*Pharmaceutical Research, Vol. 23, No. 9, September 2006 (© 2006)*
DOI: 10.1007/s11095-006-9055-9

*Research Paper*

# Correlation between Inertial Impaction and Laser Diffraction Sizing Data for Aerosolized Carrier-based Dry Powder Formulations

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*Received March 9, 2006; accepted May 5, 2006; published online August 10, 2006*

***Purpose.*** The purpose of the study was to determine whether the drug fine particle fraction (FPF) from different dry powder aerosol formulations measured by laser diffraction at a range of flow rates correlated with that measured by inertial impaction.
***Materials and Methods.*** Ten binary formulations were prepared containing 1.5% w/w salbutamol base or sulphate, blended with the sieved (63–90 μm) fraction of different sugars (regular lactose, spray-dried lactose, sorbitol, dextrose or maltose). A further six ternary formulations were prepared containing 1.5% w/w salbutamol sulphate, 97% coarse lactose (63–90 μm) and 1.5% micronised or intermediate-sized lactose (1–50 μm). The FPF particles (< 5 μm) of these formulations were measured by laser diffraction and inertial impaction at flow rates between 28.3 and 100 l $min^{-1}$.
***Results.*** When only the particles with diameter < 60 μm obtained by laser diffraction were considered the FPF (< 5 μm) could be determined and this enabled the aerosolisation of all 16 blends to be feasibly compared at flow rates ranging from 28.3 to 100 l $min^{-1}$. A significant linear correlation was found between the fine fractions measured by laser diffraction and the salbutamol fine fractions determined by inertial impaction ($r^2 = 0.934$). Such correlation was also confirmed for formulations containing added fine lactose.
***Conclusion.*** Particle size measured by laser diffraction under the employed conditions reflected the aerodynamic properties of the drug. Laser diffraction can be used as on-, in- and/or at-line measurements and controls for dry powder aerosol formulations.

**KEY WORDS:** dry powder inhaler; fine particle fraction; formulations; inertial impaction; laser diffraction.

## INTRODUCTION

The proven therapeutic advantages of employing inhalation aerosols for the delivery of locally active drugs to the airways, and the potential of the lungs as a route for systemically active drugs, vaccines and gene therapeutic agents, have led to a marked evolution of device designs and formulations of inhalation aerosols in recent years (1,2). Increasing environmental concerns over propellants, and the apparent lack of advancement of pressurized metered-dose inhalers (pMDIs) has led to an increasing popularity of dry powder inhalers (DPIs) as evidenced by the value of worldwide market now being almost equally shared between DPIs and pMDIs (3). The share of DPIs is expected to grow even further, with the imminent approval by the US Food and Drug Administration (FDA) of inhaled insulin delivered from a DPI (4). Thus DPIs will play an important role in systemic delivery of proteins and peptides, as a consequence of novel formulation techniques that ensure the preservation of the biological activity of these molecules in solid states (5).

One of the most important criteria for the development of any new dry powder aerosol formulation is the particle size distribution of the aerosolised formulation. Current compendial techniques for determining this size distribution are all based on inertial impaction principles (6). These are excellent tools for the quality control of the finished product but such techniques classify particles only into a small number of size ranges. Information provided by inertial impaction may not be sufficient for detecting some formulation differences that are associated with significant changes in the quality of the final product. In addition such techniques, based on inertial impaction are laborious, time-consuming and difficult to establish as in-line process control techniques. In line with the development of FDA's Process Analytical Technology Initiative (7), there is a need to set up robust techniques that are capable of conducting on-, in- and/or at-line measurement of particle size distribution to ensure that a predefined quality at the end of manufacturing process can be consistently achieved.

Laser diffraction may prove to be such a technique since it can generate and process data rapidly, is readily automated, can provide real-time measurement, is highly consistent, and

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can produce a number of size classes within the size range relevant to lung deposition. Laser diffraction has been successfully employed to examine aerosols for inhalation from nebulisers (8) and pMDIs (9), but its application to dry powder aerosols has been less well studied (10,11). This is because the spray from a pMDI or mist from a nebuliser is primarily composed of the drug particles or droplets. Indeed, the results obtained from laser diffraction were found to correlate well with those obtained from an Andersen cascade impactor for nebulisers (12) and pMDI (13). However, the validity of laser diffraction to measure the drug particles emitted by dry powder aerosol formulations is questionable largely due to the fact that the fine carrier particles overlap with the micronised drug in particle size which, in the absence of chemical analysis, cannot be differentiated. It was therefore the purpose of the present study to investigate how the coarse and fine lactose particles affect the fine particle fraction of the drug measured by laser diffraction. The particle size results measured by laser diffraction were compared with those obtained from an inertial impaction method to establish if there is any correlation between two techniques.

## MATERIALS AND METHODS

### Materials

Micronised salbutamol base and sulphate (Allchem International, Maidenhead, UK), regular grade α-lactose monohydrate (Borculo Whey Products, Saltney, Cheshire, UK), spray-dried lactose "Zeparox™" (Borculo Whey Products, Saltney, Cheshire, UK), Sorbitol (Fisons Lab Supplies, Loughborough, UK), Dextrose (Fisons Lab Supplies, Loughborough) and Maltose (Sigma–Aldrich Chemical Co. Ltd., Poole, Dorset UK) were obtained from the suppliers indicated. *p*-hydroxybenzoic acid ethyl ester (ethyl paraben) (Sigma, Poole, UK), methanol (HPLC grade) and hexane (Rathburn Chemicals Ltd., Walkerburn, Scotland), ammonium acetate (HPLC grade), span 80 and butan-1-ol (BDH Lab Supplies, Loughborough, UK), lecithin (BDH Lab Supplies Poole, UK) and distilled water (MilliQ grade) (Millipore, Watford, UK) were used as supplied.

### Preparation of Coarse Carrier Particles

The sieved fraction (63–90 μm) of coarse sugar carrier, comprising regular lactose, spray-dried lactose, sorbitol, dextrose 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. All the powders were stored in glass containers which were placed in a desiccator at room temperature over silica gel until further required.

### Particle Size Determination Using Wet Dispersion Laser Diffraction

The particle size distributions of the salbutamol base and sulphate were determined in liquid medium by laser diffraction, according to an independent model, using a Malvern 2600 laser diffraction sizer (Malvern Instruments, Malvern, Worcs., UK) fitted with a 63 mm lens.

A 10 ml solution of 0.5% (w/v) lecithin was prepared to which approximately half a small spatula tip of drug (*ca*. 5 mg) was added and the suspension sonicated for 10 min. The Malvern liquid cell was filled with hexane and a background measurement taken. The drug suspension was added dropwise from a pipette to the liquid cell until an obscuration of between 0.1 and 0.3 was achieved. Dispersion was maintained by a follower magnetically rotated at the bottom of the liquid measurement cell while the measurement was taken. Size measurements were carried out in triplicate on each of three freshly prepared suspension samples.

The particle size distributions of the sieved excipients were carried out according to the method as described above with the following modifications. A Malvern 2600 sizer fitted with a 100 mm lens was used and the excipients were suspended in a liquid medium of 0.1% (w/v) span 80 in buta-1-nol. The particle size distributions of the five excipients suspended in this liquid medium were calculated according to an independent model of analysis.

### Preparation of Dry Powder Formulations without Added Fine Excipients

Salbutamol base or sulphate (3.0 g) was blended with each excipient (202.5 g) to obtain a target concentration of 1.45% according to the following protocol. The drug was added to approximately 25% of the excipient followed by the addition of a further 25% of the excipient such that the drug was 'sandwiched' between the excipient to limit the adherence of micronised 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 returned to the mixer for a further 10 min mixing. The blending procedure was carried out under controlled conditions of 22–23°C and 45% RH.

The homogeneity of the blends was examined by sampling aliquots (*ca*. 27 mg) from each blend and determining salbutamol content. Each aliquot was added to a 50 ml volumetric flask and made up to volume with HPLC mobile phase containing 0.2% (w/v) internal standard. Six aliquots were taken randomly from each blend and each solution assayed in duplicate using the HPLC assay for salbutamol described below.

### Preparation of Binary Dry Powder Formulations Containing Added Fine Excipient

Blends of micronised salbutamol sulphate (SS) of volume mean diameter (VMD) 2.5 μm and coarse lactose (CL: 63–90 μm) were prepared and lactose fines were added. These fines were either "micronized lactose" (ML) with particle size range 1–20 μm (VMD 6.06 μm), or "intermediate sized lactose" (IML) with particle size range 1–50 μm (VMD 20.62 μm). The three components [drug, coarse lactose, and lactose fines (ML or IML)], were blended together in the weight ratio of 1:66.5:1

**Table I.** Composition and Details of The Mixing Order for the Blends with Added Fine Lactose

| Blend Codes | Compositions | Mixing Orders |
|---|---|---|
| $IML_{control}$ | CL, IML | CL mixed with IML |
| $IML_1$ | CL, IML, SS | CL mixed with IML then SS blended with this mixture |
| $IML_2$ | CL, IML, SS | CL mixed with SS then IML blended with this mixture |
| $IML_3$ | CL, IML, SS | SS mixed with IML then CL blended with this mixture |
| $ML_{control}$ | CL, ML | CL mixed with ML |
| $ML_1$ | CL, ML, SS | CL mixed with ML then SS blended with this mixture |
| $ML_2$ | CL, ML, SS | CL mixed with SS then ML blended with this mixture |
| $ML_3$ | CL, ML, SS | SS mixed with ML then CL blended with this mixture |

using different mixing sequences for the various components (14). Eight blends were made (Table I)

### HPLC Analysis of Salbutamol Base or Sulphate

Salbutamol base or sulphate was analysed by HPLC employing a mixture of methanol and 0.0013M 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 and the internal standard were found to be 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- and inter-day variability of the results.

### Development of a Metal Throat

The purpose-designed equipment, comprised an open-ended 'throat,' (Fig. 1) prepared from sheets of stainless steel (1 mm thick) which were welded together ensuring that the sides were parallel. The throat was constructed according to the dimensions given in Fig. 1. These dimensions were loosely based on anatomical measurements reported in the literature: distance from mouth to back of throat 10.3 cm, mouth-area diameter of 33 mm (15), and diameter of the upper trachea approximately 20 mm (15,16).

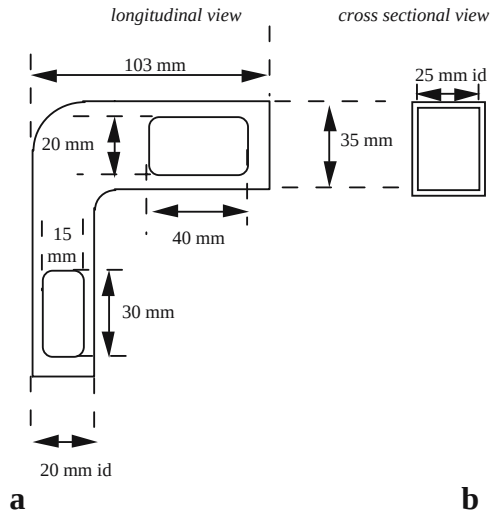


**Fig. 1.** Schematic diagram of the metal throat (not to scale).

Rectangular windows were cut in the sides of the metal throat to permit the laser beam to be passed through the dispersed aerosol. Two pairs of windows were cut-one pair (top window-40 × 20 mm) before and the other pair (bottom window-30 × 15 mm) after the 90° bend in the throat. This arrangement allowed measurement of the aerosol before and after impaction of the aerosol upon the back of the throat. The windows were covered by float glass (2 mm thick) cut to be several mm larger in each aspect than the holes in the throat. The glass was attached to the outside of the metal throat using silicone adhesive. The metal throat was positioned in front of the lens of the Malvern 2600 sizer either with the laser beam passing through the centre of the top (20 and 10 mm from the window edges in the *X*- and *Y*-axes, respectively) or the bottom (7.5 and 20 mm from the window edges in the *X*- and *Y*-axes, respectively) pair of windows.

### Glass Inhaler

A glass inhaler was designed and made in-house to introduce the powder blends into the selected air flows drawn through inertial impactors and/or other sizing devices. The device (Fig. 2a) had a 29/32 Quickfit™ socket which fitted into the glass throat of a conventional twin stage liquid impinger. A similar device (Fig. 2b) was made with a 24/29 Quickfit™ socket to connect with the teflon entrance seal at the mouthpiece of the metal throat. The small version of the glass device (Fig. 2b) only differed from the larger device in the dimensions of the Quickfit™ connections and the internal diameter. The dimensions of the sample port and bleed holes were identical in each version. The sample port allowed blend 'doses' to be loaded *in situ*, isolated from the air flow by occluding the port and then released into the air flow once the flow had stabilised through the two 'bleed' holes at the end of the device. The bleed holes also served to reduce the specific internal resistance of the device.

These glass devices were employed to examine the *in vitro* aerosol performance of salbutamol blends, which was independent of the influence of any commercial dry powder inhaler. However, it was accepted that the blends' performance would be dependent upon the conditions e.g., turbulence, produced in this simple device.

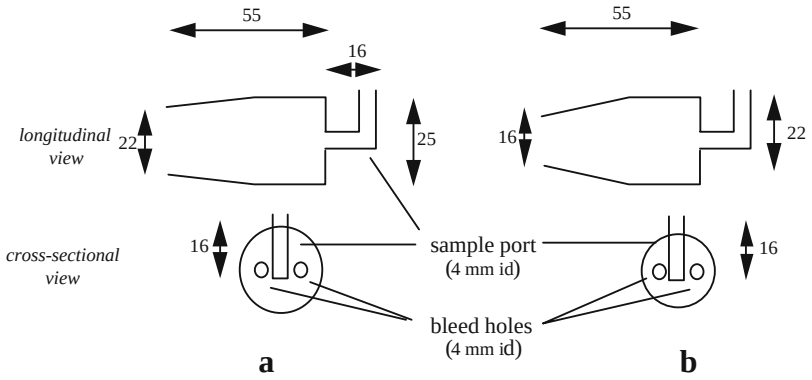


**Fig. 2.** Diagram of glass inhaler device showing the dimensions of (a) the large version which fitted into the glass throat of a twin stage impinger (BP 2002), and (b) the small version which fitted into the teflon entrance seal of the metal throat.

### Particle Size Measurement by Multi-stage Liquid Impinger

A multi-stage liquid impinger (MSLI) (Astra Draco, Lund, Sweden) was employed to examine aerodynamic particle size distribution of dry powders at 30, 60 and 100 l $min^{-1}$. The impinger was assembled with a filter paper (Qualitative 1, Whatman Labs. Div., Maidstone, Kent) in stage 4 and 20 ml mobile phase containing the internal standard (MPIS) dispensed into each of the remaining three stages. The larger glass device (Fig. 2a) was inserted into the throat of the impinger. Eight 'doses' of salbutamol blend (approximately 200 mg blend) were drawn through the apparatus for each deposition experiment. Each dose of blend was filled into the sample port of the device. This port was covered by the operator's finger while the pump was switched on. Once the air flow through the apparatus had stabilised, the port was uncovered releasing the dose. The pump was switched off after 4 l of air had been drawn through the device. This procedure was repeated for each of the eight doses.

Once all the doses had been discharged, each stage of the impinger was washed individually using the MPIS and the washing solution was then made up to a fixed volume (50 or 100 ml) with the same solvent for analysis of salbutamol content using the HPLC. The fine particle fraction of salbutamol was obtained by calculation from the drug deposited on stages 3 and 4, and expressing this as a percentage of the total dose recovered from all washings of the complete apparatus.

### Validation of the Laser Diffraction Method

Monodisperse aerosol particles were generated in order to validate the sizing by laser light scattering of dry powder aerosol particles as they were drawn through the metal throat. The monodisperse aerosol generating equipment (MAGE) employed was a modified Sinclair–La Mer generator with a forced air circulation thermostat and gas flow by-pass (17). In this study the nuclei (tiny salt particles on which stearic acid condensed to generate monodisperse particles), were produced by atomisation of an aqueous solution of sodium chloride (0.9 g $l^{-1}$) using a nitrogen flow rate of 210 l $h^{-1}$ and the by-pass valve fully open. Stearic acid aerosols were generated over a temperature range of 180–260°C.

Aerosols were generated after allowing the system to equilibrate from the ambient temperature to the selected MAGE temperature setting over 1.5–2 h. Once the nebuliser was started particles were very quickly produced by the MAGE but it took 10–20 min for a stable monodisperse aerosol to be generated. After this equilibration time the particle size of the generated aerosols in flight were examined by a Malvern 2600 laser diffraction sizer. The particles were produced in such a concentration that it was necessary, particularly at high temperatures with stearic acid, to divert only part of the generated aerosol through the laser beam to achieve optimum obscuration (0.1–0.3). The remaining undiverted aerosol was drawn into a filter by a vacuum cleaner. Size measurements of the aerosol were made repeatedly throughout the 20–30 min of aerosol generation to monitor reproducibility and stability of monodispersity during throat validation experiments.

The aerosols were characterised by analysing the log-normal particle size distributions, particularly by the calculated geometric mean diameters (GMD) and their related geometric standard deviations (GSD). The GSD is a convenient index of the spread of the particle size distribution. There is no hard and fast rule governing monodispersity, but it is generally accepted that an aerosol distribution with GSD < 1.2 is monodisperse (18).

To test the validity of sizing fine particles through this apparatus, measurements were made by laser diffraction of monodisperse stearic acid particles generated the MAGE. Particles of stearic acid were generated at temperatures of 170 and 220°C to create particles of two geometric mean diameters within the particle size range of micronised salbutamol. Background readings were taken over 2,000 sweeps, which was the number of times a reading was taken of light falling on each of the concentric photodiode detectors, before each recorded sample measurement. The monodisperse particles were sized according to four different methods (A, B, C and D):

**A** Particles issuing directly from the MAGE were simply diverted through the path of the laser beam without any intervening apparatus. Particles were sized on manual instruction over 500 sweeps of the diffraction detector elements.

**B** Particles were drawn through the metal throat *via* an open-ended glass tube and sized on manual instruction over

500 sweeps of the diffraction detector elements. The glass tube had the same coned Quickfit socket (24/29) and internal diameter (22 mm) as the glass device (Fig. 2b). The glass tube did not have the device's sample port as it was simply used to divert the stream of MAGE particles through the throat.

**C** Particles were drawn through the metal throat *via* the glass tube (as used in method B) and sizing triggered externally by a pulse sensor to measure over five sweeps of the diffraction detector elements (simulating the measurement time of a discrete aerosol dose).

In addition, however, a fourth method (D) was designed to mimic the actual passage and measurement of a discrete aerosol dose.

**D** A pulse of particles was drawn through the metal throat by switching the pump on and off. Size measurement was initiated manually before the particles passed through the laser beam and lasted for 200 sweeps to ensure the complete 'dose' was measured. No external triggering system was employed.

Background readings were taken over 2,000 sweeps of the diffraction detector elements before each sample measurement. Methods B, C, and D were used to size the two size ranges of MAGE particles drawn through the top and bottom windows of the metal throat at both 28.3 and 80 l $min^{-1}$. All particle size distributions were calculated based on a log-normal mode of distribution analysis. The distributions were accepted when the geometric standard deviation was less than 1.25, i.e., indicative of monodispersity. The geometric mean diameter (GMD), geometric standard deviation (GSD) and span of each distribution were noted.

### Sizing of Salbutamol Blends through the Top Window

The glass inhaler was attached to the uncoated metal throat through which the blend aerosols were drawn under constant air flow to waste *via* a methanol/water mixture trap and filter system (Fig. 3). A Malvern 2600 sizer with 63 mm lens was positioned such that the laser beam passed through the top window of the throat, to determine the size distribution of the aerosol as soon as it emerged from the device. Thus, the sample (approximately 30 mg) was loaded into the device sample port which was then occluded. The vacuum pump was started to pull the calibrated flow rate. Once the flow had become steady after a few seconds, the background measurement was initiated over 1,000 sweeps of the diffraction detector elements. After the measurement command had been manually initiated to record 200 sweeps of the diffraction detector elements, the port was uncovered to release the sample into the air flow.

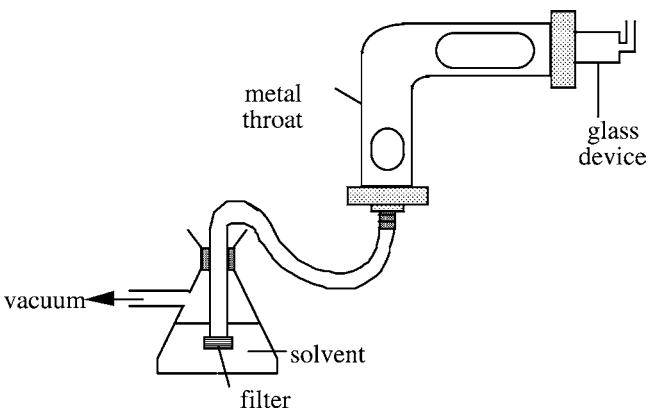


**Fig. 3.** Schematic diagram of the apparatus used to generate aerosols of salbutamol blend under constant air flow for characterisation by laser diffraction.

All ten binary blends were examined using the method described above and each blend was tested after aerosolisation at a flow rate of 28.3, 60, 80 and 100 l $min^{-1}$ from the glass inhaler. Ten to 15 measurements were taken for each blend.

### Sizing of Salbutamol Blends through the Bottom Window

The internal surface of the metal throat was coated with a pressure-sensitive double-sided adhesive tape, such that one side adhered to the metal throat and the other side provided an adhesive layer exposed so as to capture particles that might impact on the surface. Aliquots (approximately 30 mg) of the five salbutamol base blends were drawn from a glass device through the coated metal throat at 60 l $min^{-1}$ with the laser beam directed through the top window. After three aliquots had been discharged through the throat, the adhesive tape was removed, the throat cleaned and a fresh piece of tape applied. The experiment was carried out in triplicate. This was then repeated with the laser beam directed through the bottom window.

## RESULTS

### Formulation Development

The size distributions determined by wet dispersion laser diffraction confirmed the existence of particles >10 μm and even suggested a bimodal distribution in the case of the salbutamol base sample (data not shown). The drug was not thought to have an optimal particle size for inhalation and was subsequently remicronised. The remicronised base and sulphate gave similar volume mean diameters (and associated spans) of 2.42 μm (1.01) and 2.58 (1.05), respectively. Thus, the remicronised salbutamol base and sulphate were considered to be of a suitable size to be used in dry powder aerosol formulations.

When measured by wet dispersion laser diffraction, different types of sugar gave a similar mean size and span of distribution. The VMD (span) values were 81.0 μm (0.7), 78.9 μm (0.8), 75.5 μm (0.6), 78.2 μm (0.8), 80.9 μm (0.5) for the Regular lactose, Zeparox™, Sorbitol, Dextrose and Maltose, respectively. Similarity in particle size is likely to be due to the fact that these sugars have been subjected to similar sieving treatments.

The mean recovery of salbutamol base and sulphate from each formulation was between 98–101% with all relative standard deviation (RSD) values ≤ 3%. All individual recovery data were comfortably within 90–110% target, suggesting that homogenous blends had been obtained with each sugar carrier. These highly consistent data were also indicative of the reproducibility and accuracy of the overall mixing, sampling and HPLC analysis.

### Validation of Laser diffraction Using Monodisperse Aerosols

Mean values of GMD from MAGE particles measured by methods A, B, C and D are summarised in Table II. The

**Table II.** Mean GMDs from Log-Normal Distributions of MAGE Particles Generated at 170 and 220°C and Measured by Sizing Methods A, B, C and D through Top and Bottom Throat Windows

| Temperature of Steric Acid | Flow Rate (l min$^{-1}$) | Window Positions | Mean GMD (% CV) (μm) | | |
|---|---|---|---|---|---|
| | | | B | C | D |
| 170°C | 28.3 | Top | 2.12 (1.56) | 2.11 (0.93) | 2.13 (1.12) |
| | | Bottom | 2.10 (2.39) | 2.09 (1.77) | 2.10 (0.52) |
| | 80 | Top | 2.12 (1.52) | 2.09 (0.93) | 2.12 (1.39) |
| | | Bottom | 2.09 (1.60) | 2.07 (1.02) | 2.10 (0.87) |
| | – | Method A | | 2.11 (0.98) | |
| 220°C | 28.3 | Top | 3.38 (3.11) | 3.40 (1.44) | 3.38 (1.69) |
| | | Bottom | 3.36 (3.03) | 3.32 (0.66) | 3.34 (1.12) |
| | 80 | Top | 3.31 (3.44) | 3.30 (2.47) | 3.40 (1.31) |
| | | Bottom | 3.32 (1.66) | 3.37 (2.11) | 3.41 (0.98) |
| | – | Method A | | 3.38 (1.39) | |

three-way analysis of variance carried out on the MAGE particles generated at 170°C revealed that overall there were no significant differences in the GMDs caused by flow rate changes ($p = 0.179$) or sizing method changes overall ($p = 0.055$). Although not significantly different from the control method A ($p < 0.02$, Student's *t*-test), the GMDs by method C were markedly lower than those of methods B or D. Sizing of these particles (at 170°C) was affected by the position of measurement within the throat (i.e., the top or bottom window) ($p = 0.007$). The mean GMDs measured *via* the bottom window were lower than those *via* the top window independent of the method of measurement used (all $p < 0.05$, Student's *t*-test). However, only the mean GMD measured at 80 l min through the bottom window by method C was different from that by method A.

The three-way ANOVA of GMDs for MAGE particles generated at 220°C revealed that there were no significant "inter-method," "inter-position" or "inter-flow rate" differences in the data ($p = 0.109$, $p = 0.670$ and $p = 0.551$, respectively). The differences in mean GMD found between window positions for the MAGE particles at 170°C, were not detected for the larger particles at 220°C, although differences caused by an interactive effect of position and flow rate were suggested ($p = 0.057$).

In general the laser diffraction sizing of aerosol particles of this size range did not appear to be significantly affected by any of the variables examined in the measuring technique. However, in deciding upon the method of particle size measurement to be used in future aerosol characterisation, a number of findings and experiences produced in this work were considered:

- Although not significant ($p = 0.055$, three-way ANOVA), some "inter-method" differences in the size data for particles generated at 170°C were noted. On closer examination these seemed to arise from the data generated from method C which employed the external triggering system and the short timespan of only five detector element sweeps.
- Some practical problems were experienced with the external triggering system in that the measurement could be triggered prematurely by minor vibrations e.g., vacuum pump, or could occur late and miss the front of the aerosol plume.
- Differences were found between sizing *via* the top and bottom windows of the throat for MAGE particles at 170°C; the GMDs measured *via* the bottom window being lower than those of the control (A) ($p < 0.05$, Student's *t*-test).

As a consequence of these findings it was decided to examine the aerosol through the top window, to initiate the measurement manually before the aerosol was released into the air flow, as in method D, and to stop the measurement after 200 readings had been taken of the laser detector.

### Sizing of Aerosolised Salbutamol Blends through the Top Window

In an attempt to highlight any differences at the lower end of the particle size distribution (i.e., particles $d_v < 10$ μm) the light scattering data recorded in the first inner ring of the detector that captured the diffracted light from large particles was removed or 'killed' for the overall scattered light and the particle size distribution recalculated. This recalculated distribution was termed the "kil (1,0) distribution." For a lens of focal length 63 mm, this meant that only particles $d_v \leq$ approximately 60 μm would be included in the distribution. Since the "kil (1,0) distribution" eliminated any interference from larger particles, any small difference in the therapeuti-

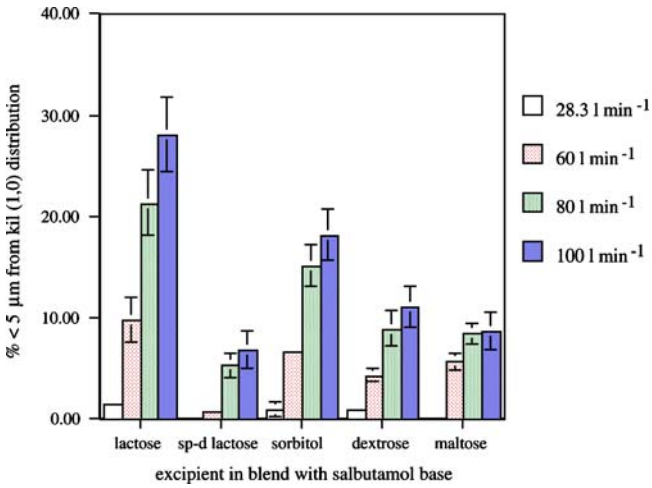


**Fig. 4.** Fine aerosol fractions (<5 μm) measured in salbutamol base blends at 28.3, 60, 80 and 100 l min$^{-1}$ by laser diffraction [mean (sd), $n = 15$] sp-d lactose is spray-dried lactose (Zeparox™).

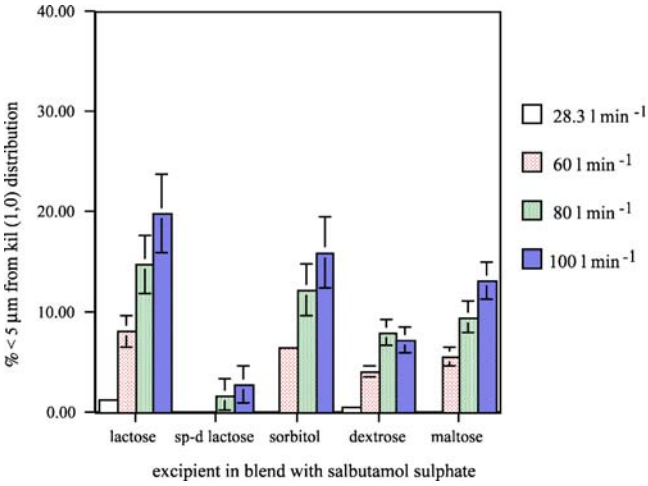


**Fig. 5.** Aerosol fine fractions (<5 μm) measured in salbutamol sulphate blends at 28.3, 60, 80 and 100 l min$^{-1}$ by laser diffraction [mean (sd), $n$ = 15] sp-d lactose is spray-dried lactose (Zeparox™).

cally important finer fractions (e.g., <10 μm) could now be detected by the technique.

The fine aerosol fractions from different blends, generated in the four flow rates (28.3, 60, 80 and 100 l min$^{-1}$) and measured by laser diffraction are represented graphically in Figs. 4 and 5 for formulations containing salbutamol base and salbutamol sulphate, respectively. Both salbutamol base and sulphate blends displayed increasing mean fine fraction with increased flow rate, for all blends. At flow rates ≥80 l min$^{-1}$ the carrier excipients within the base blends (Fig. 4) can be placed in order of increasing mean fine fraction as follows: spray-dried lactose < maltose ≤ dextrose < sorbitol < regular lactose. This trend was found to be repeated for the sulphate blends (Fig. 5) with the exception of the maltose blend which generated a fine aerosol fraction larger than that of the dextrose blend at each flow rate ($p < 0.05$, Student's $t$-test). The fine fractions from the base blend aerosols seemed to be greater than those from the corresponding sulphate blends but these differences were only significant at flow rate above 60 l min$^{-1}$ for the blends containing regular lactose, spray-dried lactose and dextrose. However, the fine fractions from the base and sulphate blends containing sorbitol were not statistically different by virtue of the large standard deviations calculated on the mean fine fractions for this particular excipient ($p > 0.1$, Student's $t$-test).

**Table III.** Volume Mean Diameters (μm) Obtained from the kil (1,0) Distributions of Control Excipients Measured by Laser Diffraction Through the Top and Bottom Throat Windows at 60 l min$^{-1}$ [Mean (sd), $n$ = 9]

| Control Excipient | Top Window | Bottom Window |
|---|---|---|
| Regular lactose | 43.5 (0.3) | 43.3 (0.4) |
| Spray-dried lactose | 44.0 (0.3) | 43.8 (0.8) |
| Sorbitol | 43.8 (0.8) | 43.1 (0.5) |
| Dextrose | 43.9 (0.5) | 43.6 (0.4) |
| Maltose | 43.8 (0.2) | 43.6 (0.4) |

**Table IV.** Aerosol Fine Fractions (% < 5 μm) Obtained from the kil (1,0) Distributions of Salbutamol Base Aerosols Measured by Laser Diffraction through the Top and Bottom Throat Windows at 60 l min$^{-1}$ [mean (sd), $n$ = 9]

| Excipient in Blend | Top Window | Bottom Window |
|---|---|---|
| Regular lactose | 9.81 (1.6) | 11.55 (1.7) |
| Spray-dried lactose | 0.41 (0.2) | 0.52 (0.3) |
| Sorbitol | 5.41 (0.8) | 7.42 (1.4) |
| Dextrose | 3.74 (0.2) | 5.07 (1.3) |
| Maltose | 4.96 (1.0) | 5.88 (1.5) |

## Sizing of Aerosolised Salbutamol Blends through the Bottom Window

The mean VMDs of the control measured *via* the top and bottom windows are detailed in Table III. The fine fractions of the blends measured *via* the top and bottom windows are detailed in Table IV.

The particle size distributions of the controls revealed no fines when measured *via* the top or bottom throat windows. The mean VMDs for each excipient (Table III) measured *via* the top and the bottom throat windows were not significantly different from each other ($p > 0.1$, Student's $t$-test). On the other hand, the mean fine fractions of all the salbutamol base blends measured through the bottom window were found to be greater than those through the top window (Table IV) although the increases were only found to be significant for the blends containing lactose, sorbitol and dextrose ($p < 0.05$, $p < 0.005$ and $p < 0.001$, respectively, Student's $t$-test).

## *In vitro* Deposition Profiles of Salbutamol Base from Various Formulations at Different Flow Rates

The *in vitro* deposition profiles of salbutamol base in the multistage liquid impinger are detailed in Tables V, VI, VII for aerosols generated at 30, 60 and 100 l min$^{-1}$, respectively. For each of the salbutamol base blends, the fine fraction increased with increasing flow rate ($p < 0.001$, ANOVA). These increases were more apparent with some blends (e.g., lactose) than others (e.g., dextrose), a similar finding to that obtained from the laser diffraction data. At an air flow rate of 30 l min$^{-1}$, the fine aerosol fractions from the lactose, sorbitol and dextrose blends were not significantly different ($p = 0.089$,

**Table V.** Cumulative Percent Deposition of Salbutamol Base in Different Stages of a Multi-Stage Impinger at 30 l min$^{-1}$ [Mean Percent of Recovered Dose (sd), $n$ = 3]

| Excipient Insalbutamol Base Blend | Stage 2 (<18.37 μm) | Stage 3 (<9.62 μm) | Stage 4 (<4.38 μm) |
|---|---|---|---|
| Lactose | 6.44 (0.7) | 6.26 (0.6) | 5.75 (0.6) |
| Spray-dried lactose (Zeparox™) | BD | BD | BD |
| Sorbitol | 7.30 (1.0) | 7.19 (1.0) | 6.41 (0.7) |
| Dextrose | 8.14 (1.6) | 8.13 (1.6) | 7.88 (1.4) |
| Maltose | 2.03 (0.2) | 2.03 (0.2) | 1.88 (0.1) |

BD-levels below the limit of detection for the HPLC assay.

**Table VI.** Cumulative Percent Deposition of Salbutamol Base in Different Stages of a Multi-Stage Impinger at 60 l $min^{-1}$ [Mean Percent of Recovered Dose (sd), $n = 3$]

| Excipient in Salbutamol Base Blend | Stage 2 (<13.0 μm) | Stage 3 (<6.8 μm) | Stage 4 (<3.1 μm) |
|---|---|---|---|
| Lactose | 16.63 (4.9) | 15.03 (4.0) | 13.97 (2.7) |
| Spray-dried lactose (Zeparox™) | 4.88 (0.8) | 4.74 (1.0) | 4.4 (1.1) |
| Sorbitol | 15.82 (2.7) | 15.01 (1.3) | 11.74 (1.0) |
| Dextrose | 13.04 (0.5) | 12.81 (3.7) | 11.16 (2.3) |
| Maltose | 12.30 (1.0) | 10.50 (0.8) | 8.60 (0.5) |

ANOVA), but each of the fine fractions of these blends was significantly greater than that of the maltose blend ($p < 0.05$, Student's *t*-test). There was no detectable fine aerosol fraction from the Zeparox™ blend at 30 l $min^{-1}$. At higher flow rates, differences among the mean fine fractions from different blends became more apparent. At 100 l $min^{-1}$, the fine fractions measured for the lactose, sorbitol and dextrose blends displayed marked differences ($p = 0.001$, ANOVA). These findings were again very similar to those discovered with laser diffraction.

### Correlation between Laser Diffraction and Inertial Impaction

In Fig. 6, the fine fractions for salbutamol blends measured by inertial impaction have been compared with and plotted against the fine fractions measured by laser diffraction. Regression analysis of the data demonstrated a good correlation between the two techniques with significant linearity ($r^2 = 0.934$).

### Sizing of Aerosolised Blends Containing Added Fine Lactose

From each 'virgin' particle size distribution [kil (0,0)] the fine fraction was noted (Table VIII). The kil (0,0) distribution was used instead of the kil (1,0) distribution used previously since the excipient particle size fraction which would be removed by excluding scattered light detected on the

**Table VII.** Cumulative Percent Deposition of Salbutamol Base on Different Stages of a Multi-Stage Impinger at 100 l $min^{-1}$ [Mean Percent of Recovered Dose (sd), $n = 3$]

| Excipient in Salbutamol Base Blend | Stage 2 (<10.07 μm) | Stage 3 (<5.27 μm) | Stage 4 (<2.4 μm) |
|---|---|---|---|
| Lactose | 40.74 (3.9) | 37.50 (3.2) | 21.50 (2.7) |
| Spray-dried lactose (Zeparox™) | 19.82 (2.2) | 15.67 (0.3) | 8.59 (0.7) |
| Sorbitol | 32.82 (0.6) | 29.42 (0.8) | 16.71 (1.1) |
| Dextrose | 27.72 (1.8) | 24.66 (1.3) | 13.51 (2.5) |
| Maltose | 18.11 (2.7) | 14.21 (2.1) | 6.58 (0.7) |

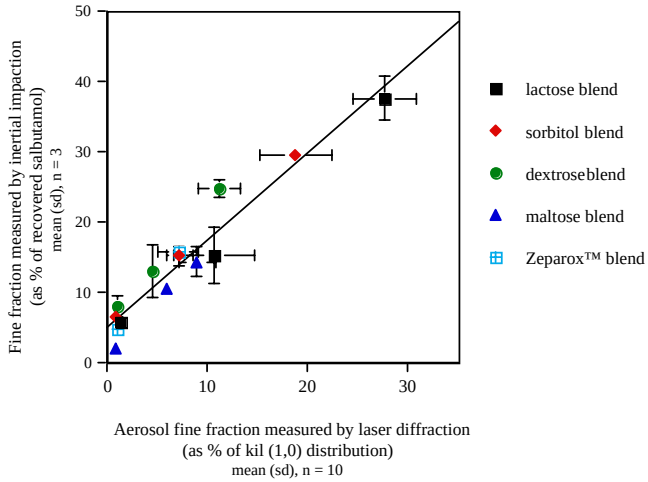


**Fig. 6.** The salbutamol fine fractions (<6.8 μm) obtained by inertial impaction plotted as a function of corresponding aerosol blend fine fractions (<6.8 μm) obtained by laser diffraction, for aerosols generated at 30, 60 and 100 l $min^{-1}$ from blends of salbutamol base with lactose, sorbitol, dextrose, maltose and spray-dried lactose (Zeparox™).

inner ring was not anticipated to be the same in ML blends as in IML blends. The fine fraction was represented by % <6.48 μm, to enable comparison of this data at 60 l $min^{-1}$ to be made with the inertial impaction fine fraction results at 60 l $min^{-1}$ (15). The fine fractions measured in control aerosols ($ML_{control}$ and $IML_{control}$) were also determined (Table VIII). The two controls containing lactose fines ($ML_{control}$ and $IML_{control}$) generated aerosols at 60 l $min^{-1}$ with mean fine fractions of 9.1 and 4.1%, respectively (Table VIII).

The fine fractions from blends $ML_1$, $ML_2$ and $ML_3$ were found to be significantly greater than those from blends $IML_1$, $IML_2$ and $IML_3$ ($p < 0.001$, ANOVA). This was not unexpected since the weight ratio of particles <6 μm: total blend particles in ML blends was double that found in IML blends. This was confirmed by the fine fraction (%) of $ML_{control}$ being markedly greater that $IML_{control}$ ($p < 0.05$ Student's *t*-test). The salbutamol fine fractions can therefore be calculated by subtracting the total fine fraction from the

**Table VIII.** Aerosol Fine Fraction of the Controls ($ML_{control}$ and $IML_{control}$) and Blends Containing Added Lactose Fines (VMDs 20.62 and 6.06 μm) Determined by Laser Diffraction and a Twin Stage Liquid Impinger (TSLI) at 60 l $min^{-1}$ [Mean (sd)]

| Blends | Laser Diffraction* | | TSLI** |
|---|---|---|---|
| | Total fine fraction (%) | Salbutamol fine fraction (%) | Salbutamol fine particle fraction (%) |
| $IML_{control}$ | 4.1 (0.7) | | |
| $IML_1$ | 6.0 (0.4) | 1.9 (0.1) | 9.58 (0.9) |
| $IML_2$ | 5.8 (0.6) | 1.7 (0.2) | 9.66 (0.9) |
| $IML_3$ | 4.7 (0.9) | 0.6 (0.1) | 7.56 (0.2) |
| $ML_{control}$ | 9.1 (0.7) | | |
| $ML_1$ | 12.0 (1.5) | 2.9 (0.4) | 14.48 (1.2) |
| $ML_2$ | 10.6 (0.5) | 1.5 (0.1) | 8.09 (2.1) |
| $ML_3$ | 10.1 (0.7) | 1.0 (0.1) | 9.34 (1.9) |

*$n = 10$; **$n = 3$ (15).

fine fraction of the control assuming that the presence of salbutamol did not alter lactose fine fraction. The resultant fine fractions are also listed in Table VIII.

The various blends containing added fine lactoses were previously tested using a twin stage liquid impinger from a Rotahaler® at 60 l $min^{-1}$ (14). The salbutamol sulphate fine fraction of each blend (% <6.4 μm of recovered salbutamol) was determined in triplicate and is reported in Table VIII. When salbutamol fine fraction data at 60 l $min^{-1}$ derived by laser diffraction were compared with those obtained by inertial impaction a significant linearity was revealed ($r^2 = 0.794$).

## DISCUSSION

Laser diffraction is probably the most widely used technique for particle size analysis in the pharmaceutical industry. It has been used at nearly all stages of product development, manufacture and quality control of pharmaceutical dosage forms. However, due to the unique characteristics of dry powder aerosol formulations, particle size measurements using laser diffraction need to be conducted in a well-controlled manner in order to obtain results that reflect the true performance of the formulations.

Dry powder aerosol formulations are typically composed of micronised drug (down to less than one micron) blended with a coarse carrier (up to several hundred microns). Lorenz-Mie theory should be employed in order to obtain accurate measurement of particles at both lower and upper ends of the distribution. According to this theory, the scattering of light by particles approaching the wavelength of incident radiation is a function of the equivalent sphere diameter of the particle, the angles of scattering, the wavelength of the incident light, differences in refractive indices including light absorption coefficient between the particles and the medium in which they are dispersed (19). Thus, there are several restrictions associated with the use of Lorenz-Mie theory. First, the calculation is based on the assumption that the spherical particles have smooth surfaces and therefore any differences in particle morphology between the drug and carrier particles will in theory result in measurement errors. In practice, such an error is deemed insignificant if both the carrier and drug have predefined morphology and if the purpose of the measurement is to compare different formulations using the carrier and the drug instead of obtaining the "true" values of particle size distribution. For instance, the crystalline lactose monohydrate carrier normally exhibits a well-defined tomahawk shape and in most cases, the micronised drug is approximately spherical. It has been reported that the diameter of coarse lactose measured by laser diffraction can be adjusted using a single shape factor to be close to that measured by sieving (20). Therefore, differences in morphology between the drug and carrier should not restrict the use of laser diffraction for the characterisation of dry powder aerosol formulations. Second, since the results obtained by Lorenz-Mie theory are affected by the refractive index and absorption coefficient of the measured particles, care has to be taken to use the theory to obtain particle size distribution of a mixture of different components having significantly different optical properties. However, the refractive index of pharmaceutical actives and excipients is generally between 1.4–1.7 and it only needs to be known to an accuracy of ±0.2 whilst the particle absorption need only be specified to the nearest order of magnitude (0.01–0.1 for most pharmaceutical crystals and milled materials) to achieve reliable results (21). Since the coarse carrier usually constitutes over 90% w/w of dry powder aerosol formulations, using the optical parameters of the carrier particles would be expected to give results that closely resemble the particle size distribution of the mixture. It is therefore possible to use instruments based upon Lorenz–Mie theory to obtain representative measurements of particle size distribution of a typical dry powder aerosol formulations.

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. This latter theory relates the intensity of light scattered by a particle to the particle size, whereas the magnitude of the diffraction pattern is inversely proportional to the particle size. The Fraunhofer approximation does not require knowledge of the optical properties of the component particles being studied, therefore its use is recommended when mixtures are being examined (22). The instruments based on the Fraunhofer approximation operate on the assumptions that all particles are greater than 40 times the wavelength of the laser light (i.e., 25 μm when a He-Ne laser is used), and particles of different sizes scatter with the same efficiency; and particles are opaque and do not transmit any light. In reality, none of the assumptions is strictly correct and this could lead to significant errors. In the present study, particle size measurement was performed using the Malvern 2600 Particle sizer, which analyzes the Fraunhofer diffraction pattern formed when a laser beam traverses the aerosol field (23). The forward scattered light was focused on to a detector consisting of 32 light sensitive diodes. The aerosol size distribution is computed from the light energy distribution detected by the geometrically defined diode array by assuming a Rosin–Rammler distribution of droplet sizes.

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 (24). In these salbutamol blends, the distributions were likely to be skewed towards the mode that contributed the strongest peak in the diffraction pattern. This would exaggerate the fraction of large carrier particles at the expense of the aerosol fines (< 5 μm). 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. This may explain why the difference in fine particle fractions measured by two techniques is smaller when large particles (>60 μm) were excluded than when all particles were included in calculations. In order to obtain more accurate results, the "masking" effect of large particles on small particles must be minimized when instruments based on the Fraunhofer approximation such as Helos Laser Diffraction (Sympatec GmbH, Clausthal-Zellerfeld, Germany) are used to measure the particle size distribution of dry powder aerosol formulations. One approach would be to use a pre-separator to capture the majority of the large lactose crystals before the light scattering patterns of the aerosol particles are taken (25).

Despite these restrictions and limitations, however, the laser diffraction technique permitted the generation of a sizing

parameter, corresponding to the aerosol fine fraction, commensurate with the salbutamol fine fraction from the studied blends measured by inertial impaction. Within the flow rate and particle size ranges of the carrier and the drug examined in this work, the *in vitro* salbutamol fine fraction from any of these blends could be predicted from measurements obtained from the laser diffraction technique using one linear relationship.

## CONCLUSIONS

The laser diffraction technique employing a metal throat, which was validated using monodisperse aerosols, was used to examine the particle size distributions of various salbutamol blends. Each blend, containing a different bulking excipient, was prepared from components of two distinct particle size ranges: drug (< 5 μm) and excipient (63–90 μm). The laser diffraction technique was employed to generate a criterion of the distribution i.e., fine particle fraction, and this was found to correlate linearly with the drug fine fraction determined by inertial impaction ($r^2$ = 0.934). This relationship was unaffected by changes in flow rate, excipient, salbutamol form (base or sulphate) and could be used to predict the aerosol performance of these blends, in terms of salbutamol fine fraction, from the laser diffraction distribution.

When the particle size distributions of the individual blend components are not distinctly separate, the systems could be more difficult to compare in this way. However, when the appropriate controls were employed a good correlation could be found between fine fractions generated by the two sizing techniques ($r^2$ = 0.79). This study demonstrates the potential of the technique to examine such complex blends and suggests that 'better' formulations containing a higher fine fraction of drug may be identified from 'poorer' formulations more quickly and easily during their development than by obtaining data from conventional inertial impactors. Therefore, the laser diffraction technique could prove to be an important tool for initial formulation and process screening, in-process control and even quality control of the finished products.

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