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Humidity-induced changes of the aerodynamic properties of dry powder aerosol formulations containing different carriers

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Abstract

This paper presents the findings of two related studies. The aim of the first was to study any changes in the aerodynamic properties of salbutamol base powder formulations when different sugars were used as the carriers, after storage at an elevated humidity (75% RH), and whether any such changes (if any) were related to the physical properties of the carriers. The aim of the second was to investigate whether "ageing", i.e. storage of the carrier, drug and blends under desiccation for more than 2 years, affected the aerodynamic properties of salbutamol sulphate powder formulations. Different formulations were prepared, each containing 1.5% (w/w) micronised salbutamol base or sulphate blended with the sieved fraction (63–90 μm) of one of the following sugars: alpha lactose monohydrate, sorbitol, maltose and dextrose. The salbutamol base blends were then stored unprotected at 75% RH (ambient temperature) and salbutamol fine particle fractions (FPFs) were measured by laser diffraction (LD) (% < 5.2 μm) and a multistage liquid impinger (MSLI) (% < 5.3 μm), following aerosolisation at 100 l min^{-1} from a model glass inhaler, after storage of each formulation at the elevated conditions for 0, 1 and 6 days. Particle morphology and equilibrium moisture content (EMC) of each formulation prior to and after storage were also evaluated. However, the salbutamol sulphate blends containing either "fresh" or "aged" components were only characterized using LD at 60 l min^{-1}. Prior to exposure to 75% RH, the lactose blend was found to give the highest FPF of salbutamol (30% by LD and 37% by MSLI), followed by the sorbitol blend (17% by LD and 29% by MSLI), then by the dextrose blend (15% by LD and 25% by MSLI) and finally by the maltose blend (13% by LD and 13% by MSLI). Exposure to 75% RH for 6 days resulted in a small reduction of salbutamol FPF from the lactose blend but drastic diminution of salbutamol FPFs from other blends. After exposure to the high RH, the lactose blend adsorbed ca. 0.4% whilst each of the other sugars took up larger quantities of water (15–40%) and underwent a marked change in the surface texture of the particles. "Ageing" of the carriers and/or formulations did not seem to alter the aerodynamic properties of the drug. "Ageing" of micronised salbutamol sulphate prior to blending, however, was found to improve the FPF of drug. LD was capable of detecting subtle differences between the various formulations and generated FPF results that correlated with those measured by MSLI.

Keywords: Dry powder inhaler; Formulation; Excipients; Water sorption; Fine particle fraction; Laser diffraction

1. Introduction

Dry powder formulations for inhalation have been the subject of extensive research over recent years (Telko and Hickey, 2005). A typical dry powder aerosol formulation is composed of the micronised drug blended with a coarse carrier. Alpha-lactose monohydrate has been widely used as the carrier due to its ready availability, proven safety profiles and long history of use as a pharmaceutical excipient (Timsina et al., 1994). However, the selection of lactose was not based on any vigorous screening process that would have compared the advantages and disadvantages of a number of potential candidate carriers. Several studies have been conducted trying to establish the suitability of using alternative sugars to replace lactose (Tee et al., 2000; Steckel and Bolzen, 2004). In reality, there are a number of factors a formulator needs to consider carefully before taking the bold step of switching to a new carrier excipient. Apart from potential concerns from the regulatory authorities over the safety profiles of any excipient that has not been tested as extensively as lactose, the long-term stability of the final products containing the new

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excipient has to be well established at an early stage of product development. For a formulation to be suitable, it must remain stable in terms of physicochemical, pharmaceutical and microbiological characteristics during its entire shelf-life. Therefore, during initial formulation development or feasibility studies, the long-term stability profiles of a new formulation have to be reliably predicted. One approach would involve exposing the formulation to elevated humidities and/or temperatures without any protective measures and then analysing the physicochemical and pharmaceutical properties of the formulation at predetermined time intervals. The direct exposure of a formulation to accelerated conditions is a high stress procedure since the formulation will eventually be contained either in the reservoir of an inhaler or in blisters or capsules all of which will provide the formulation some degree of protection against environmental moisture. This approach would allow conclusions to be drawn expeditiously and reliably during initial formulation development without having to wait for the entire length of the stability programmes (usually 2–4 years).

It is well known that the storage of lactose-based formulations in an environment of a high relative humidity (RH) usually reduces the fine particle fractions (FPFs) of the drugs due to increased particulate cohesiveness and adhesiveness within the formulation as a result of moisture sorption (Jashnani et al., 1995). However, few studies have been conducted on the effects of moisture uptake on the aerodynamic properties of formulations using other sugars as the carriers. In addition, it is still not clear how the storage of the drug, the carrier and the blend under desiccation over a prolonged period affects the aerodynamic properties of the drug although in theory, prolonging the contact time between particles is likely to increase interparticulate forces within a formulation (Krishnan et al., 1994). Therefore, the present study investigated whether the aerodynamic properties of various dry powder formulations changed after storage at 75% relative humidity (RH) for different time periods and if any changes were related to the physical properties of the carrier particles. In order to highlight changes due to the excipients, salbutamol base, which is hydrophobic in nature, was employed in this part of the study since salbutamol base might be expected to accumulate water primarily at the surface under the experimental high humidity conditions. Due to its hydrophilic nature, salbutamol sulphate might be anticipated to absorb water within the particles, leading to a change of the aerodynamic properties of the drug which would complicate the interpretation of any changes caused by the excipients. In addition, two carrier entities that were identified as having the least and the most sensitivity to environmental moisture were investigated in more detail to determine to what extent the aerosol performance could be compromised by "ageing", i.e. prolonged storage at 0% RH, and to examine the components of the blend affecting this potential change in performance. Salbutamol sulphate was employed in this study since it is the most commonly used form of salbutamol in dry powder aerosol formulations and it is more susceptible to changes in moisture content of the air than the base. The aerodynamic properties were investigated using both a compendial technique based on inertial impaction and a laser diffraction technique. The inertial impaction technique was employed to generate data that would comply with the pharmacopoeial and regulatory requirements whilst the laser diffraction technique was used to obtain more detailed information into the performance of formulations and to identify any subtle difference in formulation that could have a profound impact on the quality of the final products.

2. Materials and methods

2.1. Materials

Micronised salbutamol base and sulphate (Allchem International, Maidenhead, UK), regular grade α-lactose monohydrate (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 as indicated. Chemicals and solvents used were *p*-hydroxybenzoic acid ethyl ester (ethyl paraben) (Sigma, Poole, UK), methanol (HPLC grade) (Rathburn Chemicals Ltd., Walkerburn, Scotland), ammonium acetate (HPLC grade, BDH Lab Supplies, Loughborough, UK) and distilled water (MilliQ grade, Millipore, Watford, UK).

2.2. Formulation development

2.2.1. Preparation and characterisation of salbtuamol and excipients

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

The particle size distributions of the salbutamol base and sulphate were determined in a 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 (BDH Laboratory Supplies, Poole, Dorset, UK) in hexane (Rathburn Chemicals Limited, Walkerburn, Scotland) 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. Suspension 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 measurements 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 (BDH Lab Supplies, Loughborough, UK) in butan-1-ol (BDH Lab Supplies, Loughborough, UK). The particle size distributions of the four excipients suspended in this liquid medium were calculated according to an independent model of analysis.

The particle surface topography and texture of the excipients before and after exposure to the elevated humidity 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, 20 mA. Several photomicrographs were produced by viewing fields, selected randomly, at several magnifications.

2.2.2. Blending process

Salbutamol base or sulphate (3.0 g) was blended with each excipient (202.5 g) to obtain a target concentration of 1.46% (w/w) according to the following protocol. 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 whole mixing 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.

2.2.3. Storage of various salbutamol base blends at 75% RH

The four salbutamol base blends and their excipient controls (lactose, sorbitol, dextrose and maltose) were stored either at room temperature in a sealed desiccator, or at room temperature under humid conditions of 75% RH in a sealed cabinet for up to 6 days. Samples were removed for examination after storage times of 24 h and 6 days.

The conditions of high humidity were created in the cabinet by a saturated aqueous solution of sodium chloride (with excess salt present), with the humidity being monitored using a small hair hygrometer within the cabinet. The relative humidity level maintained by a saturated solution of this particular salt (NaCl) was not affected by small fluctuations in room temperature over the storage time.

2.2.4. Preparation of fresh or aged salbutamol sulphate formulations

Three different samples of excipient (lactose and sorbitol) were prepared for blending. (N.B. The suffix 'e' represents 'excipient'.): (a) OLDe, 63–90 μm fraction which had been stored for 2 years at 0% RH; (b) NEW(1)e, 63–90 μm fraction which was freshly prepared from the original stored batch used in (a) for either lactose or sorbitol; (c) NEW(2)e, 63–90 μm fraction which had been freshly prepared from a new batch of sorbitol and stored for 14 days at 0% RH. The storage history of the new sorbitol batch at the suppliers was unknown but following its receipt at King's College London it was stored at 0% RH prior to preparation of the 63–90 μm sieve fraction.

Two different samples of salbutamol sulphate were prepared for blending. (N.B. The suffix 'd' represents 'drug'.): (a) OLDd, remicronised sample from original batch and stored for 2 years at 0% RH with a volume median diameter (VMD) 2.58 μm; (b) NEWd, sample from the original batch which was freshly remicronised. Particle size determination by laser diffraction of the remicronised sample gave a value for the VMD of 2.26 μm. A matrix of drug/excipient blends were freshly prepared (denoted blending "NEWb") from the components above, according to the blending method mentioned above.

2.3. Determination of moisture uptake by carrier excipents

The moisture determinations were carried out according to guidelines on the determination of equilibrium moisture content (EMC) (Handbook of Pharmaceutical Excipients, 1994a) and determination of loss on drying (LOD) (USP XXII, 1989). Two aliquots (approximately 200 mg) of each excipient (63–90 μm) were weighed accurately (w_1) on a 5-figure balance (Sartorius GmBH, Guttingen, Germany). One sample was placed uncovered in a sealed cabinet for 6 days to equilibrate at room temperature (20–23 °C) at 75% RH. The humidity conditions within this cabinet were monitored by a small hair hygrometer. After storage for 7 days, this sample was sealed, removed and reweighed (w_2). The second sample was used to determine the initial moisture content of the excipient (control) by measuring the 'loss on drying (LOD) to constant weight' (USP XXII, 1989). A drying temperature of 70 °C was selected as this ensured that the temperature did not exceed the lowest melting point (mpt) of any of the excipients (i.e. dextrose with a mpt of 83 °C being the lowest). After overnight drying the samples were sealed, removed and reweighed (w_3). The EMC of each excipient was determined in triplicate. The EMC of each excipient was calculated as follows:

$$\text{EMC of control excipients}_{(\text{relative to LOD wt})} = \frac{w_1 - w_3}{w_3} \times 100 \quad (1)$$

$$\text{EMC of excipients at 75\% RH}_{(\text{relative to LOD wt})} = \frac{w_2 - w_3}{w_3} \times 100 \quad (2)$$

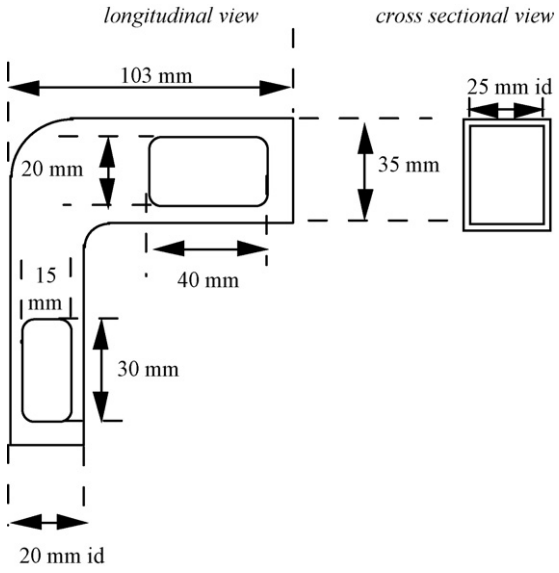

Fig. 1. Schematic diagram of the metal throat (dimensions not to scale).

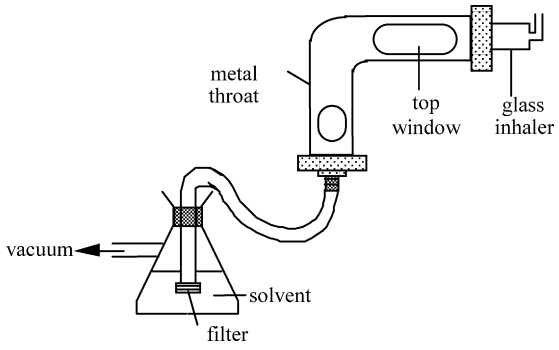

Fig. 3. Schematic diagram of the apparatus used to generate aerosols of salbutamol blend under constant air flow for characterisation by laser diffraction.

2.4. HPLC analysis of salbutamol base or sulphate

Salbutamol base or sulphate 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, FL. USA) 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.

2.5. Sizing of aerosolised salbutamol base and sulphate blends by laser diffraction

A method was adopted to characterise the particle size distributions of various dry powder aerosol formulations using laser diffraction (Zeng et al., 2006). A metal throat was designed and made in-house to receive the aerosol cloud generated from each formulation and permit particle sizing to be made by a Malvern 2600 laser diffraction sizer (Fig. 1). A Teflon™ entrance seal to the metal throat was adapted to accommodate a model glass inhaler. Two model glass inhalers were constructed in-house to introduce the powder blends into either the impinger or the metal throat at the predetermined flow rate (Fig. 2). The glass inhaler was attached to the metal throat through which the blend aerosols were drawn under a 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 so 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 inhaler.

The sample (approximately 30 mg) was loaded into the inhaler sample port, which was then occluded. The vacuum pump set up to pull the calibrated flow rate was started. Once the flow had become steady after a few seconds, the background measurement was initiated over 1000 sweeps of the diffraction detector elements. After the sample measurement command had been manually initiated to be over 200 sweeps of the diffraction detector elements, the sample port was uncovered to release the sample into the air flow. A high flow rate of 100 l min^{-1} was selected for the blends stored at 75% RH in an attempt to generate sufficiently high fine particle fraction (FPF) values for

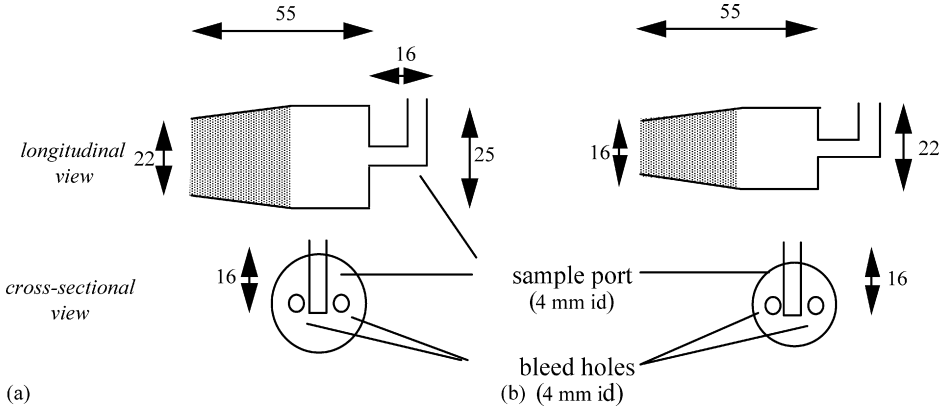

Fig. 2. Diagram of glass inhaler device showing the dimensions of (a) the large version which fitted into the glass throat of a twin stage impinger, and (b) the small version which fitted into the teflon entrance seal of the metal throat.

statistical comparison since FPFs were expected to be reduced considerably after storage at the elevated conditions. In contrast, a medium flow rate of 60 l min^{-1} was selected for the blends stored under desiccation since it was considered to be suitable for the blends that had not been exposed to elevated humidities. 10–15 samples of each blend were examined and the mean fine fraction for each blend calculated from particle size distributions. This was repeated for each storage condition. All aerosol samples were measured on the same day. Each excipient alone (control) was sized in this way following storage under identical conditions to those of the test blends.

2.6. Sizing of aerosolised salbutamol base blends by inertial impaction

Aerodynamic particle size of salbutamol base of each blend was also determined using a multistage liquid impinger (MSLI, Astra Draco, Lund, Sweden) fitted with a twin stage liquid impinger (TSLI) glass throat at a constant air flow of 100 l min^{-1}. The mobile phase (20 ml) containing the internal standard (MPIS) was introduced into each of the first four stages of the impinger. The larger glass inhaler (Fig. 2a) was inserted into the throat of the impinger. Each dose of blend (approximately 200 mg blend) was filled into the sample port of the inhaler. This port was covered by the operator's finger and the pump was switched on. Once the airflow through the apparatus had stabilised, the port was uncovered thus 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 the eight doses had been released, each stage of the impinger was washed individually using the MPIS and the washing solution was then made up to a fixed volume (50 ml) with the same solvent for assay. The concentration of salbutamol in each of the samples was analysed using the HPLC method outlined above. Each blend sample was tested in triplicate.

3. Results

3.1. The active and carrier particle size distribution and blend homogeneity

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 VMDs (and associated spans) of 2.42 μm (1.01) and 2.58 μm (1.05), respectively. Thus, the remicronised salbutamol base and sulphate were of a suitable size to be used in dry powder aerosol formulations. The particle size measurements of the sieved fraction (63–90 μm) of each sugar produced VMDs of: lactose 81.0 μm (0.7), sorbitol 75.5 μm (0.6), dextrose 78.2 μm (0.8) and maltose 80.9 μm (0.5). It was therefore found that the different types of sugar produced particles after sieving with a similar mean size and span of distribution.

The mean recovery of salbutamol base and sulphate from each formulation was between 98 and 101% with all relative standard deviation (R.S.D.) values ≤ 3%. All individual recovery data were comfortably within 90–110% target, suggesting that homogenous blends were 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.

3.2. Aerosol fine fractions of salbutamol base blends by laser diffraction

Samples of the excipients alone (controls) were drawn through the metal throat. The control particle size distributions indicated that there was no evidence of fines (e.g. <5 μm) at any of the flow rates used. By comparing the particle size distributions of the control and its respective blend containing salbutamol, the differences 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 (data not shown). The particles <5 μm were considered most likely to be free salbutamol. 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 re-calculated 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.

The VMDs of the excipient controls stored under desiccation or at 75% RH for 24 h and 6 days (Table 1), were not found to be statistically different ($p = 0.195$, ANOVA). It could be concluded therefore that no measurable change in the excipient control particle size distribution (as described by the mean

Table 1
Volume median diameters (VMDs) (taken from laser diffraction kil (1,0) particle size distributions) of excipient controls stored under desiccation and stored at 75% RH for 24 h and 6 days before being aerosolised at 100 l min^{-1} (mean (S.D.), n = 10–15)

Excipient	Excipient stored under desiccation (μm)	Excipient stored at 75% RH for 24 h (μm)	Excipient stored at 75% RH for 6 days (μm)
Lactose	43.8 (0.2)	43.6 (0.2)	43.8 (0.2)
Sorbitol	43.4 (0.6)	44.1 (0.1)	44.3 (0.6)
Dextrose	43.6 (0.4)	43.6 (0.2)	43.6 (0.2)
Maltose	43.9 (0.1)	43.9 (0.2)	44.1 (0.2)

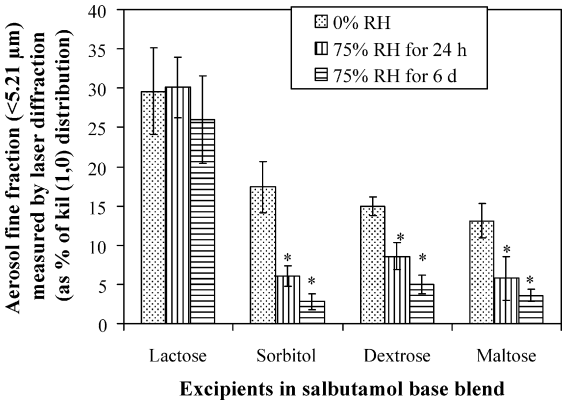

Fig. 4. Aerosol fine fraction (<5.21 μm) measured by laser diffraction at 100 l min^{-1} from salbutamol base blends which had been stored under desiccation, at 75% RH for 24 h, and at 75% RH for 6 days (mean (S.D.), $n = 10$–15). *Significant difference in fine fraction from that of desiccated sample ($p < 0.05$, Student's t-test).

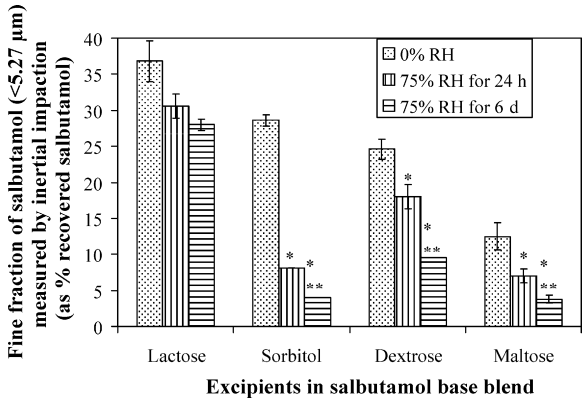

Fig. 5. Fine fraction of salbutamol base (<5.27 μm) measured by MSLI at 100 l min^{-1} from salbutamol base blends which had been stored under desiccation, at 75% RH for 24 h, and at 75% RH for 6 days (mean (S.D.), $n = 3$). *Significant difference in fine fraction from that of desiccated sample ($p < 0.05$, Student's t-test); **Significant difference in fine fraction from that of 75% RH sample for 24 h ($p < 0.05$, Student's t-test).

VMD) was detected by this technique following storage of any of the excipients at 75% RH for up to 6 days. Any measurable differences in the fine fraction distribution of each test blend, with storage under similar conditions, would most likely be due directly to a change in the fine particle fraction itself rather than indirectly through a change in the size of the excipient carrier particles.

Fig. 4 presents graphically the mean fine fractions measured in each formulation from blend samples stored at room temperature under desiccation, at 75% RH for 24 h and at 75% RH for 6 days. Following 24 h storage of each blend at 75% RH, there was a significant reduction in measured mean fine fraction, relative to that measured from the corresponding blends stored under desiccation, for each blend (with the exception of the lactose blend) ($p < 0.05$, Student's t-test) (Fig. 4). The reduction was greatest for the sorbitol blend, for which a mean reduction of approximately 70% was noted after storage at 75% RH for 24 h. Although there was an apparent reduction in mean fine fraction of the lactose blend following storage at 75% RH, the difference was not significant even after 6 days ($p > 0.1$, Student's t-test).

3.3. Aerosol fine particles fractions of salbutamol base blends by inertial impaction

The fine fraction of salbutamol base can be considered as that salbutamol base which was deposited on both stages 3 and 4 of the impinger expressed as a percentage of the total salbutamol base recovered (%, <5.27 μm at 100 l min^{-1}). The mean salbutamol base fractions obtained in this way are displayed in bar chart form in Fig. 5. It was found that, following storage of the blends at 75% RH for 24 h, the salbutamol base fine fraction for each blend (with the exception of the lactose blend) was significantly reduced ($p < 0.05$, Student's t-test) from that measured in aerosols of the blend stored under desiccation. This reduction was most noticeable in the sorbitol blend aerosols. Further storage for 5 days at 75% RH produced a further reduction in mean fine fraction ($p < 0.05$, $n = 3$, Student's t-test).

Overall the differences in blend fine fractions (<5.27 μm) detected by inertial impaction were very similar to those (<5.21 μm) detected by laser diffraction (Section 3.2). In particular both techniques revealed an overall trend in fine fraction differences among different blends (in order of increasing fine fraction) of maltose blend < dextrose blend < sorbitol blend < lactose blend or maltose blend ≤ sorbitol blend < dextrose blend < lactose blend after storage under desiccation or at 75% RH for 24 h, respectively. There was a drastic reduction in sorbitol blend aerosol fine fraction but a small change in lactose blend aerosol fine fraction with storage at 75% RH for 24 h.

3.4. Moisture uptake by different carrier excipents

The results of EMC determinations for excipients stored at 75% RH for 6 days and the excipient controls stored under desiccation are detailed in Table 2. The relative increase in excipient EMC under these humid conditions is also shown. As can be seen from Table 2, no real change was found in EMC of lactose with storage at 75% RH for 6 days ($p > 0.80$, Student's t-test), but the remaining three excipients each displayed a significant increase in moisture content under these storage conditions ($p < 0.05$, Student's t-test). Having been stored desiccated, the EMC of the control sorbitol sample was unexpectedly high (12.5%) compared to those obtained for the other excipients.

Table 2
Equilibrium moisture contents (EMCs) of carrier excipients stored under desiccation (controls) and at 75% RH for 6 days (mean (S.D.), $n = 3$)

Excipient	Storage at 75% RH for 6 days	Storage under desiccation (control)	Increase in % mean EMC
Lactose	2.7 (1.2)	2.3 (2.1)	0.4
Sorbitol	38.6 (3.5)	12.5 (3.1)	26.1
Dextrose	15.5 (2.8)	4.2 (2.1)	11.3
Maltose	32.8 (3.2)	3.9 (1.9)	28.9

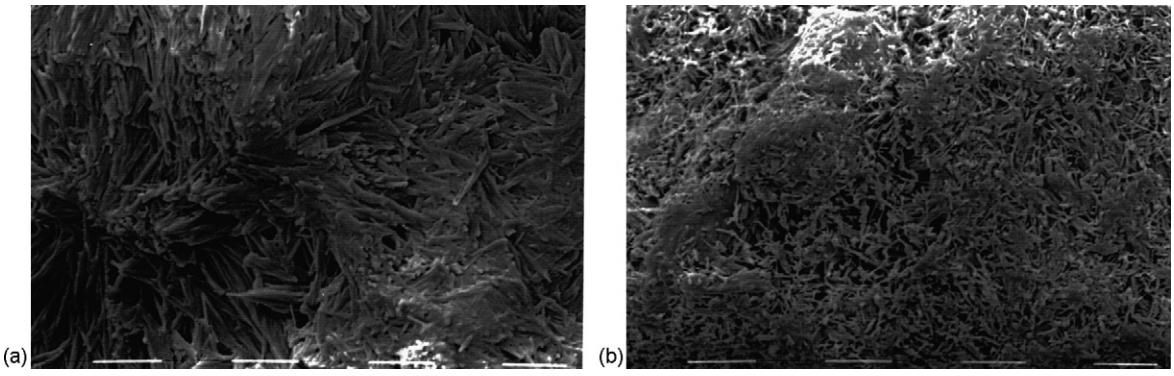

Fig. 6. Scanning electron micrograph of the surface of a sorbitol particle found within a sample of the excipient stored (a) at 75% RH and (b) under desiccation for 6 days (bar 10 μm).

3.5. Humidity-induced changes of morphology

The reduction in fine fraction generally observed with storage of these blends at 75% RH was most likely caused by a change in the surface characteristics of the excipient particles and/or the salbutamol particles through their adsorption of water. This water adsorption by the particles may simply have increased the forces of adhesion between excipient and drug particles through a surface tension effect, but it may also have caused physical changes to occur on the particles' surface. To examine the surface of the blend particles more closely, scanning electron micrographs (SEMs) were taken of each blend before and after storage for 6 days at 75% RH. The overall size and shape of the drug-coated excipient particles were examined to detect any changes due to humidity. Using higher magnification, the surface of the drug-coated excipient particles and the size and shape of the adhered drug particles themselves could be examined.

The SEM of the sorbitol stored at 75% RH for 6 days (Fig. 6) suggested that a structural change in excipient surface also arose in the humid conditions; a change which likely involved the blended salbutamol particles. The drug particles adhered to this excipient surface may have been more firmly attached as a result of the formation of solid bridges akin to those formed during crystallisation changes. Under normal conditions of low humidity, such fine particles tended to adhere to the excipient where the surface contact is greatest, i.e. within indentations or surface irregularities of the excipient (Schmidt and Walter, 1994). Under conditions of high humidity the storage of sorbitol had the effect of increasing surface roughness (Fig. 6a) which may have contributed to the increased adhesion between sorbitol and salbutamol particles. Aerosol fine fractions from dextrose and maltose blends after storage under high humidity were reduced relative to the control (0% RH). However, storage under these conditions appeared to have a smoothing effect on the surface of dextrose particles alone (Fig. 7) and to a lesser extent on the surface of maltose particles alone (Fig. 8).

3.6. Effect of "ageing" of the active, carriers and formulations on aerosol fine fractions

To minimise the time lapse between blend preparation and testing of *in vitro* aerosol performance, the aerosol blends generated at 60 l min^{-1} were sized by the laser diffraction rather than by inertial impaction. As can be seen in Table 3, the fine fractions measured from lactose blends (A–E) were not significantly different ($p = 0.081$, ANOVA). This was as expected for lactose blends, which seemed unaffected by storage conditions of 75% RH. Neither the history of blending (comparison between blends A and B) nor the history of salbutamol sulphate (comparison between blends C and E) was found to affect the fine fraction of the formulation. However, the old lactose (blends A, B and D) appeared to give consistently smaller fine fraction than the new lactose (blends C and E), which was likely to be caused

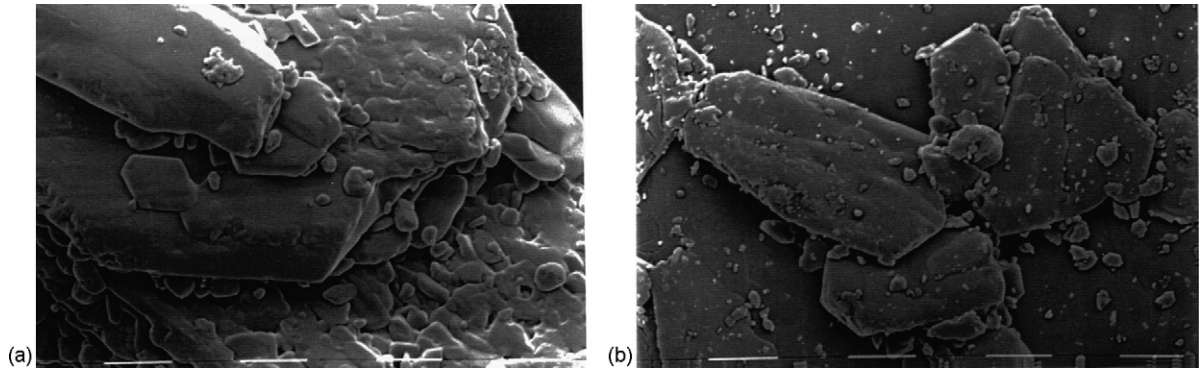

Fig. 7. Scanning electron micrograph of the surface of a dextrose particle found within a sample of the excipient stored (a) at 75% RH and (b) under desiccation for 6 days (bar 10 μm).

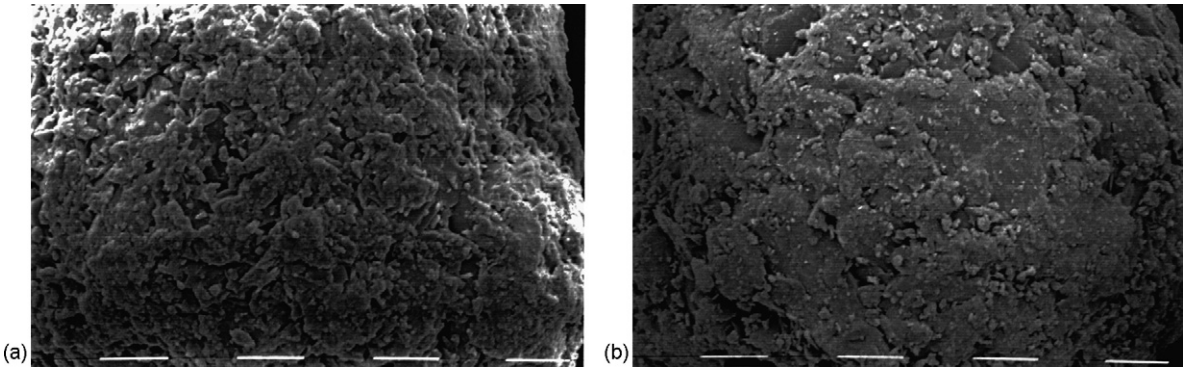

Fig. 8. Scanning electron micrograph of the surface of a maltose particle found within a sample of the excipient stored (a) at 75% RH and (b) under desiccation for 6 days (bar 10 μm).

Table 3
Fine fraction (% <5 μm) of different salbutamol sulphate/lactose blends at 60 l min^{-1} determined by laser diffraction (mean (S.D.), $n = 9$)

Blends	Salbutamol sulphate	Lactose	Blending	Fine fraction (% <5 μm)
A	OLDd	OLDe	OLDb	9.71 (1.9)
B	OLDd	OLDe	NEWb	9.52 (2.0)
C	OLDd	NEW(1)e	NEWb	10.49 (1.6)
D	NEWd	OLDe	NEWb	8.22 (1.2)
E	NEWd	NEW(1)e	NEWb	10.10 (1.8)

by normal batch-to-batch variation rather than by the history of storage of the excipient under desiccation.

As is shown in Table 4, the blends containing the old sorbitol (blends F, G & K) presented a lower fine fraction than the blends containing the new sorbitol. This was probably due to the growth in particle size of OLDe sample of sieved sorbitol in the blend during storage under desiccation. The VMD of the OLDe sorbitol sample was found to increase from 76 μm measured previously to 96 μm, suggesting that the excipient had gone through recrystallisation during storage. When this excipient sample was resieved (NEW(1)e) and subsequently blended (H), an increase in fine fraction of aerosol was measured ($p < 0.001$, ANOVA). Therefore, the reduction in fine fraction of blends G & K that contained the old sorbitol was largely attributable to increased particle size of the carrier.

The effect of blending freshly remicronised salbutamol sulphate (NEWd) with these sorbitol samples was examined in blends K, L and M. Blended in place of the older salbutamol sulphate (OLDd) (blends G, H and J), the use of freshly remicronised drug tended to reduce the fine fraction measured in the corresponding blends (K, L and M).

Table 4
Fine fraction (% <5 μm) of different salbutamol sulphate/sorbitol blends at 60 l min^{-1} determined by laser diffraction (mean (S.D.), $n = 9$)

Blends	Salbutamol sulphate	Sorbitol	Blending	Fine fraction (% <5 μm)
F	OLDd	OLDe	OLDb	5.00 (0.5)
G	OLDd	OLDe	NEWb	4.50 (1.0)
H	OLDd	NEW(1)e	NEWb	6.90 (1.2)
J	OLDd	NEW(2)e	NEWb	7.20 (1.1)
K	NEWd	OLDe	NEWb	3.00 (1.5)
L	NEWd	NEW(1)e	NEWb	5.60 (0.9)
M	NEWd	NEW(2)e	NEWb	4.80 (0.9)

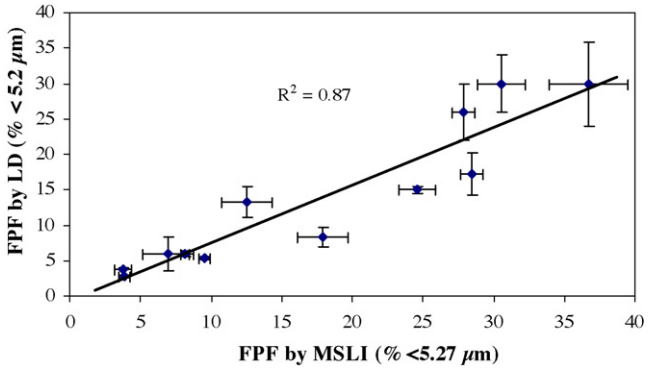

Fig. 9. Relationship between fine particle fraction (FPF) measured by the multistage liquid impinger (MSLI) and laser diffraction (LD).

3.7. Correlation between laser diffraction and inertial impaction

In Fig. 9, 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 ($R^2 = 0.87$).

4. Discussion

The lactose monohydrate, sorbitol and dextrose carrier particles (63–90 μm) were of similar overall shape but their surfaces differed. The lactose particles appeared smoother than the particles of the other two carriers. Since the adhesion of drug to carrier is a surface phenomenon, these morphological differences may have a significant effect on the ability of the air flow to overcome these adhesive forces and generate a dispersed aerosol. It has been reported in several previous studies that increasing the surface smoothness of the carrier particles increased the fine particle fraction of the drug (Zeng et al., 2000). The smoother surface of lactose may have contributed to higher fine particle fraction of salbutamol, when compared with other sugars. In

contrast, the surface of maltose particles were particularly pitted and indented although these particles were spherical, with improved flow properties by comparison to the other three excipients. The maltose particles gave the appearance of having been spray-dried but this could not be confirmed with the suppliers. The adhesive forces between salbutamol and this carrier would be understandably greatest as a consequence of the mechanical interlocking of salbutamol within the clefts of the carrier. Thus, maltose produced the lowest fine particle fraction of salbutamol due to its surface being the roughest. Whilst the initial fine particle fraction from each formulation can be correlated with the surface texture of the carrier of similar size distribution, the stability profile of the formulation is largely determined by how much moisture the formulation takes up and how moisture sorption affects the interparticulate forces within each formulation.

Water can interact with crystalline solids in four different ways: (1) adsorption on the surface of the particles, (2) incorporation into microporous regions by capillary condensation, (3) formation of a crystal hydrate, and (4) deliquescence (Ahlneck and Zografi, 1990). At a low RH, a crystalline material only adsorbs water onto its surface and moisture uptake is a function of specific surface area of the material and the RH of the environment (Van Campen et al., 1983). As the RH is increased, a complete monolayer of water molecules will be formed on the surface of the crystalline solid particles (Hancock and Shamblin, 1998). Since most crystalline solids have low specific surface areas (<1 $m^2\ g^{-1}$), they will adsorb less than 0.1% (w/w) water vapour for a complete monolayer to be formed on its surface. Further increase in the RH will result in dissolution of water-soluble particles in adsorbed water vapour and a saturated solution will form on the surface of the particles (Van Campen et al., 1983; Kontny et al., 1987; Kontny and Zografi, 1995). If the environmental vapour pressure is kept higher than the vapour pressure of the saturated solution, then water vapour will continue to condense on the particle surface to dilute the saturated solution. The diluted solution will allow more molecules to be dissolved in the surface-adsorbed water, eventually leading to deliquescence of the solid. The RH at which deliquescence first occurs is characteristic of the individual sugar and its storage temperature, and it is commonly termed the 'critical relative humidity' (RH_0). Typically poor water-soluble compounds have RH_0 values around 90% at 25 °C and when solubility increases, RH_0 decreases (Kontny et al., 1987; Kontny and Zografi, 1995). Table 5 lists the RH_0 and other physical properties of salbutamol base and various crystal sugars used in this study.

Salbutamol base exists predominantly as crystalline form although it might have a minute amount of amorphous content on its surface induced by micronisation. Since it did not show an RH_0 at all RHs below 95% (Jashnani and Byron, 1996), it would only take up water on its surface at 75% RH, which may lead to crystallisation of any amorphous content on particle surface. It has long been recognized that water condensation on the surface of the drug and/or carrier particles can markedly increase the magnitude of cohesive forces (between micronised powder particles) and adhesive forces (between drug and carrier particles) (Jashnani et al., 1995; Price et al., 2002). Thus, increased cohesiveness and adhesiveness within the formulation were the main causes for the reduction in fine particle fraction observed for all formulations after exposure to 75% RH.

Crystalline lactose monohydrate contains ca. 5% water of crystallisation and has an RH_0 of ca. 99% at 25 °C. After exposure to an RH that is substantially lower than its RH_0, the material is expected to take up moisture on its surface only and this may explain the small difference (ca. 0.4%) in EMC values between the lactose samples stored under desiccation and at 75% RH. Since the surface adsorbed water was less than 0.5%, the measured EMC values of lactose monohydrate (2.3–2.7%, w/w) were attributable to partial dehydration of water of crystallisation during measurement. Due to relatively small water sorption by both the drug and the carrier, there was only a small but insignificant ($p > 0.05$) reduction in fine particle fraction of salbutamol from the formulation containing lactose monohydrate after exposure to 75% RH.

Dextrose displayed an EMC of 4.2% (w/w) after drying at 70 °C overnight, conditions that would have removed all water of crystallisation from dextrose monohydrate. Yet, the EMC of the material stored under desiccation was less than half of the theoretical content (9%) of water of crystallisation, suggesting that the material was a mixture of anhydrous dextrose and its monohydrate. Since this was not a completely pure crystalline material, its particle surface had many micropores that would allow water molecules to penetrate the particles. Water absorption may eventually result in the conversion of anhydrous dextrose into dextrose monohydrate since the hydrated form represents a lower thermodynamic state than the anhydrous form (Hancock and Shamblin, 1998). In addition, since the environmental RH was very close to the RH_0 of dextrose monohydrate, some degree of deliquescence was expected to occur, resulting in extra moisture uptake by the particles. The change of crystal form and dissolution of the sugar into the surface-adsorbed moisture layer might have acted to smooth the particle surface,

Table 5
Summary of the physical properties of various sugars pertinent to moisture uptake

Materials used	Molecular formula	RH_0 at 25 °C	Water of crystallisation	Interaction with moisture
Salbutamol base	$C_{13}H_{21}NO_3$	NA[a]	None	Adsorption; crystallisation of amorphous regions if any
Lactose monohydrate	$C_{12}H_{22}O_{11}{\cdot}H_2O$	~99%	5%	Adsorption; crystallisation of amorphous regions if any
Dextrose monohydrate	$C_6H_{12}O_6{\cdot}H_2O$	~81%	9%	Adsorption; deliquescence
Anhydrous sorbitol	$C_6H_{14}O_6$	~70%	6.2% in $C_6H_{14}O_6{\cdot}(2/3)H_2O$	Adsorption; incorporation into microporous regions;
Spray dried maltose	$C_{12}H_{22}O_{11}$	NA[b]	5% in $C_{12}H_{22}O_{11}{\cdot}H_2O$	formation of crystalline hydrate and deliquescence

[a] Not available for salbutamol base since it did not show a sharp increase in moisture sorption between 0 and 95% RH investigated (Jashnani and Byron, 1996).
[b] Not available for spray dried, amorphous material since it absorbs water at the entire range of RHs.

as was evident from the SE micrographs before and after exposure to the elevated humidity (Fig. 7). However, since these changes happened in the presence of adhered salbutamol particles, they would definitely have increased the interparticulate forces between the drug and carrier particles through complicated mechanisms such as capillary condensation and solid bridging, leading to drastic reduction in fine particle fraction of salbutamol.

After storage under desiccation, the sorbitol was found to contain ca. 12.5% water, which was twice the theoretical content of (6.2%) of water of crystallisation of sorbitol 2/3 hydrate, suggesting that there was free water in the sugar. This was not thought to be possible since the material had been previously stored under desiccation. This high water content was almost certainly the result of moisture uptake by this highly hygroscopic excipient in the laboratory during weighing and control sample preparation. The increase in EMC of sorbitol when stored at 75% RH is, however, in line with that reported in the literature (Handbook of Pharmaceutical Excipients, 1994b). After exposure to 75% RH which is higher than its RH_0, sorbitol can adsorb water on its surface, absorb water into its crystals, convert from anhydrous to hydrated form under the influence of water and become deliquescent. Consequently, the sorbitol was shown to take up large quantities of water (up to 39%) and undergo marked change in crystal shape and surface texture (Fig. 6), either of which can cause dramatic reductions in the fine particle fraction of the drug.

The maltose particles were shown to be spherical in shape with many clefts on the particle surface (Fig. 8), suggesting that it was a spray-dried material. Spray drying usually produces particles that are amorphous, porous and spherical. Such material can take up water on the particle surface and absorb water inside particles even at a low RH (Naini et al., 1998). Large quantities of water vapour can be taken up by spray-dried particles because water molecules are absorbed into the bulk of the amorphous material, rather than being restricted to the particle surface. For instance, in this study maltose was found to take up ca. 33% water after exposure to 75% RH for 6 days. Once the glass transition temperature of the amorphous material is reduced to below room temperature by the absorbed water, then the amorphous material will be converted into crystalline form (Hancock and Shamblin, 1998). This may explain why the maltose particles underwent marked changes in surface textures after exposure to 75% RH (Fig. 8).

Only the aerosolisation of salbutamol base from these stored blends was examined, but its replacement by salbutamol sulphate would be anticipated to generate further differences in aerosol blend performance. Salbutamol sulphate, with a water solubility of 250 mg ml^{-1}, is considerably more water soluble than the base and as such its particle size or cohesiveness would be expected to be more significantly affected by moisture than the base. Increasing the relative humidity has been shown to diminish aerosol performance of micronised salbutamol sulphate more markedly than micronised salbutamol base (Jashnani et al., 1995). Storage of micronised salbutamol sulphate for 12 h at 75% RH was shown to reduce markedly the fine particle fraction of salbutamol after aerosolisation of the pure drug at 60 l min^{-1} from a modified Turbohaler™ (Young et al., 2003). Therefore, environmental moisture would be expected to exert more severe effects on formulations containing salbutamol sulphate than those containing salbutamol base.

The effect of prolonged storage time under desiccation (at room temperature) was examined for lactose and sorbitol blends with salbutamol sulphate, but no changes in performance were detected. The storage of micronised salbutamol sulphate prior to blending, however, was found to improve the performance of the sorbitol blends relative to blends prepared from freshly micronised salbutamol sulphate. The process of micronisation can induce disorder in crystalline materials to create some amorphous material within the structure (Briggner et al., 1994). These amorphous regions can absorb moisture more readily than the surrounding crystalline material and, in a dry powder blend for inhalation, have the potential to detrimentally affect aerosol performance. Furthermore, immediately following micronisation, the drug is expected to contain high electrostatic charges on its surface, which may increase the cohesiveness and adhesiveness of the drug particles. During storage, the static charge will gradually dissipate and the amorphous content may slowly convert into crystalline form. This may explain why the old salbutamol sulphate was more readily dispersed than the new salbutamol sulphate. It is interesting to note that such a phenomenon was more pronounced in the formulations containing sorbitol than those containing lactose. This is an important finding since, to date, there has been little information on how storage and handling history of micronised materials affect the quality of the final products. Further studies on this topic are deemed necessary in order to minimise the batch-to-batch variation commonly associated with commercial dry powder inhaler products.

5. Conclusions

After exposure to 75% RH for up to 6 days, the dry powder formulation containing micronised salbutamol base and the 63–90 μm sieved fraction of sorbitol, or dextrose, or spray dried maltose as the carrier was found to take up a large quantity of water which resulted in a marked change in surface textures of the particles and drastic reduction in the fine particle fraction of salbutamol base. The high moisture sorption by each of these materials was caused by either its critical relative humidity close to or less than the environmental relative humidity, or the material being amorphous and/or highly porous. Therefore, in order for a sugar to be suitable as the carrier for dry powder aerosol formulations, it should ideally be crystalline, non-porous and have a high critical relative humidity (e.g. >90% RH). Even if the hygroscopic formulation is eventually protected against moisture, it is still a challenging task to keep the formulation from moisture during the entire manufacturing process and powder handling, as in the case of sorbitol in the study. In contrast, the formulation containing lactose monohydrate was found to take up moisture on its surface only and there was only a small reduction in fine particle fraction after storage in 75% RH. Moreover, the lactose formulations produced substantially higher fine particle fractions of salbutamol than formulations

containing other sugars as the carrier. Lactose monohydrate is therefore a far better carrier than other sugars investigated in the study. Storage of the carrier and formulation over desiccation for up to 2 years did not seem to alter the aerodynamic properties of the drug. The storage of micronised salbutamol sulphate prior to blending, however, may improve the performance of the blends relative to those prepared from freshly micronised salbutamol sulphate. The laser diffraction technique has been shown to be capable of detecting subtle differences between various formulations and of generating fine particle fraction results that correlated with those measured by inertial impaction. It can thus be concluded also that exposing dry powder formulations to elevated RHs, followed by particle size characterisation using laser diffraction and inertial impaction, is a fast and reliable approach for identification of stable dry powder aerosol formulations.

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