler™ Doses Aerosolised by Different Square Wave Air Flow Profiles, and Measured by Laser Diffraction (mean(sd), $n = 10 - 15$)

Profile	Square wave air flow Profile conditions: Time to PFR (s)	FIR (l min^{-1} s^{-1})	PFR over 2 s (l min^{-1})	Terbutaline FPF (% <5 μm)
A6	0.5	120	60	18.2 (10.5)
A7	0.3	200	60	18.4 (6.7)
A8	0.2	300	60	29.8 (3.6)
A9	0.1	600	60	45.5 (4.7)
A10	0.1	300	30	9.2 (2.6)
A11	0.1	1000	100	59.2 (8.7)

Table VII. Terbutaline Fine Particle Fractions (% <5 μm) Emitted by a Bricanyl Turbohaler™ Aerosolised by Volunteer Inhalation Flow Profiles, and Measured by Laser Diffraction (*mean*(*sd*), $n = 10 - 15$)

	Volunteer Inhalation Profile Conditions		
Profile	FIR over Initial 0.2 s (l min^{-1} s^{-1})	PFR (l min^{-1})	Terbutaline FPF (% <5 μm)
C1	160	60	19.0 (5.6)
C2	200	55	15.0 (5.6)
C3	400	90	52.5 (10.5)

Volunteer Inhalation Profiles

The terbutaline fine particle fractions measured in Bricanyl Turbohaler™ doses using volunteer inhalation profiles C1, C2 and C3 are summarised in Table VII. The conditions of profile C3 were significantly different from profiles C1 or C2 and as such generated an aerosol containing a significantly higher fine particle fraction.

Profiles C1 and C2 had both very similar PFRs and FIRs over the initial 0.2 s. However the profiles only appeared to differ over the 1 s of profile following the initial 0.2 s. After 1.2 s, profile C1 accelerated to peak flow at an approximate rate of 120 l min^{-1} s^{-1}, while profile C2 rose at only approximately 20 l min^{-1} s^{-1} over the same time period. These transient differences, however, did not seem to affect the measured aerosol fine particle fraction ($p>0.1$, ANOVA), suggesting that the initial 0.2 s was more important than the remainder of the acceleration phase.

There was no difference in aerosol fine particle fraction produced by these profiles and by the corresponding square wave profiles under similar conditions of FIR and PFRs (profiles A6 and A7).

DISCUSSION

Dry powder aerosol performance is still only routinely assessed *in vitro* at constant air flow rates, principally because of the limitations of the inertial impaction testing apparatus currently used to characterise aerosols aerodynamically. However, an understanding has been gained of the sensitivity of dry powder formulations and their inhaler devices to patient inhalation parameters other than PFR, such as the initial FIR to reach that peak (5,12). This has led to the desire to assess aerosol performance *in vitro* using air flow profiles more closely resembling those generated by patients (17). The variable air flows generated by *in vivo* inhalation profiles, however, present problems in the assessment of aerosols by compendial impaction techniques which require operation at constant air flow rates.

In the Electronic Lung™, the aerosol generated by the inhalation profile is drawn through impaction sizing apparatus at a constant flow rate to achieve a sizing assessment *in vitro*. This procedure subjects the aerosol to an additional and potential air flow transition of unknown turbulence, which would be unnecessary if the aerosol could be sized as soon as it emerged from the inhaler device. The LD technique has considerable potential in this area since it has been shown to be capable of characterising aerosols quickly immediately after they emerge from the device, a situation far more representative of the aerosols actually inhaled by a patient. Most importantly, the aerosols can be sized by LD as they travel in any varying air flow profile rather than being further constrained by a constant air flow rate. Therefore the Electronic Lung™ was used in this work in combination with LD detection. However, without the need to size by inertial impaction, a simpler piece of apparatus could be designed to draw a volume of air through the device at varying air flow rates akin to a typical inhalation profile.

The results obtained using salbutamol blends demonstrated the importance of assessing the performance sensitivity of dry powders to inhalation profiles rather than determining fine particle fraction at constant air flow rates only. Some formulations may prove highly sensitive to the air flow pattern generated in reaching a particular PFR i.e, the imposed FIR profile might be a key factor, a phenomenon not always apparent from *in vitro* testing at constant flow (12).

The aerosol fine particle fractions obtained from both salbutamol blends and Bricanyl Turbohaler™ doses through the Electronic Lung™ were found to be affected not only by the PFR but also by the initial phase of acceleration to reach that peak. It has been reported that during the initial 0.3 s of an inhalation profile the FIR could affect the *in vitro* fine particle fraction obtained from some dry powder inhalers (5). Aerosol emission could occur even during this short period before the peak inspiratory flow rate had been reached. This was also the finding from LD measurements made in this work, using air flow profiles with a phase of acceleration taken over 0.5 s. This observation emphasises the advantage of characterising the aerosol by LD immediately after it emerges from the device, without the need to subject the aerosol to a different constant flow rate as required by inertial impaction techniques.

It was found that a high PFR did not necessarily guarantee a high aerosol fine particle fraction. Even in air flow profiles reaching a peak flow of 60 l min^{-1} the *in vitro* fine particle fraction obtained from a Bricanyl Turbohaler™ could be low (19.0%) if the initial FIR was insufficiently high. A similar conclusion was reached after a separate study which showed that the fine particle output from a Pulmicort Turbohaler™ could be halved if the initial FIR to 60 l min^{-1} was reduced (12).

Such variations in PFR and FIR could be found using patient inhalation profiles through the same dry powder inhalers. This implies that the changes in *in vitro* aerosol fine particle fractions could occur *in vivo* with potential clinical repercussions. Only further *in vivo* testing could elucidate the significance of such *in vitro* fine particle fraction variations.

The aerosol fine particle fractions from salbutamol blends measured by the LD technique were found to correlate linearly with the salbutamol fine particle fractions from the same blend measured by inertial impaction under variable flow profiles. Good correlation has also been demonstrated between the two techniques when they were employed to measure aerosol particle size distribution from both carrier-free formulations (15) and different carrier-based powder formulations under different constant flow

rates (14). However, the LD technique offers a number of advantages:

- Aerosols can be sized more quickly than by conventional methods, and examined immediately after they emerge from the inhaler device mouthpiece.
- The technique could give detailed information on the complete size distribution of an aerosol in flight and record any changes which may occur during the flight e.g., before and after a bend in the throat. Inertial impaction techniques cannot provide such data.
- The technique provides flexibility in characterising aerosols under many air flow conditions e.g., constant flow rates and variable flow patterns, such as those representing realistic patient inhalation profiles.

It can therefore be concluded that drug FPF from either carrier-free or carrier-based formulations is determined by both FIR and PFR, and LD is a viable technique to measure the performance of different dry powder aerosol formulations at realistic inspiratory flow profiles.

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