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Development of a laser diffraction method for the determination of the particle size of aerosolised powder formulations

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Abstract

Impactor data are an essential component of marketing authorisation for new dry powder aerosol formulations. However such data are time-consuming to obtain and therefore impede the rapid screening of pilot formulations. In this phase of development it would be of considerable benefit to employ a technique where data acquisition was more rapid, such as laser diffraction, to predict the fine particle fraction. It was the aim of this study to investigate whether this is a feasible premise. Five different formulations were prepared, each containing 1.5% (w/w) micronised salbutamol base (volume median diameter: 2.42 μm) blended with the sieved fraction (63–90 μm) of one of the following sugars: regular crystalline lactose, spray dried lactose "Zeparox™", sorbitol, maltose and dextrose monohydrate. A Perspex™ box was constructed to contain particles released from a glass inhaler and allow the particles to be measured by laser diffraction at different flow rates. After being validated using monodisperse aerosols, this assembly was then employed to measure the particle size distributions of each powder formulation and its respective sugar carrier at flow rates ranging from 28.3 to 100 l min^{-1}. Aerodynamic particle size distribution of salbutamol base from each formulation was also measured after aerosolisation at 28.3 l min^{-1} from the glass inhaler into an Andersen cascade impactor. The flight of monodisperse particles with diameters (2–6 μm) in the desired size range of dry powders for inhalation could be contained and the size distribution determined by laser diffraction using the assembly at all flow rates investigated. Treatment of the particle size distributions measured by laser diffraction, i.e. examining only the aerosol particles with diameter <60 μm, highlighted the fine fraction (<5 μm) and enabled the aerosolisation of different blends to be feasibly compared at a range of different flow rates. The blends containing the following excipients could be placed in the following order of increasing fine fraction: spray-dried lactose < dextrose ≪ maltose < lactose < sorbitol. At 28.3 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 ($R^2 = 87.4\%$, $p = 0.02$, ANOVA). Therefore, the laser diffraction technique could prove to be an important tool for particle size characterisation of dry powder aerosol formulations.

Keywords: Laser diffraction; Inertial impaction; Salbutamol; Dry powder inhaler formulations; Fine particle fraction; Aerodynamic particle size distribution

1. Introduction

Dry powder inhalers (DPIs) have emerged within the last decade as an effective replacement for conventional pressurised metered dose inhalers (pMDIs) for the delivery of drugs to the lung. The increasingly important role of DPIs in treating lung diseases, coupled with their considerable potential for delivering drugs to the systemic circulation, has resulted in extensive investigation into nearly all aspects of DPIs that could affect the safety and efficacy of this type of medicinal devices. From the pharmaceutical perspective, these studies can be broadly categorised into those related to the formulation of the pharmaceutical powder (Zeng et al., 2000; Telko and Hickey, 2005), device design (Smith and Parry-Billings, 2003) and those related to *in vitro* and *in vivo* deposition (Newman and Busse, 2002). The quality of a DPI product is determined by the formulation, device design and interaction between them. Since different devices could be used to deliver the same molecule such as salbutamol, it is still common practice to screen a large number of candidate formulations before identifying the most suitable formulation from a specific or selected device. The aerodynamic particle size distribution (APSD) of aerosolised drug is an essential parameter to be tested and controlled for formulation screening and subsequent quality control of the final product.

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The APSD is routinely measured by sizing techniques that are based on inertial impaction. The principle of classification by inertial separation is well established and several different types of devices such as the multi-stage liquid impinger and Andersen cascade impactor have been adopted by European and US Pharmacopoeias. Impingers and impactors have been widely employed as the "gold standard" for both product development and quality control of the finished products. However, such impaction techniques are invariably laborious and time-consuming to operate and are not the best choice for screening many candidate formulations during the early stage of product and process development. Moreover, impingers and impactors can only classify particles into a small number of size ranges which may not be sufficient for detecting subtle formulation differences that could have an important influence on the performance of the finished product. Therefore, alternative techniques need to be identified to cope with the limitations of inertial impaction techniques (de Boer et al., 2002a).

Laser diffraction may prove to be such a technique since it is fast, reproducible and above all, offers a much higher number of size classes for the relevant fine particle fraction than can be obtained from inertial impaction. Laser diffraction has been successfully employed to examine aerosols for inhalation from nebulisers (Clark, 1995) and pressurised metered dose inhalers (Moren, 1981), but its application to dry powder aerosols has been little studied (Olsson et al., 1988; Everard et al., 1995). 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 when both techniques were used to test nebulisers (Ziegler and Wachtel, 2005) and pMDI (Smyth and Hickey, 2003). However, the use of laser diffraction to characterise dry powder aerosol formulations is limited for two reasons. First, most dry powder aerosol formulations are composed of micronised drug blended with a coarse carrier. Since the carrier almost always contains small particles that are similar in size to the drug, it is difficult, if not impossible, to differentiate between drug and fine carrier particles, by analysing laser diffraction patterns of the particles only. Second, particle size measurement by laser diffraction is based on the assumption that the particles are spherical. For micronised particles, deviation from sphericity is negligible whilst for large crystalline carrier particles, the shape factor should be taken into account in order to obtain an accurate measurement. Nevertheless, laser diffraction may still prove to be a valuable tool for characterising dry powder formulations under well-controlled conditions (de Boer et al., 2002a; de Boer et al., 2002b). So far, there has been no reported study seeking to find a correlation between the fine particle size distribution of dry powder aerosol formulations measured by laser diffraction and that obtained by cascade impaction. It was therefore the aim of the present study to develop and validate a laser diffraction technique to characterise particle size distributions of various dry powder aerosol formulations and to compare the results with those obtained from an inertial impaction method. In order to prepare a range of formulations, the dispersion properties of dry powder formulations containing different types of carrier entities were investigated and aerosolisation was effected at different flow rates.

2. Materials and methods

2.1. Materials

Micronized salbutamol base (Allchem International, Maidenhead, UK), regular grade α-lactose monohydrate (Borculo Whey Products, Saltney, Cheshire UK), spray-dried lactose "Zeparox^{TM}" (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) (Rathburn Chemicals Ltd, Walkerburn, Scotland), ammonium acetate (HPLC grade) (BDH Lab Supplies, Loughborough, UK) and distilled water (MilliQ grade) (Millipore, Watford, UK) were obtained from the indicated suppliers.

2.2. Formulation development

2.2.1. Preparation of excipients and blends

The sieved fraction (63–90 μm) of coarse sugar carrier, comprising the 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 required.

Salbutamol base (3.0 g) was blended with each excipient (202.5 g) to obtain a target concentration of 1.45% according to the following process. The drug was added to approximately 50% of the excipient, in a 'sandwich' arrangement to limit the adherence of micronised drug to the glass blending jar, and 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 the salbutamol content thereof. Each aliquot of blend was added to a 50 ml volumetric flask and made up to volume with HPLC mobile phase (see below) containing 0.2% (w/v) internal standard. Six aliquots were taken randomly from each blend and each solution assayed in duplicate according to the HPLC assay for salbutamol.

2.2.2. Physical characterisation of salbutamol and excipients

The particle surface topography and texture of each coarse carrier were assessed qualitatively using scanning electron

microscopy (Philip SEM 501B scanning electron microscope, Eindhoven, Holland). The sample powder was dispersed on a conductive, double-sided, adhesive tape on an aluminium sample stub. The particles were then coated with ~15–20 nm gold using a sputter coater (Polaron E5100, Polaron Equipment, Watford, UK) using an electrical potential of 2.0 kV and a current of 20 mA. Several photomicrographs were produced by viewing fields, selected randomly, at several magnifications (Philips SEM501B scanning electron microscope, Eindhoven, the Netherlands).

The particle size distribution of salbutamol base was 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) soy lecithin (Laboratory Grade, BDH Laboratory Supplies, Poole, Dorset, UK) in hexane (Rathburn Chemicals Limited, Walkerburn, Scotland) was prepared to which approximately 5 mg of drug 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. The suspension of drug was maintained by using 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 determined using the method described above with the following modifications. The Malvern 2600 sizer was fitted with a 100 mm lens and the excipients were suspended in a liquid medium of 0.1% (w/v) span 80 (BDH Lab Supplies, Loughborough, UK) in butan-1-ol (BDH Lab Supplies, Loughborough, UK). The particle size distributions of the five excipients suspended in this liquid medium were calculated according to an independent model of analysis.

2.3. HPLC analysis of salbutamol base

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.

2.4. A model glass inhaler

A glass device was designed and made by King's College London (Srichana et al., 2005) to introduce the powder blends into the selected air flows drawn through inertial impactors and/or other sizing devices. The device included a 29/32 Quickfit™ socket fitting to fit the glass throat of a conventional twin stage impinger (BP 2002) or alternatively with a glass spacer attached to a Perspex™ box (Fig. 1). This glass device was employed to examine the *in vitro* aerosol performance of salbutamol blends, independent of the influence of any commercial dry powder inhaler. The glass device was purposely designed to have a low air resistance which allowed a flow rate of >100 l min^{-1} to be generated at a pressure drop of 4 kPa so that the performance of the formulations could be highlighted. However, it was accepted that the blends' performance would be dependent upon the conditions, e.g. turbulence, produced in this simple device.

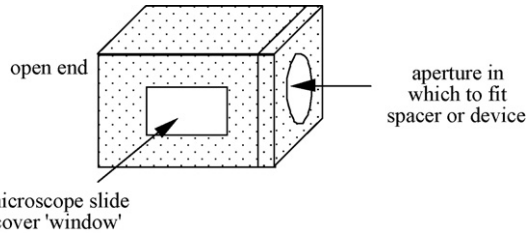

Fig. 1. Schematic diagram of the Perspex™ box used for the testing of dry powder aerosols by laser diffraction.

2.5. Particle size measurement of aerosolised powder by inertial impaction

A cascade impactor (Andersen Samplers Inc., Atlanta, Georgia, USA) was assembled with a glass adaptor (14/23 male Quickfit™ socket) on top of the preseparator to accept the glass throat of the twin-stage liquid impinger (TSI) (BP 2002). A backup filter (Qualitative 1, Whatman Labs. Div., Maidstone, Kent) was held by a rubber O-ring in the filter holder at the base of the impactor stack. To minimise particle bounce on the plates during testing, the preseparator and collection plates were coated in hexane containing 1% (w/v) silicone fluid 200/100 cps (BDH Laboratory Supplies, Poole, Dorset, UK) and left to air dry in a fume hood.

The glass device was inserted into the throat. Eight 'doses' of salbutamol blend (approximately 200 mg blend in total) were drawn through the apparatus at 28.3 ± 0.5 l min^{-1} 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 airflow through the apparatus had stabilised, the port was uncovered releasing the dose. The pump was switched off after 8 s to allow 4 l of air to be drawn through the apparatus. This procedure was repeated for each of the eight doses.

The apparatus was then disassembled for washing. The preseparator and each of the stages with its associated collection plate were rinsed thoroughly with filtered mobile phase containing internal standard (MPIS). Five solutions (50 ml) were prepared from the washings of the preseparator, stage 0, stages 1 and 2, stages 3 and 4, and stages 5–7 with the backup filter. Salbutamol was extracted from the backup filter by immersion in 20 ml MPIS and sonication for 3 min. The glassware was also rinsed with MPIS and solutions prepared using the washings from the glass device (50 ml) and from the throat and adaptor

(100 ml). The salbutamol content in each solution was determined using the HPLC assay described above. The fine particle fraction (<5 μm) was obtained by summing drug deposition on stages 3–7 (with filter) and calculating this as a percentage of the total dose recovered from all washings.

2.6. Particle size measurement of aerosolised powder by laser diffraction

2.6.1. The holding chamber for aerolised powder

An open-ended Perspex™ box with parallel sides (Fig. 1) was constructed from Perspex™ sheets (approximately 4 mm thick) to hold the aerosolised powder and permit the powder to be measured by laser diffraction. One end of the box was left open and the other covered leaving an aperture (approximately 15 mm diameter) to accept an inhaler device directly or via a glass spacer. The Perspex™ did not allow sufficient transmission of light from the laser for the detector to measure an appropriate signal level after the beam had passed through the box. Two windows were therefore cut on opposing sides of the Perspex™ box and covered internally with microscope cover slips (25 mm × 45 mm).

The box was positioned as close to the Malvern lens as possible with the central axis of the box lying approximately 25 mm from the lens. It was important to work within the operational distance of the lens as vignetting, or loss of scattered light at high scattering angles, can occur as the distance from lens to particles is increased. The effects of this loss were most apparent in the computation of the distributions involving fine particles since these particles are detected by light scattered at wide angles. Changes in the size distribution for particles 2–6 μm have been measured when the distance from lens to particles is increased (Clark, 1995).

The box was set up in-line with the sizing apparatus, as shown in Fig. 2, to provide a controlled constant airflow through the equipment. A glass spacer was included to help aerosolise the powder blend before the measurements were made (Olsson et al., 1988). The laser beam of the Malvern 2600 sizer fitted with a 63 mm lens was directed centrally through the box windows, positioned to give minimal disturbance to the instrument alignment and background noise. Air flow rate was controlled by a variable vacuum pump connected downstream of a TSI glass throat and suitable inertial impactor. At the entrance to the Perspex™ box an external trigger pulse sensor (Malvern Instruments Ltd., Malvern, Worcs., UK) was positioned to detect the presence of the passing aerosol and hence initiate size measurement automatically if required.

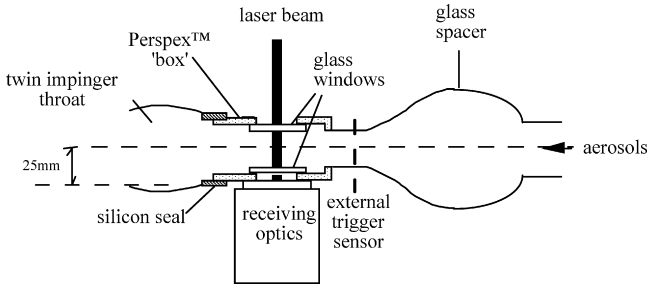

Fig. 2. Schematic plan view of the apparatus employed to size dry powder aerosols by laser diffraction through the Perspex™ box.

2.6.2. 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 Perspex box or throat apparatus. The monodisperse aerosol generating equipment (MAGE) employed was a modified Sinclair-La Mer generator (Sinclair and La Mer, 1949) with a forced air circulation thermostat and gas flow by-pass (Horton et al., 1991). In this study the nuclei were produced by atomisation of an aqueous solution of sodium chloride (0.9 g l^{-1}) using a nitrogen flow rate of 2101 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 from a 'cold start' after allowing the system to equilibrate 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 the 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 to waste by means of a modified vacuum cleaner containing a filter. 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 the calculated geometric mean diameters (GMD) and their related geometric standard deviations (GSD). The GSD (σg) is a convenient index of the spread of the particle size distribution. Although there is no hard and fast rule governing monodispersity, it is generally accepted that an aerosol distribution with GSD < 1.2 is monodisperse (Willeke and Baron, 1993).

To test the validity of sizing fine particles through this apparatus, measurements were made by laser diffraction of MAGE-generated monodisperse stearic acid particles. Particles of stearic acid were generated at temperatures of 180, 200, 220, 240 and 260 °C to create particles of different GMDs within the particle size range of micronised salbutamol. Background readings were taken over 2000 sweeps before each sample measurement. The monodisperse particles were sized according to three different methods (A, B and C):

A. Particles issuing directly from the MAGE were simply diverted through the path of the laser beam without any intervening apparatus and sized under manual instruction over 200 sweeps of the diffraction detector elements.
B. Particles were drawn through the Perspex™ box apparatus at 60 l min^{-1} and sized under manual operator instruction over 200 sweeps.

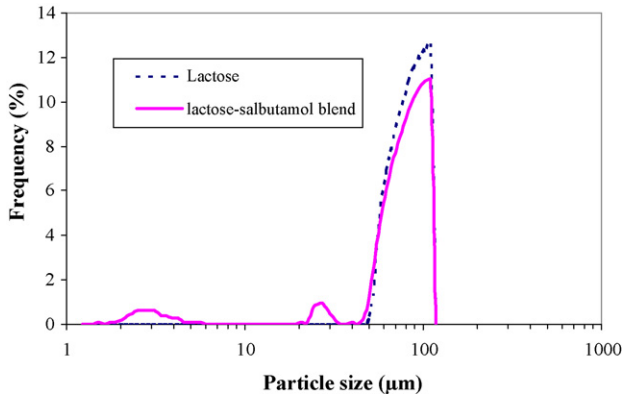

Fig. 3. Particle size distributions based on an independent model of an aerosol dose of the lactose (control) and the blend of salbutamol base/lactose at 60 l min^{-1} through the Perspex^{TM} box apparatus, measured by laser diffraction.

C. Particles were drawn through the Perspex^{TM} box apparatus at 60 l min^{-1} and sizing over five sweeps triggered externally by a pulse sensor (simulating a discrete powder dose).

Six particle size measurements were made over approximately 10 min of aerosol generation at each temperature by each of the three methods (A, B and C). The sizing data were presented as log-normal distributions.

2.6.3. Sizing of aerolised salbutamol blends

Doses of the blend (approximately 27 mg) were dispensed into the sample port of the glass device and introduced into the air through the Perspex^{TM} box apparatus. Method C was employed to size the aerosols using the external trigger and the shorter time of measurement over five sweeps. This time of measurement was selected to minimise exposure to potential background interference. Once the flow had stabilised after a few seconds, the port was uncovered to allow the powder to be drawn into the air flow. The aerosol was drawn through the spacer, triggering the initiation of particle size measurement. Measurements of 10 aliquots at each air flow rate were taken for each salbutamol base blend and each excipient alone (control). Constant flow rates of 28.3, 40, 60 and 80 l min^{-1} were employed to generate the aerosols.

Typical particle size distributions (based on an independent model) of the lactose and the blend of salbutamol base/lactose, drawn through the apparatus at 60 l min^{-1} are shown in Fig. 3. A log-normal particle size distribution was used to characterise the MAGE particles since monodispersity was anticipated and subsequently confirmed. However for the salbutamol blends and the excipients alone an independent model was employed to analyse the light scattering data without assuming a particular distribution model.

3. Results

3.1. Formulation development

The salbutamol base supplied by the manufacturer produced a bimodal distribution with some particles >10 μm (data not shown). The drug was not thought to have an optimal particle size for inhalation and was subsequently remicronised. The remicronised salbutamol base gave a volume median diameter (VMD) of 2.42 μm with a span of 1.01. Thus, the remicronised salbutamol base was of a suitable size to be used in dry powder inhaler 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^{TM}, sorbitol, dextrose and maltose, respectively. Similarity in particle size is likely to be due to the fact that these sugars had been subjected to similar sieving treatments.

The mean recovery of salbutamol base from each formulation was between 98–100% with all relative standard deviation (R.S.D.) values $\leq$3%. All individual recovery data fell comfortably within 90–110% target, suggesting that homogenous blends were obtained with all sugar carriers. These highly consistent data were also indicative of the reproducibility and accuracy of the overall mixing, sampling and HPLC analysis.

3.2. Validation of the laser diffraction method

Table 1 summarises the mean geometric mean diameters (GMDs) and relative standard deviation (%R.S.D., $n=6$) for the monodisperse aerosol sized by each method. All geometric standard deviations (GSDs) of these distributions were less than 1.25 confirming the monodisperse nature of these particles. These particles were largely spherical and inherently monodisperse with GMDs well within the declared sizing limits of the 63 mm optical lens. The measured particle size distributions of these particles were found to be accurate, with errors in GMD measurements in the order of 10%, as reported previously in the sizing of narrow unimodal systems of similar particle size (Annapragada and Adjei, 1996).

Statistical analysis of variance on the GMDs (Minitab^{TM} 10.2 for Windows) revealed no statistical differences in the data obtained by any of the three methods ($p=0.635$, two-way ANOVA). It was concluded that the sizing of the MAGE aerosols (with GMDs 2.18–5.97 μm) was not affected neither by the inclusion of the Perspex^{TM} apparatus nor by the method of sizing.

Table 1
Mean geometric mean diameters (GMDs) from log-normal particle size distributions of MAGE-generated particles obtained by three sizing methods (A, B and C) employing laser diffraction

Temperature (°C)	Geometric mean diameter (GMD) (μm) mean (%R.S.D.)			
	Method A[a]	Method B[a]	Method C[a]	Mean[b]
180	2.17 (0.63)	2.19 (1.17)	2.17 (0.86)	2.18 (0.97)
200	2.53 (2.96)	2.47 (1.70)	2.50 (2.00)	2.50 (2.34)
220	3.46 (1.80)	3.47 (1.84)	3.48 (1.19)	3.47 (1.56)
240	4.97 (1.33)	4.95 (1.60)	4.97 (1.24)	4.97 (1.33)
260	5.99 (1.26)	5.97 (1.51)	5.95 (1.02)	5.97 (1.24)

[a] Mean for $n=6$.
[b] Mean for $n=18$.

Table 2
Mean volume median diameters (VMDs) measured by laser diffraction from 'virgin' excipient particle size distributions (i.e. distributions calculated from all recorded scattered light)

Excipient	VMD (μm) from 'virgin' distribution[a]			Mean[b]
	28.3 l min^{-1}	60 l min^{-1}	100 l min^{-1}	
Lactose	82.3 (5.8)	84.1 (4.8)	85.3 (3.8)	83.9 (4.7)
Zeparox™[c]	83.7 (4.7)	82.2 (5.7)	82.2 (5.9)	82.7 (5.1)
Sorbitol	81.0 (7.5)	82.3 (8.4)	80.5 (7.9)	81.2 (7.4)
Dextrose	80.8 (6.4)	78.6 (9.8)	81.0 (9.0)	80.1 (8.1)
Maltose	83.3 (4.6)	83.3 (3.3)	84.4 (3.7)	83.5 (3.7)

[a] Mean (R.S.D.) for $n = 6$.
[b] Mean (R.S.D.) for $n = 18$.
[c] Spray-dried lactose.

3.3. Analysis of particle size data by laser diffraction for the aerosolised powder

3.3.1. The original, particle size distribution

Fig. 3 shows the particle size distribution of aerosolised lactose and the lactose-salbutamol blend measured by the laser diffraction method. The differences between the blend and control in particle size distribution <50 μm were visibly apparent at a flow rate of 60 l min^{-1}. Two peaks (<5 and 20–30 μm) in the aerosol blend distribution were not present in the control distribution. The particles <5 μm were most likely to be free salbutamol. At lower flow rates, the fraction <50 μm was much reduced and differences between blend and control less obvious. It can be seen in Fig. 3 that some particles were larger than 100 μm in diameter although the lactose had been sieved through aperture sizes between 63 and 90 μm. This is because these lactose particles may have presented in their shortest diameters (the width) to the sieve during sieving process, resulting in the sieved particles being larger than the aperture size (90 μm).

Table 2 details the mean VMDs (with %R.S.D.) determined from six original or 'virgin' distributions for each excipient. By comparing results in Table 2 with those in Section 3.1, it is obvious that the dry dispersion method gave similar results to the wet dispersion method. This similarity further confirmed the validity of the dry dispersion method using the Perspex™ box. However, the dry dispersion method did appear to give slightly larger VMD than the wet dispersion method. This is not surprising since the wet dispersion is expected to break-up any aggregates more efficiently thereby resulting in smaller particles being measured when the results are compared with those from the dry dispersion method.

3.3.2. The recalculated, "kil (1,0) distribution"

In an attempt to highlight any differences at the lower end of the particle size distribution (i.e. particles with volume diameter $d_v < 10$ 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 re-calculated. 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.

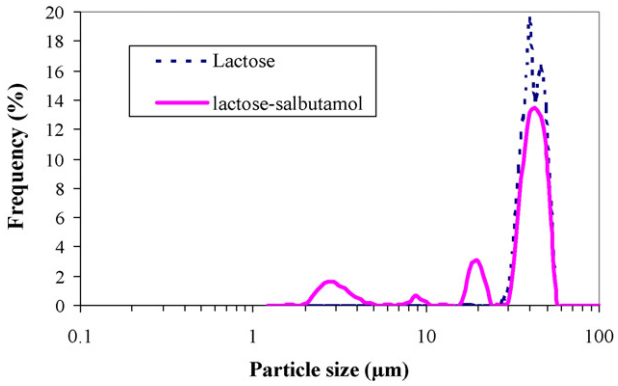

Fig. 4. The kil (1,0) particle size distribution (distributions recalculated from scattered light recorded on only the outer 30 rings of the detector elements) based on an independent model of an aerosol dose of the lactose (control) and the blend of salbutamol base/lactose at 60 l min^{-1} through the Perspex™ box apparatus, measured by laser diffraction.

Fig. 4 shows the kil (1,0) distribution of the results for Fig. 3. It can be seen that the smaller end of the particle size distribution has been amplified in the kil (1,0) distribution. Since the "kil (1,0) distribution" eliminated any interference from larger particles, any small difference in the therapeutically important finer fractions (e.g. < 10 μm) could now be detected by the technique.

Table 3 details the corresponding mean VMDs (with %R.S.D.) determined from the recalculated distributions (kil (1,0) distributions) of the same data. The statistical data revealed that inter-excipient and inter-flow differences in VMDs from the distributions were not significant (three-way ANOVA, $p = 0.186$ and 0.905, respectively).

The VMDs clearly changed when the distribution ('virgin' or recalculated) was modified ($p < 0.001$) but most importantly these differences among excipient controls were not significant when the interaction of excipient with distribution ($p = 0.297$) or excipient and flow rate with distribution ($p = 0.970$) was examined. In other words, the VMDs of the recalculated excipient distributions were no more variable than the VMDs of the excipient 'virgin' distributions. Re-calculating the size distributions and examining the VMDs from the re-calculated kil (1,0) distributions did not reveal further differences among the excipients

Table 3
Mean volume median diameters (VMDs) measured by laser diffraction from recalculated kil (1,0) excipient particle size distributions (distributions recalculated from scattered light recorded on only the outer 30 rings of the detector elements, i.e. with the light recorded on the inner ring omitted)

Excipient	VMD (μm) from recalculated (kil (1,0)) distribution[a]			Mean[b]
	28.3 l min^{-1}	60 l min^{-1}	100 l min^{-1}	
Lactose	43.7 (0.5)	43.8 (0.6)	43.7 (0.5)	43.7 (0.5)
Zeparox™[c]	44.0 (0.4)	43.9 (0.6)	43.9 (0.5)	43.9 (0.5)
Sorbitol	43.8 (0.7)	43.4 (1.8)	43.6 (0.7)	43.7 (1.1)
Dextrose	43.4 (1.0)	43.7 (1.1)	43.6 (0.9)	43.6 (1.0)
Maltose	43.8 (0.5)	43.9 (0.5)	43.8 (0.5)	43.9 (0.5)

[a] Mean (R.S.D.) for $n = 6$.
[b] Mean (R.S.D.) for $n = 18$.
[c] Spray-dried lactose.

that were not already apparent from the 'virgin' size distributions.

Based on the results of this statistical analysis, the distributions of all aerosol blends generated subsequently were recalculated in this manner, i.e. the scattered light on the inner ring of the detector was removed from the recorded data, and the kil (1,0) distribution recalculated according to an independent model. The exclusion of particles larger than 60 μm from the calculation is similar to the use of pre-separator in Andersen cascade impactor for measuring aerodynamic particle size of dry powder aerosol formulations. In a separate study which used laser diffraction to measure fine particle fraction of dry powder aerosol formulations, a pre-separator was used to collect large carrier particles before the particles were allowed to pass through the laser detector (de Boer et al., 2002b). Furthermore, for a technique to compare performance of different formulations or different batches of one formulation, it is not important whether the method generates the "true" values, providing that the measured value is a true reflection of the sample performance and is reproducible. It is therefore justifiable to exclude the particles larger than 60 μm from the calculation in order to highlight any subtle difference in fine particle fraction relevant to aerosol delivery to the lung.

3.4. Sizing of aerosolised salbutamol blends using the laser diffraction

When the distributions of the salbutamol blend aerosols were recalculated in this way, in addition to particles with d_v 30–60 μm which were common to the control powders, finer particles with $d_v < 30$ μm were also revealed in the distribution (as shown in Fig. 4 for the salbutamol/lactose blend). These finer particles appeared to fall into three particle size ranges: particles with d_v <5 μm, particles with d_v 5–15 μm, and particles with d_v 15–30 μm. The original particle size distributions were therefore re-calculated using the same approach in order to highlight the effects of carrier type and flow rate on these three particle size ranges. Fig. 5 presents these data graphically for each of the five salbutamol base blends. The cumulative fraction of particles with d_v <30 μm is also plotted against flow rate in the figure.

For the lactose blend (Fig. 5a) the fine fraction (% <5 μm) appeared to increase with increasing flow rate from that obtained at 28.3 l min^{-1} although the increase only became significant after reaching a flow rate of 60 l min^{-1} ($p < 0.001$, Student's t-test). The increases in fine fraction were not mirrored by marked decreases in the other two size fractions, 5–15 and 15–30 μm, which might have suggested the breakdown of agglomerates of the drug particles in these size ranges. It is more likely that the increase in the percentage of fine particles is due to increased detachment of fine drug from the coarse excipient carrier particles.

The fine particle size fractions measured from the spray-dried lactose blends (Fig. 5b) were significantly lower than those from the regular lactose blends (Fig. 5a) at any selected corresponding flow rate ($p < 0.001$, Student's t-test). A trend of increasing fine fraction with increasing flow rate was observed for this blend but the fraction was only increased significantly over that obtained at 28.3 l min^{-1} when an air flow rate of 60 l min^{-1} was reached or exceeded.

For the sorbitol blend (Fig. 5c) the fine fraction at 60 l min^{-1} was significantly greater than that at 28.3 or 40 l min^{-1} (both $p < 0.001$, Student's t-test) and increased in a similar fashion to that of the lactose blend. This increase in fine fraction from the sorbitol blend is partly accounted for by a decrease in the largest size fraction (15–30 μm) as the flow rate changed from 40 to 60 l min^{-1} ($p < 0.025$, Student's t-test).

Changes in the particle size distribution of the dextrose blend aerosols (Fig. 5d) with changing flow rate were minor and only evident in fine fractions measured once the flow rate had been increased from 40 to 60 l min^{-1} ($p < 0.05$, Student's t-test).

The particle size distributions and changes therein of the maltose blend (Fig. 5e) were very similar to those of the spray dried lactose blends (Fig. 5b), although a significant increase in fine fraction was only achieved by increasing the flow rate from 40 to 80 l min^{-1} ($p < 0.005$, Student's t-test).

Based on these results it was decided to concentrate on the aerosol fraction of the distribution with $d_v < 5$ μm since this was the size range which most affected the aerosol fraction with $d_v < 30$ μm and more importantly this was the size range which is considered to be "respirable". This portion of the distribution would be expected to be more easily compared to data obtained from *in vitro* deposition studies employing inertial impactors. Bearing this in mind, the fine fractions (aerosol particles with $d_v < 5$ μm) from the recalculated particle size distributions (Fig. 5) were replotted in histogram form (Fig. 6). From Fig. 6, the blends containing the following excipients could be placed in the following order of increasing fine fraction: spray-dried lactose < dextrose ≪ maltose < lactose < sorbitol. This trend was replicated at each flow rate.

3.5. Sizing of salbutamol blends by inertial impaction

In an attempt to compare some of the laser diffraction data in Fig. 6 with sizing of the aerosols by more conventional methods, aerosols were characterised by inertial impaction. The *in vitro* deposition studies were therefore conducted using the conventional apparatus of pre-separator, cascade impactor and glass throat. Aliquots of all five salbutamol base blends were drawn through this apparatus at 28.3 l min^{-1}. The particle size distribution data from these studies are detailed in Table 4. The aerosol performance of each set of salbutamol blends was found to be highly excipient dependent. For example, the use of Zeparox™ in the blend markedly reduced the aerosol salbutamol fine fraction generated at 28.3 l min^{-1} in comparison to other blends.

3.6. Correlation between laser diffraction and inertial impaction

In Fig. 7, the fine fractions for salbutamol base 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 ($R^2 = 87.4\%$, $p = 0.02$, ANOVA).

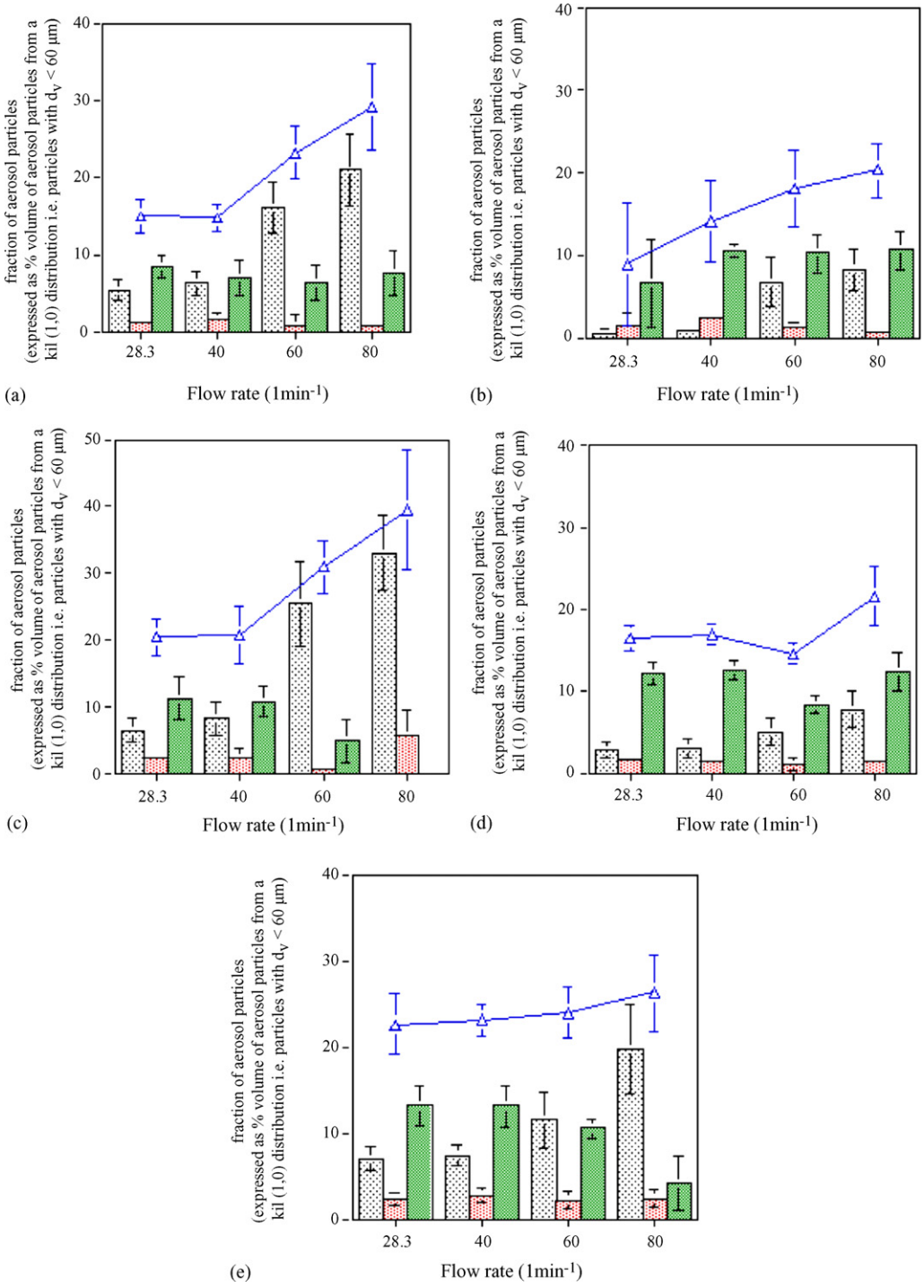

Fig. 5. Characterisation of salbutamol base blend aerosol in constant air flow rates of 28.3, 40, 60 and 80 l min^{-1} by laser diffraction through a Perspex^{TM} box (mean ± S.D., $n = 10$). (a) Crystalline lactose blend; (b) spray-dried lactose (Zeparox^{TM}) blend; (c) sorbitol blend; (d) dextrose blend; (e) maltose blend ((▨) <5 μm; (▩) 5–15 μm; (▩) 15–30 μm; (—△—) <30 μm).

4. Discussion

The differences found in the aerosol performance of the different powder blends most likely arose from the differing forces of adhesion which existed between particles of drug and carrier. A combination of intrinsic physicochemical properties, particle size, shape, surface area, and morphology affects the forces of interaction between drug–drug and drug–carrier particles and these can subsequently change the fine particle fraction of the drug (Telko and Hickey, 2005).

The lactose monohydrate, sorbitol and dextrose monohydrate carrier particles (63–90 μm) were of similar overall shape but the nature of the surfaces differed (Fig. 8). Similar differences in performance between the blends of lactose and dextrose with disodium cromoglicate have been reported (Braun et al., 1996). The lactose particles appeared smoother than the particles of the

Table 4
Salbutamol deposition from different blends in each stage of Andersen cascade impactor (ACI) at 28.3 l min^{-1} (expressed as % of the total salbutamol recovered from the apparatus, mean (S.D.), $n = 5$)

ACI stages	Particle size range (μm)	Crystalline lactose blend	Spray-dried lactose blend	Sorbitol blend	Dextrose blend	Maltose blend
Glass throat		25.0 (5.5)	20.8 (2.5)	23.2 (9.9)	19.1 (3.4)	22.4 (6.9)
Pre-separator	>10	53.4 (3.1)	55.5 (2.9)	45.6 (8.5)	55.8 (3.6)	49.4 (3.8)
Stage 0	9.0–10	12.6 (4.1)	17.6 (1.2)	17.9 (2.8)	17.0 (1.6)	15.9 (2.6)
Stages 1 and 2	4.7–9.0	1.1 (0.9)	3.2 (0.5)	2.3 (0.9)	2.3 (0.3)	3.3 (0.8)
Stages 3 and 4	2.1–4.7	3.5 (0.5)	1.3 (0.1)	4.6 (0.9)	2.3 (0.6)	5.2 (0.6)
Stages 5–8	0–2.1	4.1 (0.6)	1.5 (0.2)	6.7 (1.6)	3.3 (0.6)	3.8 (0.3)

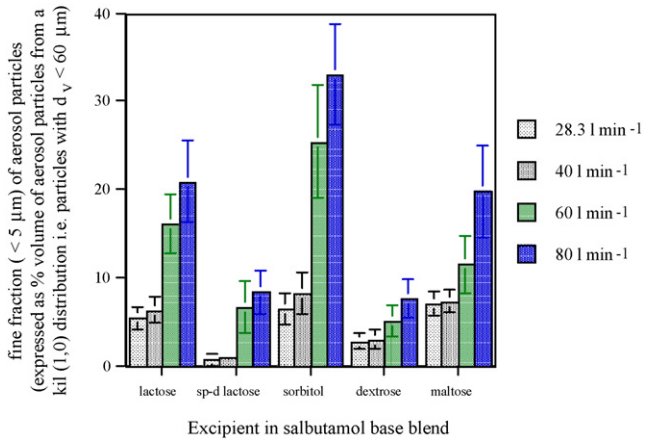

Fig. 6. Aerosol fine fraction (% with $d_v < 5$ μm) of five salbutamol base blends measured by laser diffraction through a Perspex^{TM} box at 28.3, 40, 60 and 80 l min^{-1} (mean ± S.D., $n = 10$), sp-d lactose; spray-dried lactose (Zeparox^{TM}).

other two carriers. The smoother surface of lactose may partially contribute to the higher fine particle fraction of the lactose blend, as compared with the blends containing other sugars, since it has been reported in several previous studies that increasing the surface smoothness of the carrier increased the fine particle fraction of the drug (Zeng et al., 2001).

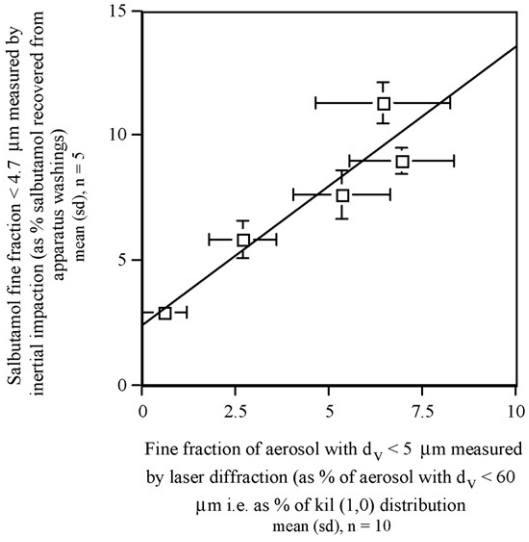

Fig. 7. Salbutamol fine fractions (% <4.7 μm) of five salbutamol base blends measured by inertial impaction plotted against the aerosol fine fractions (% <5 μm) of the same blends measured by laser diffraction at 28.3 l min^{-1}.

The carrier particles of maltose monohydrate and Zeparox^{TM} were more spherical, with improved flow properties by comparison to the other three excipients, but the surfaces of these particles were particularly pitted and indented. The maltose particles resembled spray-dried particles but this could not be confirmed by the suppliers. The adhesive forces between salbutamol and these carriers would be understandably greater as a consequence of the increased surface area available for contact between the two species (drug and carrier), and the aerosol performance, in terms of fine particle fraction, poorer as a result. The deep clefts within the surface of the Zeparox^{TM} particles might be expected to result in a stronger adherence of the drug particles than to the maltose and even flow rates of 60 l min^{-1} were insufficient to generate from the Zeparox^{TM} containing formulation an aerosol with fine particle deposition akin to that of the maltose blend. Similarly poor dispersion from salbutamol blends with spray-dried lactose has also been reported previously (Chawla et al., 1993).

A number of problems exist in the measurement of the drug–lactose blends using the laser diffraction technique, giving rise to potential sources of error. First, the drug and carrier particles differ in shape, refractive index and absorption coefficient. A carrier and drug particle may have the same equivalent volume diameter but give different light-scattering angles, leading to different laser diffraction diameters. Conversely, the same light-scattering pattern from a carrier and a drug particle does not necessarily indicate that they have similar equivalent diameters. Second, particles could exist in the blend in various forms, e.g. discrete active particles, active–active agglomerates, active particles bound to carrier particles, and combined active–carrier agglomerates. Without chemical analysis, it is almost impossible for the laser diffraction to differentiate between the drug and carrier particles. Third, the particle size ranges of the constituent particles (active and carrier) were significantly different and were at the extremes of the declared sizing limits for the 63 mm lens. Combined with the markedly different mass fractions of these blend components (1:67.5 of active:carrier), these factors were likely to account for the greatest errors in distribution sizing. Through the inversion algorithm for scattered light data, the Malvern sizer has a strong tendency to bias the distribution towards one particular mode, usually the larger mass fraction (Annapragada and Adjei, 1996). 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

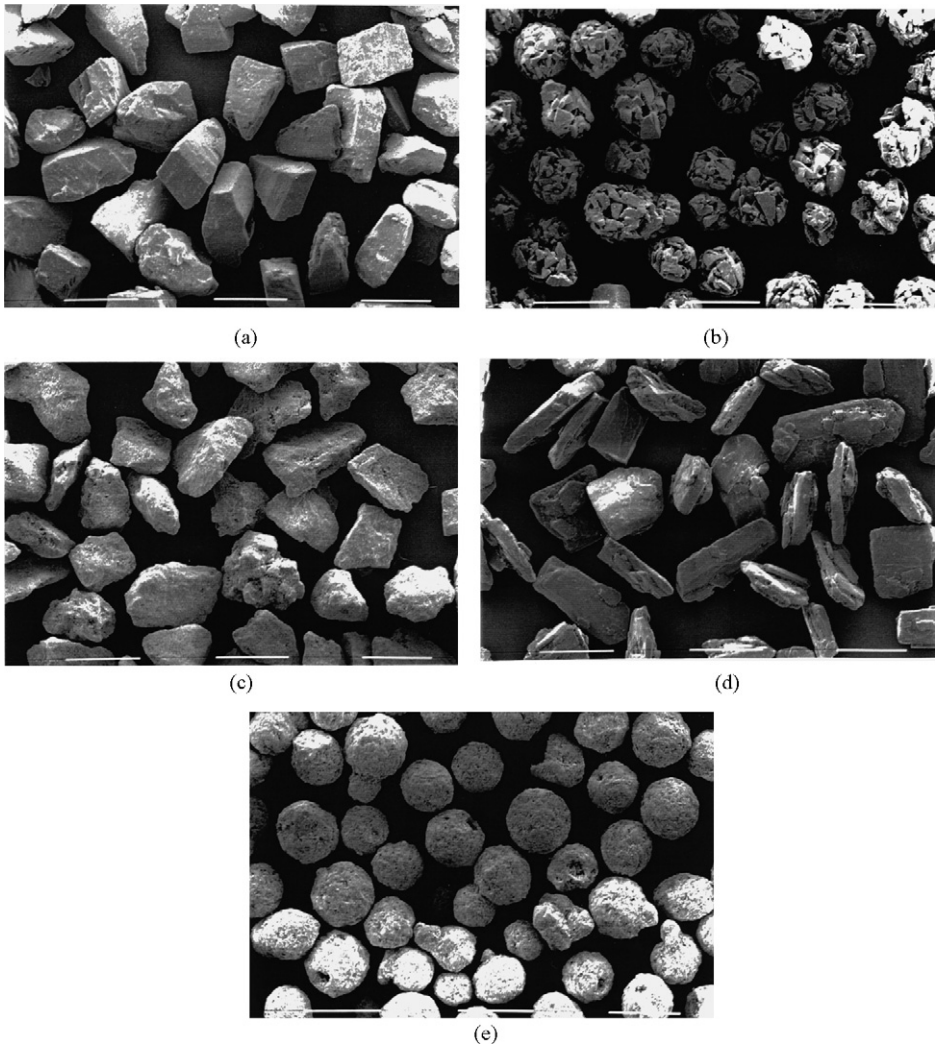

Fig. 8. Scanning electron micrographs of different coarse carriers: (a) crystalline lactose; (b) spray-dried lactose (Zeparox^{TM}); (c) sorbitol; (d) dextrose; (e) maltose.

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. In some cases, the mode of fine particles may not even be present in the fitted distribution if its contribution to the scattered light is too low (Annapragada and Adjei, 1996). This may account for the absence of fines in Zeparox^{TM} blend aerosols at low flow rates by laser diffraction. In order to minimize the effect of larger particles on the measurement of finer particles, the larger carrier particles should ideally be removed before the scattering patterns of the particles are taken (de Boer et al., 2002b).

These points aside, 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 those 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. 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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