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Research Paper

Influence of Humidity on the Electrostatic Charge and Aerosol Performance of Dry Powder Inhaler Carrier based Systems

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Abstract. To investigate the influence of storage relative humidity (RH) on the aerosolisation efficiency and tribo-electrification of carrier based dry powder inhaler (DPI) formulations using the next generation impactor (NGI) *in vitro* methodology and the electrostatic low pressure impactor (ELPI). Micronised salbutamol ($d_{0.5}$ 1.48±0.03 μm) was blended with 63–90 μm sieve fractioned α-lactose monohydrate carrier and stored at a range of humidities (0–84% RH). The aerosolisation efficiency after storage for 24 h periods was investigated using the NGI. The same experiment was conducted using the ELPI, with corona charger switched off, to measure the net charge vs. mass deposition profile. Significant variations in the aerosolisation efficiency of the formulation were observed with respect to storage RH. In general, the fine particle fraction aerosol performance measured by NGI and ELPI (fraction with mass median aerodynamic diameter <4.46 and 4.04 μm, respectively) followed a positive parabola with aerosol performance increasing over the range 0–60% RH before decreasing >60% RH. Analysis of the ELPI charge data suggested that the micronised salbutamol sulphate had an electronegative charge when aerosolised from lactose based carriers, which was most electronegative at low RH. Increased storage RH resulted in a reduction in net charge to mass ratio with the greatest reduction at RH >60%. The aerosol performance of this binary system is dependent on both electrostatic and capillary forces. The use of the ELPI allows a degree of insight into how these forces affect formulation performances after storage at different RH.

KEY WORDS: aerosol; DPI; dry powder inhaler; electrostatics; ELPI; humidity; inhalation.

INTRODUCTION

Dry powder inhalers (DPIs) have become a popular method of delivering local and systemic medicaments to the respiratory tract. In simple terms, most DPI formulations contain a micronised drug blended with a larger inert excipient such as α-lactose monohydrate. The inert excipient acts as a carrier (resulting in improved flow for metering and packaging), as a diluent and aids removal of drug from the device during patient inhalation. This formulation system requires the drug to be liberated from the carrier during the aerosolisation process to allow respiratory deposition while the carrier, with its higher mass, impacts on the oropharynx and is swallowed.

In general however, the efficiency of this process is relatively poor, with most conventional formulations producing *in vitro* respiratory deposition efficiencies <20% (1).

The primary reason for poor aerosolisation efficiency in DPI systems may be related to the inter-particulate forces between the drug and carrier. Since the micron size drug particles have a high surface area to mass ratio the force available for particle detachment from the carrier during inhalation is relatively small. To put such a statement into perspective, the force acting on a 1 μm spherical particle (adhered to a planar surface such as DPI carrier) in a 10 m s^{-1} laminar air flow (comparable to the axial velocity in a Cyclohaler™ (2)) may be approximated as 2 nN while the adhesion force of the particle-carrier (using empirical calculation (3)) is 100 nN. Although, highly simplified in this case, an understanding of particle interactions in DPI systems is of clear importance in improving respiratory deposition.

It is generally considered that three forces play a competitive part in the adhesion of drug to carrier: ubiquitous Van der Waal's, capillary and electrostatic forces. The interplay of these forces will be dependent on the physical–chemical properties of the contiguous surfaces such as roughness and surface chemistry. Variation in surface roughness for example will alter the contact area between contiguous surfaces and thus alter the total van der Waal's force of interaction. Also variation in roughness may alter the propensity for surface charging or the degree of water condensation. Clearly these factors play an important role and will be influenced by environmental factors such as temperature and relative humidity.

Relative humidity (RH) plays a major role in DPI performance since the adsorption of water on the surface of a powder will have a significant impact on the capillary forces, conductivity and in some cases surface chemistry. Previous studies, investigating the influence of humidity on the aerosolisation performance of 'drug only' DPI systems, have suggested the aerosolisation efficiency to be dependent

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on the physical–chemical nature of the active compound (4–6). For instance, Jashnani *et al.*, reported that the aerosolisation efficiency for different salts of salbutamol was dependent on the salt form and the relative hydrophilic nature of the compounds (4). Similarly, work conducted by Young *et al.*, demonstrated that the aerosolisation performance of different drug molecules (triamcinolone acetonide (TAA), salbutamol sulphate and di-sodium cromoglycate (DSCG)) after storage at different humidities (15–75% RH) could be related to the molecules under investigation (6). For example, DSCG, a highly hygroscopic material, showed the greatest decrease in performance at high humidity, while salbutamol sulphate performance decreased at elevated humidities in excess of 60% RH (6). In comparison, TAA (a hydrophobic steroid) actually showed a small but significant increase in performance which was related to electrostatic charging at lower humidity (6).

Other studies, using drug-carrier formulations have suggested similar findings (7–10). For example work by Maggi *et al.*, suggested lactose-DSCG systems had reduced performance when exposed to elevated storage conditions (10), while Vos *et al.*, suggested similar findings after exposing salbutamol sulphate-lactose carrier formulations to 100% RH (9). Recent work by Young *et al.*, suggested the performance of similar salbutamol sulphate-lactose carrier formulations to significantly decrease at humidities above 60% RH (7).

To gain a greater fundamental understanding of how environmental RH influences particulate interactions, a multitude of bulk powder and individual particle characterisation methods are available. For example, atomic force microscopy (11–15) has been utilised to directly measure the force of adhesion between individual drug particulates as a function of RH, and have been successfully correlated with *in vitro* performance (11). Other methods include, single particle levitation to investigate how RH may affect the physical form during aerosolisation (16), centrifugal models, to assess drug detachment from DPI materials under different environmental conditions (17,18), and more recently bulk electrostatic characterisation (19). Interestingly electrostatic charging in DPI systems is commonly overlooked, and the mechanisms of charging, at different humidity, and its influence on DPI performance is not clearly understood.

As previously discussed, the electrostatic charge associated with a pharmaceutical aerosol may have significant effects on the aerosol performance and subsequent respiratory deposition. Methods of electrostatic measurement, specific to aerosols, include the flow through Faraday pail (20), the electrical single particle aerodynamic relaxation time (E-SPART) apparatus (21), cyclone charging apparatus (22–24), grid probe apparatus (25,26), bi-polar aerosol apparatus (27), and the electrostatic low-pressure impactor (ELPI) (28). In general, these techniques have been successfully used to assess the electrostatic charging characteristics of a range of pharmaceutical aerosol systems. For example, Byron *et al.*, has investigated the magnitude and charge polarity of a series of respirable sized respiratory drugs (20), while Philip *et al.*, utilised the E-SPART to investigate how modification of the surface chemistry of DPI particles influenced charging and aerosol performance (21). In other studies, Bennett *et al.*, (24) and Murtomaa *et al.*, (25,26), investigated the influence of physical properties, such as the addition of fines and size/morphology of DPI carrier materials on tribo-electrification, while Rowley further investigated these systems in terms of device, cyclone air flow and carrier size (23).

Interestingly, although the environmental conditions during previous investigations have generally been carefully monitored, little work has been conducted to investigate the influence of humidity on the charging phenomena with respect to DPI aerosol performance. Indeed, work by Rowley *et al.*, investigated the electrostatic behaviour of pharmaceutical materials with respect to humidity (22), however, only recently have these studies focussed specifically on materials used in DPI formulations (19). A direct study investigating the relationship between *in vitro* DPI aerosolisation performance, tribo-electrification and humidity, to the author's knowledge, remains un-investigated.

As part of an ongoing study, the authors investigate the *in vitro* aerosolisation performance of a conventional DPI carrier formulation, (equivalent to Ventolin™ Rotacaps™ (GSK)), as a function of storage humidity. Furthermore, variations in the drug aerosol performance with respect to RH was further investigated in terms of bulk sorption properties, particle size and the charge distribution in the aerosol cloud using an ELPI impactor.

MATERIALS AND METHODS

Materials

Crystalline α-lactose monohydrate (lactose) (Lactochem® crystals), obtained from Borculo Domo, (Zwolle, The Netherlands) were sieved through a nest of stainless steel sieves to produce a 63–90 μm fraction. Micronised salbutamol sulphate was supplied by Aventis (UK). Water was purified by reverse osmosis (MilliQ, Millipore, Molsheim, France). All solvents were obtained from Biolab (Victoria, Australia) and were of analytical grade.

Particle Size Analysis

Particle size distributions of the 63–90 μm sieve fractioned lactose and micronised salbutamol sulphate was determined using laser scattering (Mastersizer X, Malvern, UK) using a 300RF lens and automated small volume dispersion sampling unit containing chloroform (refractive index 1.444). Approximately 200 mg of lactose or salbutamol sulphate was dispersed in 10 ml of chloroform and sonicated for 5 min, to aid dispersion. The dispersed sample was added drop-wise to the sample cell until an obscuration between 10 and 30% was obtained. Size distributions were based on 2,000 sweeps for each sample, with refractive indexes of 1.533 and 1.553 for lactose, and salbutamol sulphate, respectively. Each sample was analysed in triplicate.

Dynamic Vapour Sorption

Dynamic Vapour Sorption (DVS) was used to investigate the relative moisture sorption into samples of 63–90 μm sieve fractioned lactose and micronised salbutamol sulphate.

Samples (ca. 100 mg) were added to glass sample pans which were placed in the sample chamber of a commercially available DVS (DVS-1, Surface Measurement Systems Ltd, London, UK). Each sample was dried over nitrogen at 0% RH before exposure to 10% RH increments for two 0–90% RH (25°C). Equilibrium moisture content at each increment was determined by a dm/dt of 0.0002% min^{-1}.

Salbutamol Sulphate Analysis

The concentration of salbutamol sulphate in experimental samples was obtained by high performance liquid chromatography (HPLC): Waters 717+ autosampler, 600 pump, 486 detector set at 276 nm, 600 controller with Millennium software and NovaPak 3.9×150 mm C_{18} column (all Waters Ltd, Sydney, Australia). Standards and samples were prepared in purified water. Mobile phase was 60:40 methanol:water containing 0.1% w/v sodium dodecyl sulfate. Linearity was obtained between 0.5 and 12.5 μg ml^{-1} (R^2=0.999) with a retention time of approximately 5 min. Collected samples were diluted appropriately to fit within this region. The limit of detection was 0.05 μg ml^{-1}.

Preparation and Storage of the Blend

A 67.5:1 (w/w, excipient/drug) formulation (approximately 3 g) containing of 63–90 μm sieve fractioned lactose and micronised salbutamol sulphate was prepared in a mortar by geometrical hand mixing. The final blend was transferred to a glass vessel (the powder was approximate 2/3 of the total vessel volume) and mixed for 1 min using a Whirlimixer (Fisons, UK). Finally, the blend was transferred to a Turbula (Bachofen, Basel, Switzerand) which was operated at 46 rev min^{-1} for 30 min. Content uniformity (30 mg samples), taken prior to storage, across the blend indicated a coefficient of variation less than 5% (n=10).

Small (ca. 500 mg) samples of each blend was stored, on open Petri dishes, in separate airtight containers, at 25°C, with saturated salt solutions to produce the following specific humidities (29): phosphorous pentoxide (0% RH); calcium chloride (30% RH); sodium bromide (58% RH); sodium chloride, (75% RH); potassium chloride (84% RH). Humidity in the containers was confirmed using portable humidity probes (Vaisala, Melbourne, Australia). Each sample was stored for 24 h prior to analysis. Based on DVS data and previous particle adhesion studies (12), this was determined sufficient for moisture equilibration.

In vitro Analysis: Next Generation Impactor

The effect of storage humidity on the aerosolisation performance of micronised salbutamol sulphate from 63–90 μm lactose sieve fractioned lactose was determined using Apparatus E, the next generation impactor (NGI) (British Pharmacopoeia). The method followed that specified for DPIs stated in the pharmacopoeia (Appendix XXI F, http://www.pharmacopoeia.co.uk). All *in vitro* measurements were conducted at 60 l min^{-1}, (obtained using a Rotary vein pump and solenoid valve timer) which was set using a calibrated flow meter (TSI 3063, TSI instruments Ltd, Buckinghamshire, UK). Prior to testing, all eight collection cups were coated with silicon oil to eliminate particle bounce and the NGI pre-separator was accurately filled with 15 ml of purified water.

Approximately 30±3 mg of the humidity-equilibrated formulation was weighed into a size 3 gelatine capsule (Capsugel, Sydney, Australia) which was placed into the sample compartment of a Cyclohaler DPI (Novartis, Surrey, UK). The DPI was connected to a mouthpiece adapter, inserted into a united state pharmacopoeia (USP) throat (connected to the NGI), and tested for 4 s at 60 l min^{-1}. Temperature and humidity throughout the testing was 25°C and 45% RH. The entire experimental procedure (capsule weighing and aerosolisation) was conducted rapidly to avoid prolonged exposure to ambient conditions (<5 min), following the same procedure as described previously (7). After actuation, the device, capsule, throat, pre-separator and all sample stages were washed into separate volumetrics using water. Each humidity-equilibrated blend was tested in triplicate and was randomised for storage humidity.

In vitro Analysis: Electrostatic Low Pressure Impactor

The effect of storage humidity on the aerosolisation performance and static charge distribution of micronised salbutamol sulphate from 63–90 μm lactose sieve fractioned lactose was determined using the electrostatic low pressure impactor (ELPI) (28,30). Briefly, the ELPI consists of a 13 stage impactor, which, when operated at 30 l min^{-1}, produces aerodynamic cut-off diameters between 0.03 and 10 μm. In addition, each impaction stage is electrically isolated and connected to a digital Femto ampmeter, which measures current as a function of time. Subsequently, the charge distribution of an aerosol cloud as it transverses the impactor can be measured as a function of aerodynamic diameter and can be correlated with drug mass recovery. Details relating to ELPI design and operation for measuring charge distribution in pharmaceutical aerosols can be found in references by Marjamaki *et al.*, (30) and Glover and Chan (28), respectively.

The ELPI was operated at 30 l min^{-1} (optimum flow rate for the ELPI) with the corona charger switched off. The electrometer range was set to 400 k fA. The measurement procedure followed that of the NGI, however 10 mg of the formulation was investigated in each experiment. The ELPI impaction stages were smaller than NGI stages and a 10 mg formulation mass was chosen as it produced no evidence of impactor 'overload.' Each impactor stage was coated with silicon oil prior to measurement. Each formulation was weighed into size 3 gelatine capsules and loaded into a Cyclohaler before being inserted into a USP throat (connected to the ELPI), and tested at 30 l min^{-1} for 10 s. In comparison to the NGI methodology, testing was conducted after the pump was initiated and the ELPI electrostatic baseline was zeroed. During the sample period the current on stages 1–12 of the ELPI was recorded simultaneously. Temperature and humidity throughout the testing was 25°C and 45% RH. As with the NGI study, the entire experimental procedure (capsule weighing and aerosolisation) was conducted rapidly to avoid prolonged exposure to ambient conditions (<5 min). After actuation, the device, capsule, USP throat and all ELPI stages were washed into separate volumetrics using water. Each

humidity-equilibrated blend was tested in triplicate and was randomised for storage humidity.

Statistical Analysis

Data were subjected to analysis of variance (ANOVA) (Minitab 12.1, Minitab Ltd., Coventry, UK). Significant differences between formulations were analysed using post-hoc multiple comparisons and p values of <0.05 (Fisher Pair wise) were considered to be significant. Unless otherwise stated data is represented in terms of mean and standard deviation.

RESULTS AND DISCUSSION

Particle Size Analysis

Representative particle size distributions of the micronised salbutamol sulphate and 63–90 μm sieve fractioned lactose are shown in Fig. 1.

As seen from Fig. 1, the particle size diameters of the drug and excipient fell within the size range for respiratory delivery and carrier function, with median diameters of 1.48±0.03 μm and 88.78±0.48 μm being observed for salbutamol and lactose, respectively (n=3).

Dynamic Vapour Sorption

A representative water sorption isotherm for micronised salbutamol sulphate and 63–90 μm sieve fractioned lactose is shown in Fig. 2. Analysis of the salbutamol isotherm suggested the data followed a sigmoidal Type 2 curve suggesting multilayer water sorption onto the crystal surface of a nonporous sample (31). In general, the moisture sorption isotherms for both salbutamol sulphate and sieve fractioned lactose indicated reversible moisture sorption (2-cycles), suggesting the materials to be crystalline with no detectable amorphous content. The moisture sorption of water into the salbutamol sulphate sample was greater (at all humidities) than for the sieve fractioned lactose. Reasons for such variation, in nonporous, stable crystalline material maybe

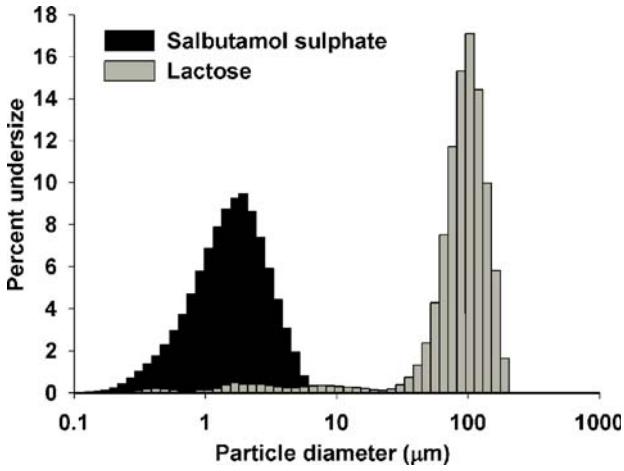

Fig. 1. Particle size distribution of micronised salbutamol sulphate and 63–90 μm sieve fractioned lactose.

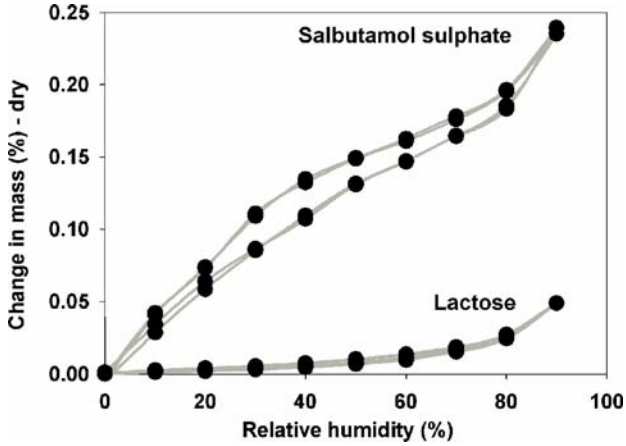

Fig. 2. Dynamic vapour sorption isotherms (2 sorption and desorption cycles) of micronised salbutamol sulphate and 63–90 μm sieve fractioned lactose.

attributed to both the surface chemistry and the relative difference in the surface area of the materials. In general, both lactose and salbutamol sulphate had similar isotherm sorption profiles to those in previous studies (6,7,13,22,32)

In vitro Deposition Studies using the Next Generation Impactor

The aerosolisation efficiency of salbutamol sulphate, from a lactose carrier blend was investigated as a function of storage humidity using the NGI impactor. Drug concentration from each stage of the impactor and device were calculated and processed to produce total dose (TD) (drug recovered from all stages of the NGI, pre-separator, throat, device and capsule), emitted dose (as total dose minus device and capsule), fine particle dose (FPD) (drug recovered from stages 3 to filter) and fine particle fraction (FPD/TD×100). At a flow rate of 60 l min^{-1}, the FPD and FPF represented the dose and percentage drug concentrations with an aerodynamic diameter less than 4.46 μm.

Statistical analysis of the total and emitted doses suggested no significant differences between formulations stored at different humidities. In general total doses of 430.27±27.40 μg and emitted doses of 384.00±27.82 μg were observed across all humidities, suggesting a constant device removal efficiency of 89%. It is important to recognise, that the relationship between total and emitted dose may not be related to the aerosolisation efficiency of drug, but the retention of formulation mass in the device and capsule. Indeed, recent studies by Young *et al.*, (33) have demonstrated that under similar conditions (device, lactose type, etc), the device efficiency remained independent of dose, thus suggesting that device losses are due to drug adhered on device retained lactose. It is interesting to note, the removal efficiency in previous studies, using salbutamol sulphate, 63–90 μm sieve fractioned lactose and a Cyclohaler operating at 60 l min^{-1} also suggested device efficiencies of 89% (33,34).

In comparison to the total and emitted dose, analysis of the FPD and FPF (Fig. 3) suggested storage humidity had a significant influence on aerosolisation performance. In general the FPD and FPF followed a parabola, with both FPD and FPF increasing with respect to increased humidity, over

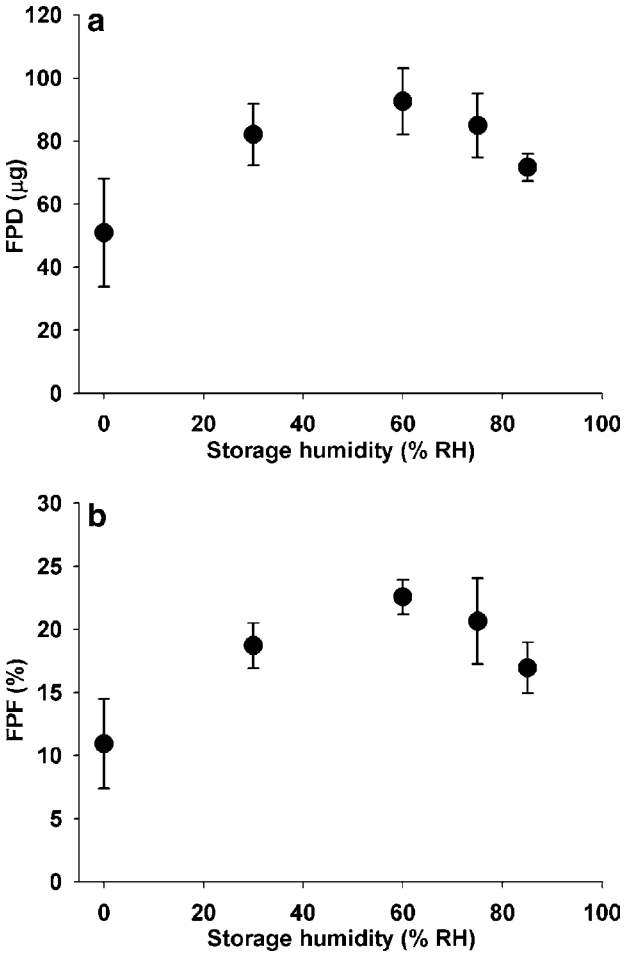

Fig. 3. Fine particle dose (*FPD*) and Fine particle fraction (*FPF*) collected by NGI of salbutamol sulphate aerosolised from 63–90 μm lactose carrier as a function of storage humidity, (n=3, *error bars indicate standard deviations*).

the range 0–60% RH, before decreasing (between 60 and 84%). In terms of aerosolisation performance, the FPF of salbutamol sulphate increased from 10.93±3.56% after storage at 0% RH to a maximum of 22.56±1.38% at 60% RH. Subsequent increase in storage humidity resulted in a decrease in FPF to 16.95±2.04% at 84% RH.

The relationship between humidity and aerosol performance may be attributed to the influence of moisture sorption on the combination of cohesive and adhesive forces within the system. For example, at elevated humidities, condensation between salbutamol sulphate and lactose carrier may result in increased capillary forces and reduced drug liberation during inhalation. Previous studies using salbutamol sulphate/carrier systems have suggested elevated humidity above 60% RH to result in a significant reduction in aerosolisation performance (7,8). Furthermore, similar studies conducted on drug only systems suggested salbutamol sulphate aerosolisation performance to decrease when the humidity was above 60% RH (5,6). Comparison of the NGI results obtained here showed good correlation with these previous studies, suggesting capillary forces to dominate aerosolisation performance at elevated humidity (>60% RH).

The mean FPD and FPF of salbutamol sulphate increased between 0 and 60% RH storage humidities suggesting a different mechanism, other than capillary forces, to influence performance. The improved aerosolisation efficiency of salbutamol sulphate as storage humidity was increased between 0% RH and 60% RH may be attributed to the dissipation of tribo-electrification-induced surface charges. Although, not clearly understood, electrostatic 'charging' of insulating materials (i.e. salbutamol sulphate, lactose and device components) may have a significant impact on powder flow, interaction and deposition in an airstream.

To further investigate the relationship between storage humidity tribo-electrification and aerosolisation performance, the experimental procedure was repeated using the ELPI impactor.

In vitro Deposition and Simultaneous Charge Studies using the Electrostatic Low Pressure Impactor

As previously discussed, the influence of storage humidity on both the aerosolisation performance and charge distribution was conducted using the ELPI. As the aerosolised particles pass into an impactor stage of the ELPI, the net

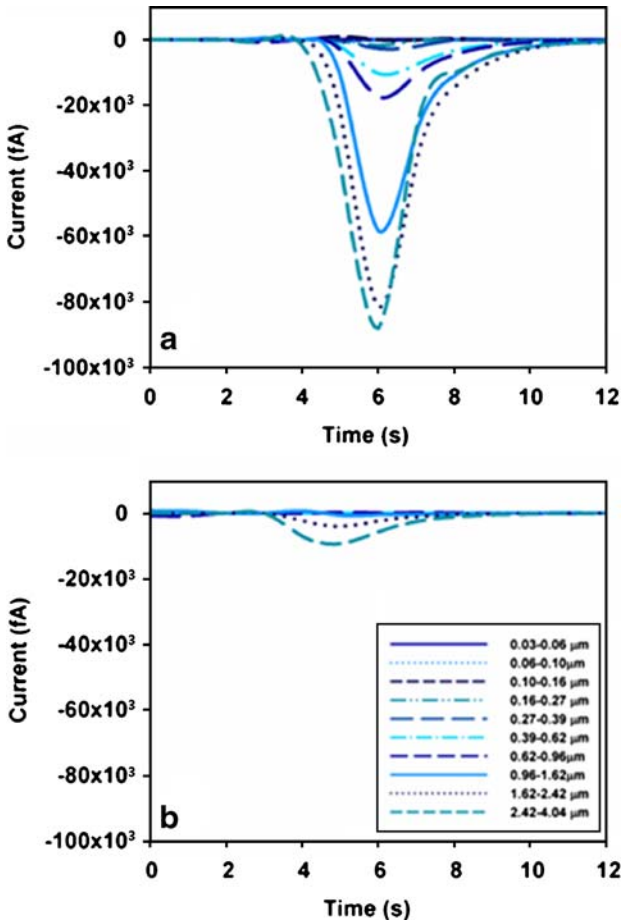

Fig. 4. ELPI Electrostatic charge distribution of salbutamol sulphate from a lactose blend after storage at (**a**) 0% RH and (**b**) 85% RH. Legend values indicate particle aerodynamic cut-off diameters for each stage.

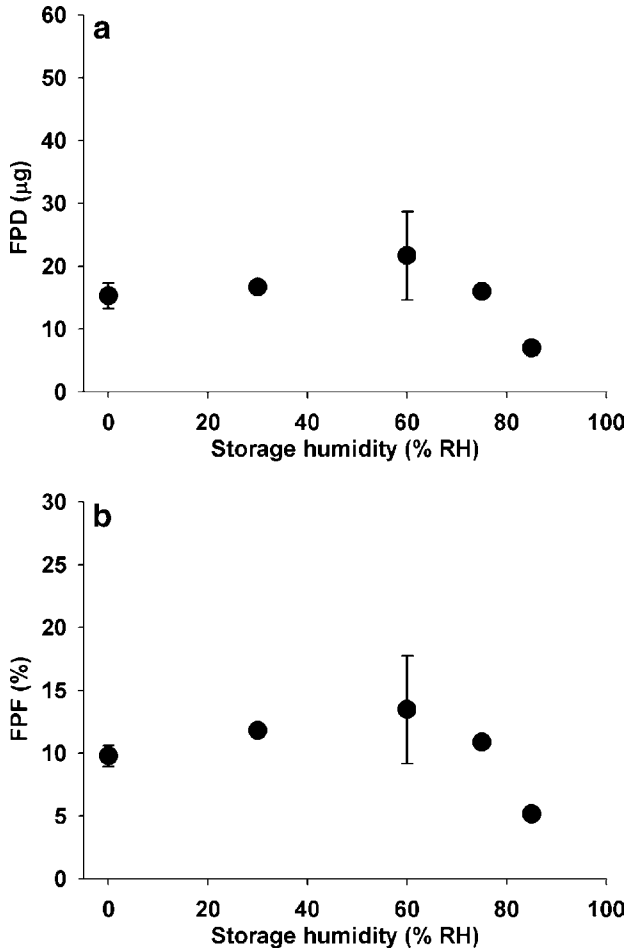

Fig. 5. Fine particle dose (*FPD*) and Fine particle fraction (*FPF*) collected by ELPI of salbutamol sulphate aerosolised from 63–90 μm lactose carrier as a function of storage humidity, (n=3, error bars indicate standard deviations).

surface charge present on the aerosol cloud induces a current in the metal of the isolated 'Faraday' impaction stage. Upon leaving a particular stage the aerosol cloud will produce an opposite charge, such that the net "image" charge will be related only to those particles that are impacted. Thus, by measuring the induced current on each stage as a function of time the net particle 'image' charge for any stage may be calculated through integration. Representative current vs. time plots for stages 1–10 of the ELPI after aerosolisation of blends stored at 0 and 85% RH are shown in Fig. 4a and b, respectively. Stages 1 to 10 represent particles with an aerodynamic diameter of ≤4.04 μm at 30 l min^{-1}. This cut off diameter is suitable for respiratory delivery and also comparable with the NGI effective aerosol cut-off diameter of 4.46 μm at 60 min^{-1}. From Fig. 4 it can be seen that the net charge on the aerosol cloud was negative. Such observations are in good agreement with previous studies which have suggested the salbutamol sulphate to negatively charge when aerosolised from a lactose carrier based formulation (20).

Furthermore, as can be seen from Fig. 4 the net charge after storage at 0% RH is greater than at 85% RH. To some extent, such observations would be expected since the adsorbed moisture in samples stored at higher humidities would allow a greater degree of electron mobility and thus reduce the charging mechanism.

Interestingly, integration of the net charge data for the formulation stored at 60% RH suggested a mean electrostatic charge of −419 pC. This value was of the same order of magnitude as that observed by Byron *et al.*, (20) (estimated at around −160 pC from graphical data), at similar uncontrolled humidities of 57 to 61% RH and using similar mass of a Ventolin™ formulation (10 mg). Variations between these two reports are most likely due to variation in the FPF between the two experiments. The original study by Byron *et al.*, would have contained different batches of drug and excipient and utilised a Dryhaler™ for charge measurement. Recent studies by Steckel *et al.*, (35) have shown the variation in lactose batch and supplier may result in variation in aerosolisation performance, while previous studies by Srichana, reported large variation in device efficiency when comparing devices delivering the 67.5:1 lactose:salbutamol sulphate formulations as used here (36).

However, it is important to note, that without aerosol mass deposition data, the net specific charge on the aerosol as a whole is of little use and the charge to mass ratio is required to gain a tangible understanding of this phenomena. Subsequently, to further elucidate the relationship between charge and aerosol performance the deposition of salbutamol sulphate on each of the stages of the ELPI, throat and device components were analysed.

Total salbutamol sulphate deposited, in the ELPI stages, device and throat, (across all measurements) gave a mean loaded dose of 148.22±14.46 μg (in good agreement with the 10 mg formulation mass). As with the NGI studies, the influence of storage humidity had no significant effect on the emitted dose, with a mean of 112.11±10.90 μg, being observed across all storage humidities. Interestingly, the overall formulation removal efficiency measured in the ELPI was less than that measured in the NGI (76% in comparison to 89%). Such observations are most likely due to the decreased flow rate and pressure drop through the device resulting in reduced formulation entrainment. However, since the emitted dose was independent of storage humidity,

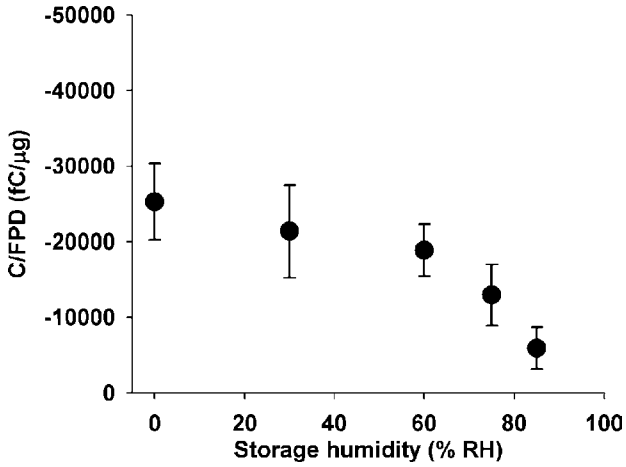

Fig. 6. Fine particle charge to fine particle dose ratio (c/FPD) plotted as a function of relative humidity, (n=3, error bars indicate standard deviations).

the liberation of salbutamol sulphate from carrier could be confidently investigated.

As with the NGI *in vitro* studies, the deposition of salbutamol sulphate in the ELPI suggested the storage humidity of the formulation prior to aerosolisation had a significant effect on the FPD and FPF (Fig. 5a and b, respectively). In general, both the FPD and FPF were in good agreement with the NGI data. Specifically, the aerosolisation efficiency followed a positive parabola, with the FPF increasing from 9.78±0.84% at 0% RH to a maximum of 13.48±4.29% at 60% RH, before decreasing at elevated humidities to 5.17±0.29% at 85% RH.

As previously discussed, reasons for the variation in aerosolisation performance with respect to storage humidity may be due the complex interplay of electrostatic tribo-electrification and the presence of capillary interactions at higher humidity. In order to investigate the relationship between salbutamol deposition and tribo-electrification, the net electrostatic charge of the respirable aerosol (aerodynamic diameter ≤4.04 μm) was divided by the respirable mass to produce the charge to mass ratio on the FPD.

As expected, the influence of storage humidity had a significant influence on the aerosol charge to mass ratio (C_{FPD}/M_{FPD}). As seen in Fig. 6, the C_{FPD}/M_{FPD} decreased with respect to increased storage humidity. Analysis of the C_{FPD}/M_{FPD} data indicated a linear decrease in tribo-electrification between 0 and 60% RH from -25.25×10^3 fC $\mu g^{-1}\pm5.05\times10^3$ fC μg^{-1} to -18.87×10^3 fC $\mu g^{-1}\pm3.43\times10^3$ fC μg^{-1}, respectively (R^2=0.994). This observation correlates well with the concurrent increase in FPF (9.78±0.84% at 0% RH to 13.48±4.29% (R^2=0.997) over the same humidity range. It is envisaged that the correlation between a decrease in negative tribo-electrification and increased FPF over the storage humidity range of 0–60% RH may be due to a reduction in charge induced particle interactions. Recent studies, utilising colloid probe microscopy have identified long range 'attractive' electrostatic forces between micronised drug particles used in inhalation (TAA) (12) and have correlated this to reduced FPF at lower humidity (6,11). Although, similar observations with salbutamol sulphate were not observed (11), it is envisaged the charging mechanism observed in this binary formulation, would be more complex than that observed under single drug–drug particle measurement. Indeed, Byron *et al.*, demonstrated very different charging profiles when comparing drug only formulations to binary mixtures (20). It is therefore envisaged that after low storage humidity the salbutamol sulphate and lactose particles will have a strong propensity for charge transfer. Subsequently, during aerosolisation the energy required to separate the drug particulates from the carrier will be greater than when charge transfer does not occur. Furthermore, upon drug carrier separation, the long range electrostatic forces present in the system will encourage inter-particle instability (through long range attraction and repulsion) reducing the likelihood of the particles reaching the lower stages of the impactor.

In comparison, storage of the formulation above 60% RH resulted in a large reduction in tribo-electrification from -18.87×10^3 fC $\mu g^{-1}\pm3.43\times10^3$ fC μg^{-1} at 60% RH to 5.94×10^3 fC $\mu g^{-1}\pm2.82\times10^3$ fC μg^{-1}at 85% RH. To put this into perspective, between 0 and 60% RH (60% RH units) a decrease in the aerosol charge of 25% was observed, while between 60 and 85% RH (25% RH units) a reduction of 67% was observed. This observation may be due to the relative increase in moisture sorption of salbutamol sulphate and lactose at relative humidities above 60% (Fig. 2). As previously discussed, it envisaged that at humidities above 60%, the condensate water would allow increased electron mobility (thus reduced tribo-electrification) but also result in increased capillary forces and adhesion of salbutamol sulphate particulates to the parent carrier. As in previous studies (6,7), a significant decrease in FPF was observed at humidities above 60% RH and is most likely due to capillary forces. It is interesting to note, however, that this study has been conducted after storage at specific humidities, in comparison to at specific aerosolisation humidities. Although the storage humidity is generally regarded as the dominating factor (22), aerosolisation humidity should be considered for future investigation.

CONCLUSIONS

As reported in previous studies, a significant variation in the aerosolisation efficiency of micronised salbutamol sulphate from a lactose carrier was observed as a function of RH. In general the aerosol performance (FPF) followed a positive parabola with FPF increasing over the range 0–60% RH before decreasing >60% RH. Similar FPF results were seen for drug deposition in the ELPI. Analysis of the ELPI charge data suggested that the micronised salbutamol sulphate had an electronegative charge when aerosolised from lactose based carriers, which was most electronegative at low RH. Increased storage humidity could be related to both reduction in tribo-electrification and increased FPF until capillary interactions dominate and further increase in RH was met with a concurrent FPF reduction. Clearly, the mechanism that dominates particle interactions in binary DPI systems is complex, with many variables that are not taken into consideration in this study, however, it is clear that the aerosolisation performance is dependent on not only capillary but electrostatic forces, and is related to the degree of moisture adsorbed on the contiguous surfaces.

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