AAPS PharmSciTech, Vol. 15, No. 6, December 2014 (© 2014)
DOI: 10.1208/s12249-014-0144-5

Research Article

Theme: Advances in Formulation and Device Technologies for Pulmonary Drug Delivery
Guest Editors: Paul B. Myrdal and Steve W. Stein

Aerodynamic and Electrostatic Properties of Model Dry Powder Aerosols: a Comprehensive Study of Formulation Factors

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Received 27 January 2014; accepted 7 May 2014; published online 18 June 2014

ABSTRACT. The impact of formulation variables on aerodynamic and electrostatic properties of dry powder aerosol particles is of great importance to the development of efficient and reproducible inhaler products. Systematic evaluation requires a well-designed series of experiments using appropriate methods. A factorial experimental design was employed. In broad terms, the conditions considered were two drugs, albuterol and budesonide, in combination with different excipients, drug concentrations, delivered doses, and metering system (capsule composition) and sampled under different flow conditions using standard entrainment tubes. Samples were collected in an electrical low-pressure impactor, to evaluate distribution of electrostatic properties, and an Andersen eight-stage nonviable cascade impactor, to estimate aerodynamic particle size distribution, concurrently. The deposition studies allowed calculation of approximate per particle charge levels for drug. The results showed very high particle charge levels, often in the 1,000–10,000 of elementary charges per particle range, orders of magnitude higher than charge levels predicted by the Boltzmann charge distribution. The charge levels are considerably higher than had previously been estimated (200e per particle).

KEY WORDS: aerodynamics; aerosol(s); electrostatics; experimental design; formulation factors.

INTRODUCTION

Dry powder inhaler (DPI) formulations and devices have characteristics that can give rise to surface charging or triboelectrification. When the patient actuates a DPI, the formulation is fluidized and dispersed; drug and carrier particles separate from each other. Charges can be transferred during this process resulting in net particle charges, which may affect particle trajectory and deposition. Particles experience significant friction during DPI discharge, which tends to amplify these effects.

Aerodynamic and electrical property experiments may be performed to elucidate these properties with respect to the aerosol behavior of the formulation. Using the electrical low-pressure impactor (ELPI), the experimental apparatus permits the simultaneous determination of particle size and charge distribution of the fine particle fraction of the emitted aerosol, which are important prerequisites for understanding formulation behavior.

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The ELPI™

The experimental apparatus relies on the ELPI™ (Dekati Ltd, Tampere, Finland), an aerodynamic particle sizer that detects particles by charge (1,2). The ELPI has primarily been used in the automotive industry (2–5) and in industrial hygiene applications (6,7). More recently, it has been applied to the evaluation of electrostatic phenomena in medicinal aerosols, including metered dose inhalers (MDIs) (8) and DPIs (9).

A detailed discussion of the ELPI is available elsewhere (10). Briefly, the ELPI is composed of a low-pressure cascade impactor and a unipolar corona aerosol charger that charges the incoming aerosol uniformly prior to entry into the cascade impactor. Operating at a reduced pressure (10 kPa on the final stage), the ELPI collects particles down to a size of 30 nm, the cutoff diameter of the bottom stage (stage 1). The cutoff diameters of the other 12 stages are 10.2, 6.56, 4.09, 2.52, 1.66, 1.02, 0.66, 0.41, 0.27, 0.17, 0.11, and 0.06 μm, respectively. As is the case in other impactors, deposition inside the ELPI depends on particle aerodynamic diameter. But unlike conventional cascade impactors, which are disassembled after operation for chemical or gravimetrical assay of the stages, an ELPI detects particle deposition by measuring the current resulting from dissipation of the particles' electrical charges. The collected particles can be measured simultaneously on all stages using a multichannel electrometer and are reported in real-time on a computer. Thus, when operated with charger

turned on, the ELPI functions as a real-time particle sizer; this is the standard ELPI configuration.

The ELPI can also be operated with the charger turned off. In this configuration, the incoming aerosol is not charged within the ELPI and the electrometer measures only native particle charges. Since each impactor stage is electrically isolated from the next, charges can be measured across individual stages, allowing the evaluation of size-specific charging with accurate determination of charge magnitude and polarity on particles in the respirable size range over time.

Background

Studies of medicinal aerosol electrostatics have been attempted before. Electrostatic charge carried by a DPI aerosol cloud has been measured using a grid probe (11); however, the particles were rather large and the system was of little pharmaceutical relevance. The effects of particle morphology and crystallinity on triboelectrification of DPIs have also been studied, but particle deposition was not considered (12). Moreover, the studies relied solely on Faraday cage charge measurements, which precluded analysis of fine particle sizes and size-dependent charge distribution.

A preliminary analysis of DPI electrostatics in a pharmaceutically relevant system was previously published by the authors (9); these studies provide the foundation for the work described here and can be summarized as follows:

1. An experimental apparatus was developed that allowed inhalers to be tested at an airflow rate of 60 L/min, as recommended by the FDA and US Pharmacopeia (13), and the electrical charge of the respirable fraction to be determined. The same setup is used in the present work.
2. Initial experiments assessed the ability of the ELPI to classify therapeutic aerosols consistently and in accord with a conventional inertial impactor, *i.e.*, the Andersen eight-stage nonviable impactor. ELPI particle size distributions were in agreement with those determined from ACI deposition measurements.
3. The particle size distributions acquired by ELPI were compared to those obtained from gravimetric analysis of the stages; agreement was good with almost superimposable particle size distributions and same d_{25}, d_{50}, and d_{75} values. The ELPI system proved more precise and reproducible in the lower particle size ranges.
4. Deposited particle charge was shown to vary significantly between different particle size fractions of a given formulation as well as between formulations.
5. The study revealed that pharmaceutical product properties such as excipient, inhaler type, and metering system significantly affect the extent to which formulations experience triboelectrification. Both charge magnitude and polarity were affected consistently.

These experiments constituted the most comprehensive study of DPI triboelectrification phenomena at the time. They demonstrated that triboelectrification is a significant phenomenon in DPI actuation. Yet, the study had several shortcomings that the present study addresses. Charging effects are confounded by particle size distribution and mass depositions since the stages of the ELPI were not assayed for drug and excipient deposition during individual actuations. Moreover, excipients contained sizeable proportions of fine particles that were deposited together with the micron-sized drug after formulation actuation. As a result, it is unclear which material contributed to charge on each stage; tribocharging could not be conclusively linked with fine particulate drug, and the magnitude of particle charge could not be established. However, this information is needed to determine whether particles carry sufficient charge to affect delivery.

EXPERIMENTAL DESIGN

The goal of the experiments was to elucidate the phenomenon of electrostatic charging in a DPI by simultaneously studying charge and mass deposition following DPI actuation. Establishing which formulation variables contribute to charging has consequences for product development. However, the number of possible variables to study in this context was potentially large. The choice of a discriminating experimental design with appropriate input factors and responses was critical.

Design Factors

Drugs: Albuterol Sulfate, Budesonide

Albuterol (USAN) or salbutamol (INN) is a short-acting β_2-adrenergic receptor agonist used for the relief of bronchospasms in asthma and chronic obstructive pulmonary disease (COPD). Albuterol (molecular weight (MW) 239.3) is a commonly inhaled medicine and frequently used as a model drug in medicinal aerosol research.

Budesonide (MW 430.5) is a glucocorticoid steroid for the treatment of asthma, noninfectious rhinitis, and allergies. Budesonide is marketed as Pulmicort® with a Turbohaler DPI by AstraZenaca (14). It was selected because it is a different therapeutic category of drug and is structurally very distinct from albuterol.

Excipients: Lactose Monohydrate, Glucose, Calcium Phosphate (CP)

Lactose is the most common excipient in DPI formulations. It was the only excipient used in the preliminary experiments and is the primary excipient in the formulations tested in this study. In addition to lactose, glucose was tested. It has previously been employed in DPI formulations (15) and is thus a relevant substance to compare. CP was chosen for study because it is a chemically different material (inorganic salt), which offers a contrast to the sugars usually employed. While it is a physiological salt that is widely used in the formulation of oral dosage forms, it has not been used in DPI formulations.

Excipient Sieve Fractions: 45–75, 75–105, and 105–150 μm

Three sieve fractions were selected because they were size ranges available for most excipients (all but the sieved lactose batches, where all particles are smaller than 75 μm). A lower limit of 45 μm was selected to allow the most complete removal of excipient fines, which might otherwise be deposited on the stages of the ELPI, confounding the analysis.

Excipient Batch-to-Batch Variability (Lactose Only): Two Milled (ML), Two Sieved (SV) Lactose Products

Since batch-to-batch variability is a well-known problem, several lactose products and batches were used to make formulations. Sieved lactose batches (SV) have undergone sieving as the only process step after crystallization while milled batches (ML) have been milled after crystallization at the manufacture site.

Drug Concentration: 0.25%, 0.50%, and 1.0%

DPI formulations are interactive powder blends containing low drug concentrations, often at the 0.5–2% mass/mass range. These concentrations ensure that drug particles remain separated from one another and, thus, maintain their primary particle size, *i.e.*, they do not aggregate significantly. Drug concentrations are an important determinant of drug/carrier particle interactions. The drug concentration was examined at three levels to evaluate particle separation and charging. Carrier particle surfaces are heterogeneous with a number of particularly energetic "active sites" (16); using different drug concentrations, these active sites may be probed. The presence of active sites would likely be manifested in particle association resulting in deposition differences that are nonlinear with respect to drug concentration. The reason for having a very low concentration, 0.25% loading, of drug in the experimental design is that particles are likely to be in closer contact with the more highly energetic active surface sites.

Dose: 10, 20, 30, and 40 mg

The dose range is relevant to DPI formulations. Nonlinear responses of charging and drug deposition to changes in dose may elucidate the fluidization and charging pattern.

Fluidization Conditions: Standard Entrainment Tubes

Preliminary studies were conducted using two different inhaler devices, the Rotahaler® (GlaxoSmithKline, RTP, NC) and the Inhalator® (Boehringer Ingelheim, Germany) single capsule inhaler, a low-pressure drop and a high-pressure drop device, respectively (17). Study results indicated a strong correlation between choice of inhaler and both magnitude and polarity of acquired charge. However, this correlation could not be firmly linked to a change in pressure drop or device resistivity because the devices also differed in material and operational principles, which confounded comparisons between the devices. To eliminate this source of variability, standardized entrainment tubes (SETs) replaced DPI devices as aerosol dispersion systems. SETs are simple steel tubes of specific dimensions in which well-characterized airflow patterns are established (18).

Product Batch-to-Batch Variability (Variability in Manufacture)

Manufacture of select batches was repeated to determine if the observed phenomena were the result of fundamental material properties (as postulated) rather than the result of environmental or random effects that might arise during manufacture.

Study Design

The mere combination of excipient, batch, and sieve fractions results in 14 different excipient building blocks. If each of these were to be tested at two levels, the total number of individual formulations would be 14×2 (drugs)×2 (conc)= 56 formulations. Testing at 2 doses and 2 fluidization conditions would require 224 experiments, which does not account for the allocation of replicates (to test reproducibility and manufacture consistency). If all levels discussed above were tested, the study would require 14×2×3×4×3×4×3=12,096 experiments (again excluding allocations for reproducibility and manufacture consistency). Efficient experimental allocation is crucially important.

In determining the appropriate statistical design, several competing objectives were weighed. Picking a standard design scheme, such as a fractional factorial or central composite design is attractive because it provides the ability to interpret all generated data simultaneously using well-established statistical tools and generate response surfaces that may allow interpolation and extrapolation. However, given the large number of factors (in particular, categorical factors such as the 14 different excipient building blocks), this design would be difficult to construct and in the end would still require sufficient trials to be impractical. Consequently, a different approach was required.

The experimental design developed was a series of experiments in a design framework rather than a purely statistical design scheme. While it contains elements of both half and full-factorial designs, it was structured to test specific (if conditional) hypotheses rather than to determine a multidimensional response surface. Using this experimental framework, the results cannot be interpreted generally but must be considered discretely. Specific hypotheses, represented by the design factors listed in Section 2.1, were tested using a two-level design with a number of intermittent or extreme points at various positions to gauge the response surface curvature where it appeared to play a role. The design, which is shown schematically in Fig. 1, is based on the assumption that most factors are independent and that marginal effects are more important than interaction effects. In all, the design required 33 formulations to be tested in 63 experiments, which provided sufficient time for the repetition of a sizable portion of experiments, thereby increasing statistical power. In Fig. 1, each circle denotes an experiment, for which three sets of electrical charge data and one set of mass deposition data were determined.

The design is built around a central set of experiments that compares each excipient in a standard formulation (0.5% albuterol in 45–75 μm sieve fraction of excipient) to the other five excipients using a common set of actuation conditions (40 mg, SET A). There are two full-factorial experimental sets using the two milled lactose batches (ML80 and ML58) aimed at gauging the effects of drug concentration (0.5% and 1.0%), fluidization conditions (SET A *vs.* SET C), dose (20 mg *vs.* 40 mg), and half-factorial designs for each of the other excipients. The purpose of collecting two full-factorial sets for ML80 and ML58 is that the differences between the batches are assumed to be minute (batch-to-batch variability), much smaller than the differences expected between the different materials. A full-factorial design has greater potential to discriminate differences. Using ML80 only, the effects of drug

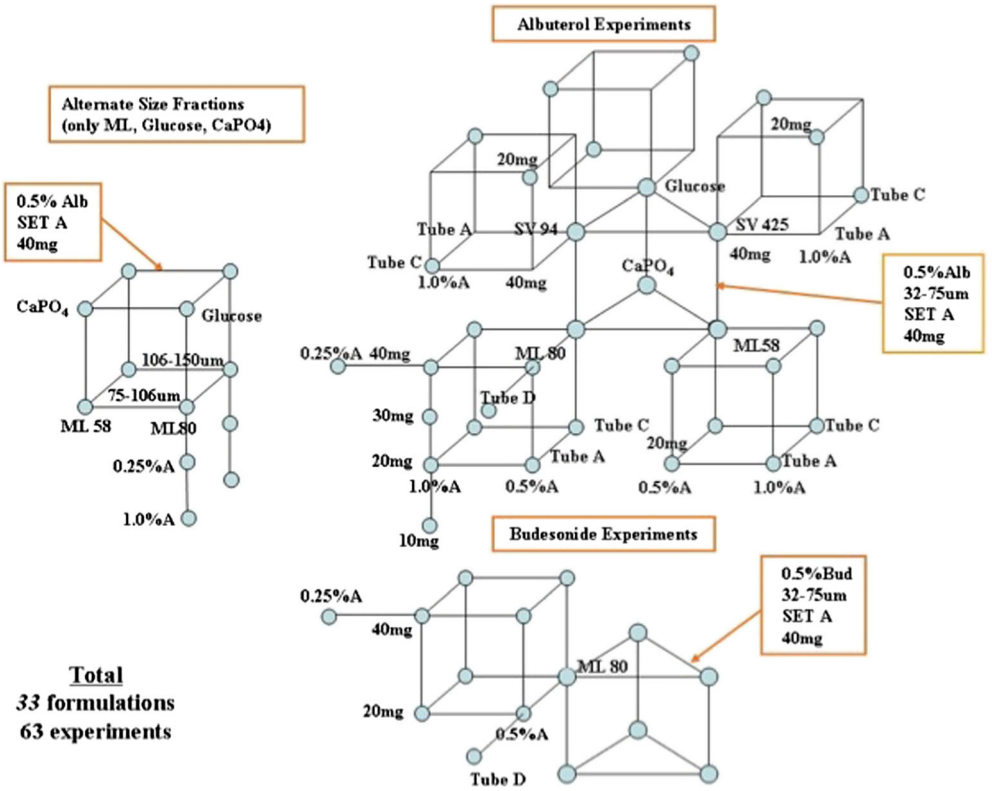


Fig. 1. Experimental framework developed to study the electrostatic effects of the variables. Each *circle* denotes a different experimental setting. The framework contains elements of factorial and central composite design but was developed to test specific hypotheses. The main elements of the design are denoted by *red boxes*. The design is described in detail in the text

concentration (0.25% albuterol), fluidization conditions (SET D), and dose (10 mg, 30 mg) were further investigated. These experiments were to provide insight on the response surface curvature with respect to these variables. The central design (triangular prism) with a full-factorial assessment around ML80 is repeated for budesonide; this should allow a comprehensive comparison of drug identity for each excipient, as well as confounded effects (interactions between drug, drug concentration, fluidization conditions, and dose) for ML80 lactose formulations. Finally, the cube on the left provides comparisons of the different sieve fractions for each of the four excipients for which the larger sieve fractions are obtainable, that is, the two milled lactose batches (ML80, ML58), glucose, and CP. Once again, using ML80 lactose, the confounding effects of sieve fraction and drug concentration may be examined. While this is one of the aforementioned conditional tests, if an effect is observed, it may well exist for some of the other excipients.

Of the 63 experiments that were conducted in this framework, 28 involve ML80 lactose (12 out of 33 formulations). Thus, a very thorough study of ML80 lactose behavior is obtained, allowing a complete examination of all possible interactions of the different variables. Examinations of the other excipients are less comprehensive.

To ensure that formulations would be tested within a week after manufacture, the order of the formulations was randomized first, and then the different actuations were randomized within blocks of ~5 formulations. Formulations were then manufactured. This ensured that formulations were not subject to different storage effects.

To evaluate the reproducibility and possible storage effects, 20 of the 63 experiments were randomly selected for retesting; several of these would be retested several times after varying periods of storage. To evaluate the variability associated with the manufacture, seven formulations were randomly selected for remanufacture and were subsequently retested using the same experimental conditions and settings. In addition, several formulations were selected to check the effect of environmental conditions, including the effect of high relative humidity (RH) and the effect of the last surface of contact.

MATERIALS AND METHODS

Materials

Excipients

Lactose monohydrate, Respitose™, was provided by DMV-Fonterra Excipients (Goch, Germany). Two batches of milled (ML001, batches 10136780 and 10138058) and two batches of sieved (SV003, batches 10190094 and 10135425) lactose were evaluated. (For convenience, the batches will be referred to as ML80, ML58, SV94, and SV425, respectively.) Dextrose (D-glucose), Lycadex™, was provided by Roquette (Keokuk, IA). Calcium phosphate, A-Tab™, was obtained from Innophos (Cranbury, NJ). To control for differences in particle size in the experiments, all excipient batches were sieved prior to use in the formulations and experiments.

Drugs

Milled albuterol sulfate (median particle size 2 μm) was obtained from Chemaco (Beetsterzwaag, NL) from drug manufactured by P.F.C. Italiana S.r.1 (Caronno Pertusella, Italy). Budesonide was obtained from Sigma-Aldrich (St. Louis, MO) and was jet-milled to a single-micron size range, verified using laser light scattering.

Dispersion Devices

To understand the effects of fluid dynamics on triboelectrification, three SET were used, giving low, intermediate, and high specific resistance values of 0.021, 0.046, and 0.140 cm $H_2O^{0.5}$/(L/min), respectively (18). The relevant properties of these SETs in comparison with the two previously tested devices are shown in Table I; SETs A, C, and D provide high, low, and very low resistivity devices, likely to provide evidence of a range of performance of the formulations in response to airflow conditions. These devices have been used previously in a series of studies intended to correlate particle dispersion with shear conditions (19–24).

Methods

ELPI Experimental Apparatus

The experimental apparatus was developed during preliminary studies to overcome two major obstacles, (1) operation at 30 L/min and (2) pre-separation of large agglomerates (of no therapeutic importance). (1) The ELPI operates at 30 L/min, but DPIs are typically tested at 60 L/min airflow rates which more closely approximates the inspiratory airflow rate; (2) unlike the ACI (ThermoAndersen, Smyrna, GA), the ELPI does not have a pre-separator capable of removing large quantities of coarse material. However, initial trials made clear that failure to remove carrier particles and agglomerates from the aerosol prior to introduction into the ELPI could result in flooding the top stages after a single inhaler actuation. The setup that was devised to address these issues is shown in Fig. 2. This approach allows a flow rate of 60 L/min at the site of actuation, segregation of coarse material, and dispersion and deposition of airborne particles in two parallel collection devices, the ELPI and ACI, each operated at 30 L/min. A SET is connected to the standard USP induction throat, which is connected to the ACI pre-separator (loaded with 15 mL water or methanol) (cutoff size of 8.6 μm at 60 L/min). A custom-manufactured aluminum connector from the pre-separator split the 60-L/min stream in two equal streams; 40 cm Tygon™ tubing (R3603) was used to connect the two outlets with ELPI and ACI, each employing a vacuum pump operating at 30 L/min. Tygon tubing is commonly used in aerosol research because, unlike other materials, Tygon has negligible electrostatic effects (25). Deposition of drug in different parts of the experimental apparatus was determined in preliminary studies; deposition in the custom-made part and tubing accounted for <5% of deposition in the ELPI and ACI. To prevent particle bounce, ACI collection plates were coated with silicone oil. Coated aluminum foils were used with the ELPI stages. The apparatus was leak tested prior to each experiment. In accordance with manufacturer recommendations, the ELPI was switched on at least 1 h prior to measurement. Inlet air was not filtered and baseline measurements of charge were collected.

Actuations

Formulations were weighed on an analytical balance, dispensed onto weighing paper and placed in front of the entrainment tube for actuation into the experimental apparatus. Preliminary studies indicated that three actuations were required to ensure sufficient material was deposited on all ELPI stages. To determine the charge contribution of excipient only, for each experimental setting, the three formulation actuations were preceded by three actuations of excipient only. In sequence, for each setting, the apparatus was turned on, the electrometer channels were zeroed, and, once the signal stabilized, the actuations proceeded in the order: placebo; active formulation; placebo; active formulation; placebo; and active formulation. All active and placebo formulations were weighed out prior to the experiment and approximately 10 s was allowed between actuations. Electrical charge across 12 stages during actuations was recorded using the ELPI V acquisition software.

Analysis of Drug Deposition on ELPI Stages

After each set of three actuations for a given formulation, the ELPI was turned off and the apparatus was disassembled and washed. The coated aluminum foils were removed from the ELPI stages and washed with 3 mL water (albuterol formulations) or 3 mL methanol (budesonide formulations). CP formulations (limited aqueous and methanol solubility) were centrifuged to separate any undissolved excipient. UV absorbance of the supernatant solutions was assessed at a

Table I. Relevant Fluid Dynamic Properties (at 60 L/min) of Standard Entrainment Tubes Used in Studies Compared with Previously Used and Commercially Available Inhalers

	Specific resistance (R_D)		ΔP	Power	Shear stress
SET	cm $H_2O^{0.5}$/(L/min)	Re	(N/m^2)	(W)	(N/m^2)
A	0.140	18,440	6,920	6.92	13.14
C	0.046	11,070	747	0.747	2.20
D	0.021	7,720	156	0.156	0.62
Rotahaler	0.040	N/A			
Inhalator	0.180	N/A			

SET standardized entrainment tube, *N/A* not available

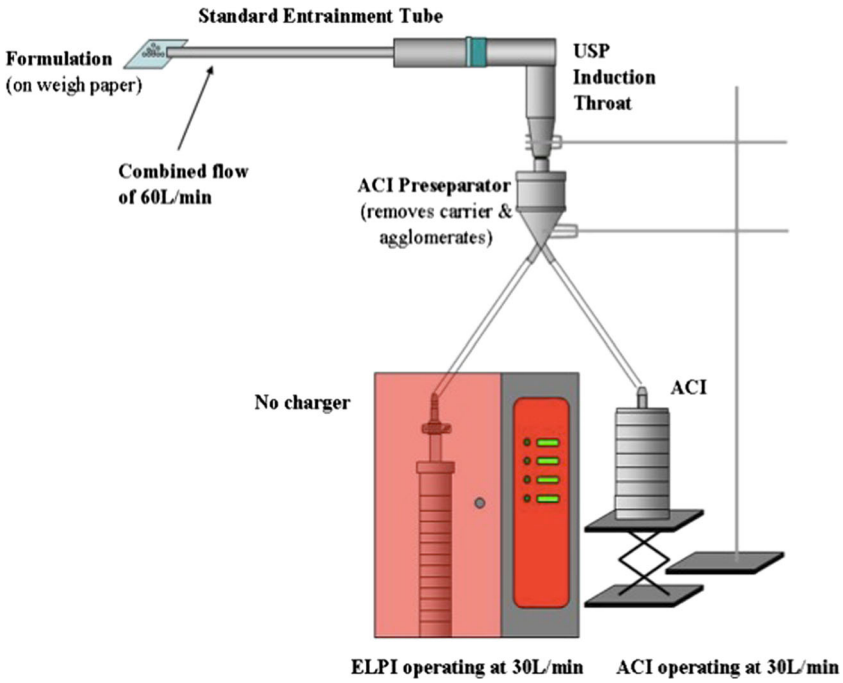


Fig. 2. Experimental apparatus for parallel sampling by ELPI and ACI (adapted from Telko *et al.*(9))

wavelength of 224.5 nm (albuterol) or 244 nm (budesonide) using a Shimadzu 160U spectrophotometer. The excipients have negligible absorbance at this wavelength.

Environmental Conditions

Triboelectrification is only observed at relative humidities of less than 65% (26). Hence, conditions must be monitored. The experiments were performed in the climate-controlled laboratory over the course of several months; the conditions during blending, storage, and actuation were constant at a temperature of 23±0.5°C and a relative humidity of 35±5%.

Environmental Effects Study

When a patient actuates an inhaler and the drug particles enter the airways, they are entering a high humidity (100% RH) environment. High RH is known to inhibit triboelectrification. However, it is unclear if the environmental conditions in the airways, to which drug particles are briefly exposed, can also dissipate the particles' charges. This was determined using a modified apparatus, which is similar to Fig. 2 with an ultrasonic humidifier (Vicks V-5100, Procter & Gamble, Cincinnati, OH) generating water vapor immediately next to the SET inlet, so that humid air instead of ambient air is drawn into the apparatus.

Surface Effects Study

Triboelectrification is a surface effect; different surfaces interact with materials and result in specific charging behavior. It is important to understand the choice of surface from which the formulation is actuated. Two formulations were actuated into the experimental apparatus from a series of different surfaces, including weighing paper, polyethylene (PE) weighing dish, aluminum weighing dish, a gelatin capsule, and carageenan capsule. Only the ELPI charge deposition data was recorded for these and was subsequently compared.

Data Analysis

Charge data were collected by the ELPI data acquisition software (ELPIVI 4.0, Dekati, Tampere, Finland). Raw data were transferred to and analyzed in Excel (Microsoft, Seattle, WA). Each actuation was isolated and charge was integrated over the time the actuation had occurred. Since there were three actuations of active formulation for each experiment, the charges were averaged and standard deviations determined. Drug deposition was determined from chemical assay of the ELPI stages and plotted in Excel.

The set of 63 experiments provides a large dataset. However, the data represents a custom design; containing elements of factorial and central composite design. Data were transferred to statistical software SPSS 16.0 for Windows (SPSS Inc., Chicago, IL) and analyzed using the general linear model multivariate analysis feature. The response elements examined included d_{25}, d_{50}, d_{75}, fine particle fraction, as well as total charge magnitude and polarity (summed across all stages), absolute magnitude of the charges recovered, and charges recovered on stages 1–3, 4–6, 7–9, and 10–12. Another response variable tested was charge/fine particle mass. Assuming the particles were perfect spheres of a uniform size at the midpoint between two stages, the average particle charge was determined and adapted as a response variable. All of the response variables were examined with respect to each of the formulation factors in a series of statistical tests.

RESULTS AND DISCUSSION

Reproducibility of ELPI Experiments

Preliminary experiments had shown good reproducibility of charge distribution profiles for a given formulation performed at constant conditions (9). This consistency was also observed in the current trials, as is highlighted in Fig. 3. Twenty milligrams of 1% albuterol in ML58 lactose, 45–75 μm sieve fraction, was actuated from SET A three times (separated by actuations of placebo formulation); the procedure was repeated 9 days later. As is evident in Fig. 3, the actuations were consistent within a given day and consistent over the course of several days. Differences in magnitude of up to 20% were common, but charge polarity and rank/order of the stages was retained in all trials (compared to other formulations and/or conditions).

Storage times of less than 2 weeks generally resulted in highly reproducible charge and particle size distributions with variability similar in magnitude to intra-experimental variability observed when experiments were performed in sequence (on the same day). In Fig. 4, a formulation was retested 5 days after the original sample was examined; this formulation (0.5% albuterol in 45–75 μm ML80 lactose) showed very good reproducibility in both mass-deposited (fine particle mass (FPM) and fine particle fraction (FPF)) and charge distributions (integrated rather than stage specific as in Fig. 4).

Variability in mass and charge deposition profiles increased after more than 2 weeks of storage (at ambient conditions). In many cases, the differences observed after storage were so dramatic that the replicates were no longer representative of the original system. In other words, many of the formulations were subject to significant storage effects. As a result of these effects, all replicates subject to storage effects, *i.e.*, those with storage times exceeding 2 weeks, were excluded from the experimental analysis.

Remanufactured Product Variability and Storage Effects

Another aspect of variability is that associated with manufacture. While the same process of manufacture is applied to all formulations, the major processing step of blending is by its nature a random process. If the variability associated with the product manufacture results in significant differences in formulation behavior, variability from other factors would be difficult to discern. In light of the observations made with respect to storage, understanding the variability of the manufacture process is essential.

Figure 5 shows the mass and charge deposition profiles for a formulation (0.5% albuterol, ML58 lactose, 75–106 μm sieve fraction) manufactured twice. Both mass and charge deposition profiles are almost superimposable, indicating a high level of reproducibility.

Figure 6 compares two batches of product, one actuated soon after manufacture (blue) as well as after 36 days of storage (red) and a remanufactured formulation actuated 2 days after manufacture (green). Clearly, the remanufactured formulation is more similar to the original formulation than to the formulation that has undergone storage. This suggests that the variability in the process is not significant but that the storage effects are significant. The large magnitude of the storage effect was an unanticipated and warrants closer investigation. However, pursuit of this objective is outside the scope of the present discussion of electrostatics.

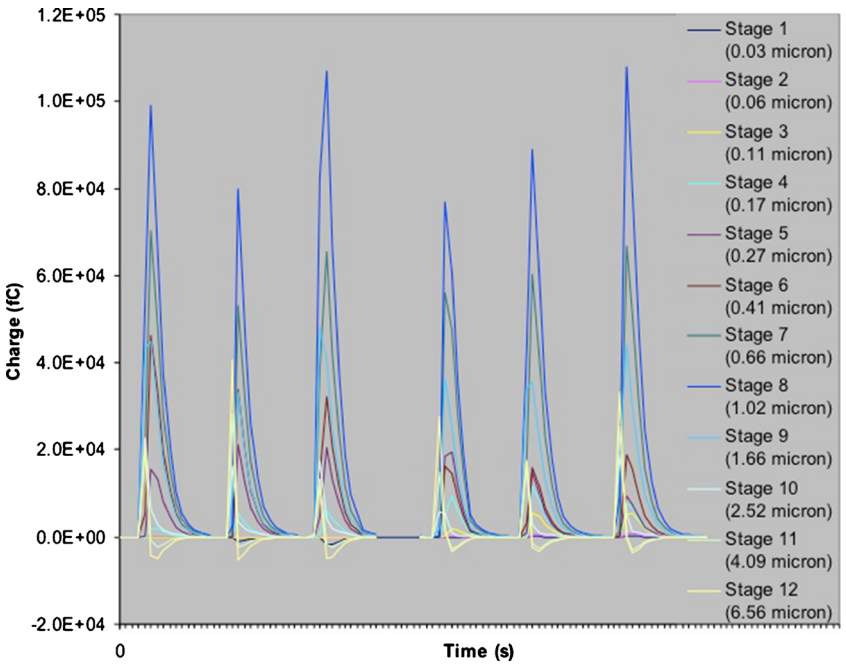


Fig. 3. Six actuations of 1% albuterol in ML58 lactose, 45–75 μm sieve fraction, and 20 mg actuated from SET A. The first three were performed on 1 day separated only by actuations of excipient, the next three performed 9 days later, also separated by excipient actuations (excipient actuations have been removed from this graph). *Double line* indicates temporal discontinuity. In each case, deposition on stages is complete in about 6 s. While there is some variability with regards to magnitude, the overall rank/order and polarity is highly preserved in each trial

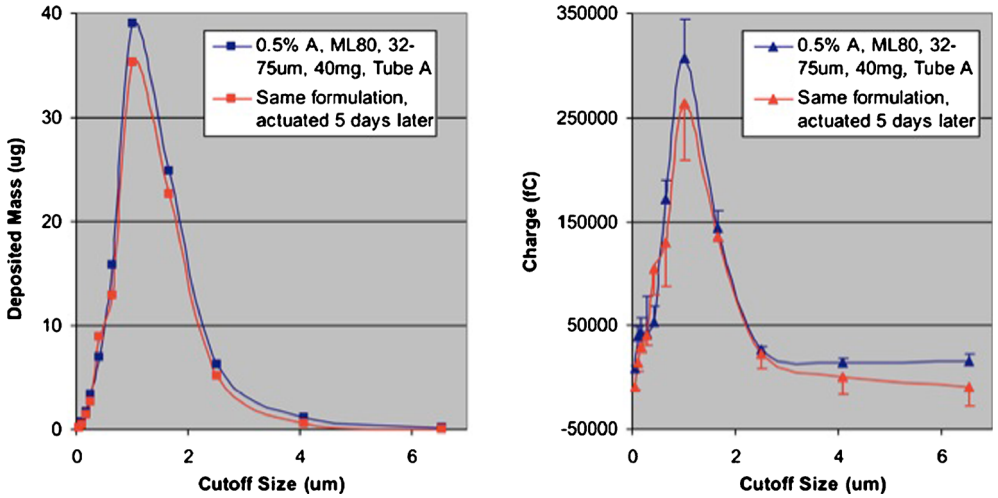


Fig. 4. Formulation (0.5% albuterol in 45–75 μm ML80 lactose, 40 mg) actuated from SET A (three times) on different days, separated by 5 days of storage. The differences in deposition (actual quantities shown) are minimal. Differences in charge distribution mirror the differences in deposition and are within a standard deviation from one another

Two formulations were actuated into the experimental apparatus from a series of different surfaces, including weighing paper, a PE weighing dish, a gelatin capsule, and a carageenan capsule. Figure 7 shows the results of one of these experiments, charge depositions over time for a formulation of 1% albuterol in ML58 lactose, 45–75 μm sieve fraction, and 20 mg actuated from SET A; the formulation had been dispensed onto each of the different surfaces and had been kept there for at least 30 min prior to actuation. The choice of surface did not appear to have a significant effect on the charging properties of the FPF within the ELPI in either case; the charging recorded on the individual stages follows the same pattern in each case, and the differences in overall magnitude were in the order of inter-actuation variability.

These observations are important because it shows that the electrical charges are the result of tribocharging, *i.e.*, charge transfers between the particles of the formulation. These charges are the result of electron transfer during particle contact during and after the blending process; the charges are exposed when the particles are separated from one another during the actuation process. This point in particular can be deduced from the fourth actuation shown, in which the blend was actuated from weigh paper after it had been treated with Zerostat® antistatic gun, which removes static charges by releasing a stream of bipolar ions. This treatment ensures that the system as a whole is uncharged. The charges, shown in Fig. 7, were integrated over time and summed across the stages; the results of this are shown in Table II. Differences in charge (simple sum and sum of absolute magnitude) are within the normal variability.

As shown in Fig. 8 and Table III, another formulation containing budesonide in sieved lactose displayed the same behavior, *i.e.*, charge distribution was independent of the surface of last contact, even as the actual charging was very different from the previous formulation. In this case, highly negative charges were recorded for all actuations, suggesting that the milled budesonide had a higher pseudo-work function than the sieved lactose leaving its particles with an excess of electrons after separation from the sieved lactose.

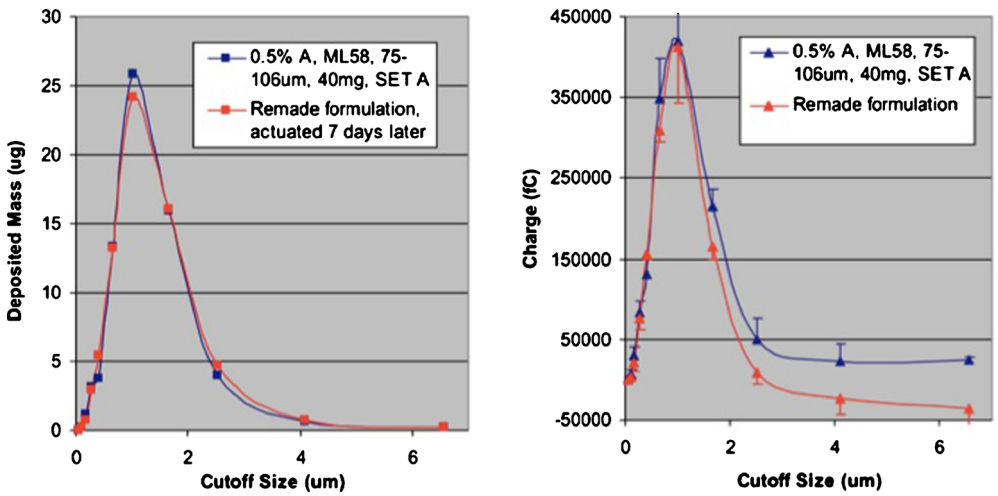


Fig. 5. Remade formulation (0.5% albuterol in 75–106 μm ML58 lactose) actuated from SET A (three times)

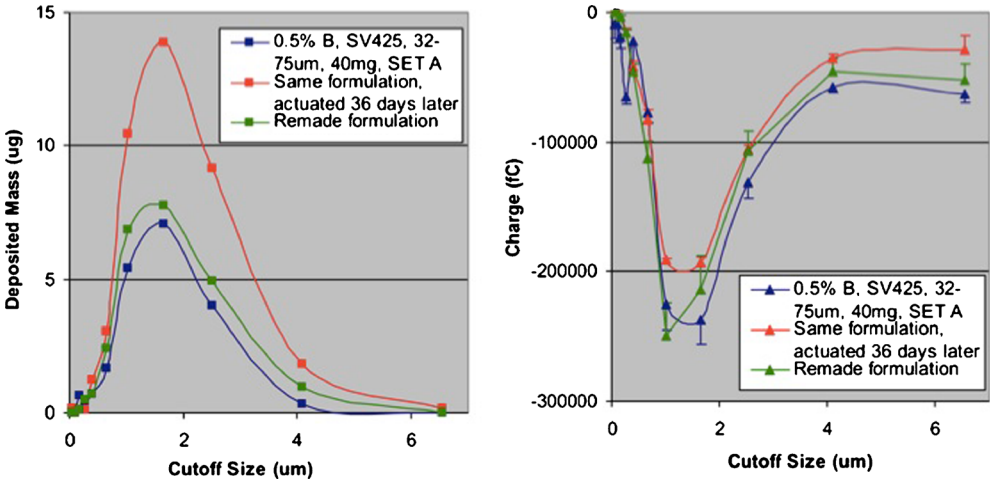


Fig. 6. Same formulation (0.5% budesonide in 45–75 μm SV425 lactose) actuated from SET A (three times) on different days, separated by 36 days of storage. Also shown remade formulation, actuated day after it was made. The remade formulation has FPF similar to the old formulation without storage, while storage results in higher deposition. Charge deposition profiles are similar for all

Environmental Effects

When drug particles enter the airways during inhaler actuation, they are entering a high humidity environment. Although high RH can inhibit triboelectrification, it is unclear if brief exposure is sufficient to dissipate particle charges. To determine the effect of high RH during aerosol transport, an ultrasonic humidifier was operated adjacent to the SET inlet, so that humid air was drawn into the apparatus. Actuations of 1% albuterol in ML58 lactose, 45–75 μm sieve fraction, and 20 mg actuated from SET A (same as Fig. 8) were performed. The charges recorded on the stages of the ELPI are shown in Fig. 9. The first two peaks show previous experiments performed at ambient humidity (35% RH), and the next three were performed at high humidity (95% RH). Then, the humidifier was turned off and another two actuations were performed at ambient humidity. While the high humidity greatly increases the noise on the stages, the actuations are essentially unchanged. Charge distribution and magnitude are the same as in a low humidity environment. This confirms that charging is a relevant phenomenon during clinical DPI use. Whether there is sufficient triboelectrification to affect particle trajectory and deposition was determined in later experiments.

Experimental Design Analysis: Effect of Formulation Factors on FPM and Particle Size Distribution

To understand how particle charge is affected by formulation and actuation factors, the charge levels need to be considered relative to the mass of the deposited aerosol particles. In the current project, charge distributions were recorded using the ELPI, then the mass deposition on each stage was determined by chemical assay. (Since the single depositions were close to the limit of detection on some stages, mass was assayed for a pooled set of three subsequent depositions of the same formulation and averaged.) As a result, we can discern how the formulation variables affect drug deposition and apply the results to the analysis of charging.

After each actuation experiment, the ELPI was disassembled and the stages assayed. The mass on each individual ELPI stage was determined, total deposited mass and FPF were calculated, and particle size distributions (PSDs) were generated. These measures are used in conjunction with the charging data, but it is also instructive to consider them individually, since they present a rich dataset of formulation effects on PSD. The PSDs are first discussed qualitatively, then in context of the statistical analysis.

Statistical Analysis: General

For the experimental design analysis, the PSD data were reduced to a smaller number of descriptive parameters

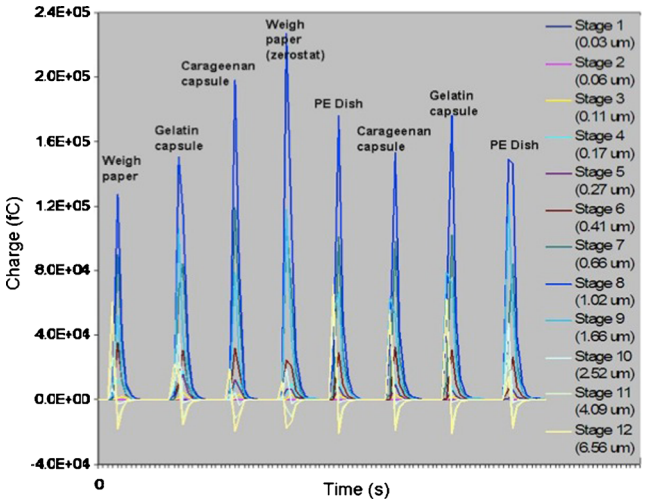


Fig. 7. Albuterol (1%) in ML58 lactose, 45–75 μm sieve fraction, and 20 mg actuated from SET A, off different surfaces. The eight actuations were performed in a row with intermittent placebo actuations. There are minor magnitude differences but the charge deposition profiles are largely the same. This supports the hypothesis that the charges observed are the result of triboelectrification during actuation, when particles have undergone charge transfer and those charges are exposed

Table II. Effect of Last Surface of Contact on Total Charge (Sum Across All Stages and Sum of Absolute Magnitudes Across All Stages) for 1% Albuterol in ML58 Lactose, 45–75 μm Sieve Fraction Formulation, 20 mg Actuated from SET A, Shown in Fig. 4. Range Is Given, Except in Last Row which Gives Actuation Average±Standard Deviation

Last surface of contact	Sum of charges (pC)	Charge magnitude (pC)
Paper (n=1)	559	663
Carageenan capsule	646–714	758–813
PE dish	781–821	886–908
Paper with Zerostat (n=1)	806	922
Gelatin capsule	829–860	934–941
Average	752±104	853±100

PE polyethylene

consisting of d_{25}, d_{50}, d_{75} (determined by linear interpolation of the particle size distribution data), and FPF, which were evaluated by multivariate statistics.

Since the drugs have different primary particle sizes, an evaluation of the entire dataset showed very strong codependency of the results on the identity of the drugs. This is expected. As a result, all significant effects contained the drug as a cofactor. To overcome this problem, the datasets for the two drugs were analyzed separately. The results of this analysis are summarized in Table V (budesonide) and Table V (albuterol).

Table V summarizes the significant effects of the formulation variables on the particle size and mass distribution of budesonide actuated from different formulations. The table follows the same format and gives the same kind of information as the subsequent result tables.

The first two rows in the table describe the data in terms of the mean (±standard deviation) and the range for the entire dataset. Subsequent rows show the results of the statistical analysis. The statistical analysis was conducted in SPSS using the general linear model (GLM) multivariate procedure. The procedure provides regression analysis and analysis of variance (ANOVA) for the dependent variables (*i.e.*, d_{25}, d_{50}, d_{75}, and FPF) by factor variables (*i.e.*, excipient, drug conc., fluidization conditions). The effects of factor variables on the means of dependent variable groupings were tested, and the interactions between factors and the effects of individual factors were probed. Only the effects of individual factors are reported in Table V and subsequent result tables because these are general and apply to different systems. The tables also provide effect size estimates based on the unbalanced full-factorial model (unbalanced because, as described above, certain factor combinations were not tested). The effect size describes the proportion of total variability attributable to a given factor. The confidence interval for each test is also given; the significance level was set to α=0.05, and only effects with lower p values are reported. For each category of factor (underlined), the cells to the right indicate whether the effect on the given response was significant. Where the effects are statistically significant, the p value is indicated and the rows below give the individual factor contribution (parameter) estimates; again, where these contributions are significantly different from the other variables of the category, the p value is indicated. One of the variables serves as a basis of comparison in each case and is assigned a contribution of 0; the effects of the other variables are given relative to it. The basis of comparison is SV94 lactose, 45–75 μm sieve fraction, 1.0% drug concentration, and the highly turbulent actuation conditions (SET A, Re=18,440). The model intercept is also given in the tables. This intercept provides the default response value in absence of the factor effects. Thus, to highlight an example from Table V, the d_{25} of budesonide after release from a 0.25% drug in ML80 formulation, actuated at Re=18,440, is expected to be 0.96–0.16+0.28+0.00=1.08 μm; this result is consistent with the raw data: the actual recorded value from experiments is 1.08±0.03 μm.

Statistical Analysis: Budesonide

Table V shows that the particle size distributions and FPFs have wide ranges. Yet, neither excipients nor drug concentrations of the formulation have a significant effect on the particle size distribution of the deposited drug. Only d_{25} is affected by the excipient, suggesting that the particle size distribution is most sensitive to formulation and actuation conditions in the smallest particle size range. The use of ML80 lactose and CP excipients results in the most dramatic shift in the d_{25} to a higher particle size (compared to SV94 lactose), suggesting relatively less separation of smaller drug particles. When considering the FPF of CP, which is significantly higher than that of the other excipients, one can see that the shift in the particle size is likely due to an overall increase in the release of drug, most of which is larger in size. Varying the drug concentration, on the other hand, does not have a statistically significant effect on the d_{25} of budesonide.

Table V confirms that d_{25}, d_{50}, and d_{75} of budesonide are all affected. The use of less turbulent (less energetic) actuation conditions, represented by a lower Re, results in the release of fewer fine particles and consequently an increase of all three particle size indicators to higher values. Remarkably, the actuation conditions did not have statistically significant effects

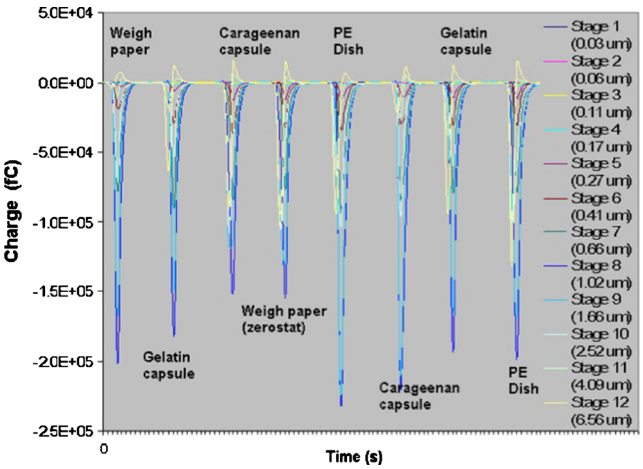


Fig. 8. Budesonide (0.5%) in SV425 lactose, 45–75 μm sieve fraction, and 20 mg actuated from SET A, off different surfaces. The eight actuations were performed in a row with intermittent placebo actuations. There are minor magnitude differences but the charge deposition profiles are largely the same. This supports the hypothesis that the charges observed are the result of triboelectrification during actuation, when particles have undergone charge transfer and those charges are exposed

Table III. Effect of Last Surface of Contact on Total Charge (Sum Across All Stages and Sum of Absolute Magnitudes Across All Stages) for 0.5% Budesonide in SV425 Lactose, 45–75 μm Sieve Fraction Formulation, and 40 mg Actuated from SET A. Range Is Given, Except in Last Row Which Gives Actuation Average±Standard Deviation

Last surface of contact	Sum of charges (pC)	Charge magnitude (pC)
Paper (n=1)	−795	834
Carageenan capsule	−1,080 to −1,086	1,125–1,136
PE dish	−1,209 to −1,426	1,254–1,447
Paper with Zerostat (n=1)	−1,077	1,129
Gelatin capsule	−822 to −959	873–1,003
Average	−1,057±205	1,100±199

PE polyethylene

on the FPF of budesonide, though this is in part the result of relatively few data points and high data variability for budesonide formulations. Changing the excipient appears to be a more reliable way of increasing the FPF than increasing the pressure drop during DPI actuation. The effects of excipient size fraction on budesonide delivery were not considered since only 45–75 μm sieve fractions were used for all excipients. (They were, however, examined for albuterol formulations.) The d_{50} value observed in the experiments (~2 μm) was in good agreement with the primary particle size measured by light scattering.

Statistical Analysis: Albuterol

The same analysis of particle size and FPF effects was conducted for albuterol; the results are shown in Table V. As explained in Section 2.2, more experiments in the experimental design involved albuterol formulations, and excipient particle size was also varied, hence the additional rows in the result table and more effects. Varying the excipient has a significant effect on each of the response variables, the entire particle size distribution, as well as the FPF. For d_{25} and FPF, the observed trends are largely in agreement with those observed for budesonide. Again, some of the most significant increases in particle size and FPF are observed for CP, indicating more efficient release of drug compared to the other excipients. Glucose provides another interesting result; the entire particle size distribution is shifted to higher particle sizes, while the FPF registers a decrease. This suggests that separation of fine particulate drug is limited, which supports prior suggestions that the small particles are most strongly affected by formulation changes. While the size fraction of the excipient does have statistically significant and consistent effects, the effects on the particle size are relatively minor. Use of the intermediate excipient sieve fraction (75–106 μm) appears to result in higher FPF in most cases. The concentration of drug has a significant effect on the FPF, with the 1% drug concentration resulting in significantly higher FPF than the 0.5% concentration. (The comparison to 0.25% is less significant, perhaps once more due to the limited number of experiments using 0.25% drug concentration.) This result supports the active site hypothesis, whereby the drug first adheres to the most energetic ("active") sites of the carrier particles and then (after active site saturation) distributes around the less energetic sites of the carrier particle, from which it can detach more easily. In contrast to budesonide, for which particle size and FPF were barely affected, albuterol formulations are significantly affected by the fluidization conditions. Using more turbulent actuation conditions (higher Re) gives reliably smaller particle size and higher FPF. This result suggests once more that primarily the separation of small particles is enhanced during actuation.

Experimental Design Analysis: Effect of Formulation Factors on Electrostatic Charging of Drug

Qualitative Analysis

As in the previous case, it is instructive to first look at the effects on drug charging qualitatively. Figure 10a–f shows the

Table IV. Effect of Excipient (All 45–75 μm Sieve Cut), Drug Concentration and Fluidization Conditions on the PSD (Using d_{25}, d_{50}, and d_{75} as Surrogate Markers), and FPF of Deposited Budesonide. In the Model, Created by SPSS Software, the Default Value Is Given by the Model Intercept and the Contribution for Each Factor Is Given in Each Column. Thus, for Example, the d_{25} of Budesonide From a 0.25% Drug in ML80 Actuated at Re=18,440 Would Be Expected to be 0.96–0.16+0.28+0.00=1.08 μm (Actual Recorded Value from Experiments Is 1.08±0.03 μm (n=2))

Factor/response	d_{25} (μm)	d_{50} (μm)	d_{75} (μm)	FPF
Data average±σ	1.38±0.28	1.99±0.22	2.73±0.31	0.056±0.061
Data range	1.04–2.05	1.75–2.56	3.34–2.39	0.002–0.219
Model intercept	0.96	1.83	2.59	0.093
Excipient	Significant (p=0.034)	Not significant	Not significant	Significant (p=0.001)
Lactose, SV94	0			0
Lactose, SV425	+0.19			−0.042
Lactose, ML58	+0.22			−0.056
Lactose, ML80	+0.28 (p=0.028)			−0.054
Glucose	+0.11			+0.037
Calcium phosphate	+0.38 (p=0.005)			+0.127 (p=0.001)
Drug concentration	Not significant	Not significant	Not significant	Not significant
Fluidization conditions	Significant (p=0.000)	Significant (p=0.000)	Significant (p=0.019)	Not significant
Re=18,440 (SET A)	0	0	0	
Re= 11,070 (SET C)	+0.25	+0.29	+0.55	
Re=7,720 (SET D)	+0.73	+0.70	+0.89	

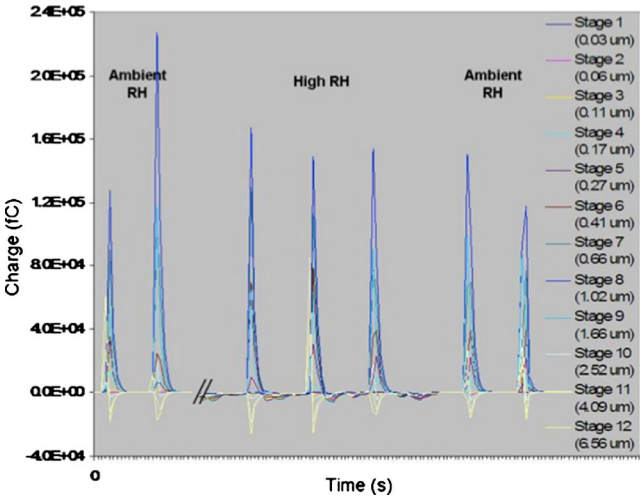


Fig. 9. Effects of concentration and actuation conditions (high-pressure drop SET A) and low-pressure drop (SET C) and very low-pressure drop (SET D) on the particle size distribution of albuterol in 45–75 μm ML80 lactose formulations

effects of varying the excipient on the mass deposition and the charge deposition of 0.5% albuterol in 40 mg of 45–75 μm sieve fraction excipient formulations, actuated from SET A (at high shear conditions). Figure 11a–f shows the same effects for budesonide formulations. The mass depositions of drug are shown in yellow diamonds (left axis), and the charge distribution (right axis) is shown in red squares.

From Fig. 11a–f, it is evident that the FPM and the deposited charges vary significantly from excipient to excipient. While the charge deposition is largely positive for albuterol drug actuated from milled lactose (subpanels a and b) and glucose (subpanel e), sieved lactose formulations (subpanels c and d) and the CP formulation (subpanel f) show bipolar charging. Clearly, the mass depositions vary, but the overall shapes of the particle size distributions are similar (as might be expected, since it is the same drug). Yet, the charge distribution is unique for each formulation. This clearly shows that the formulation has a profound effect on the charging behavior of the drug. Figure 11 makes the same point for formulations containing budesonide. The conditions are the same as in Fig. 11, *i.e.*, 0.5% budesonide in a 45–75-μm fraction of the excipient, 40 mg actuated from SET A. Note that in this case, the charge is mostly negative, with some bipolar charging observed for the formulation containing CP. Milled lactose formulations also show slight positive peaks in the 0.5-μm range, which, however, are likely due to the deposition of some residual excipient particles in that size range (actuations of excipient only are shown in blue triangles). The charge distributions of the different budesonide formulations are much more similar to one another, suggesting that the formulation has a smaller impact.

Charging Differences Between Polydisperse Particles

One striking feature observed throughout Fig. 11 is that different drug particle size fractions show differences in charging behavior. The charging behavior does not follow the particle size distribution and cannot be explained by mass depositions on the given stages. For instance, when delivered from SV425 lactose (Fig. 11c), albuterol particles smaller than ~0.5 μm charge positive, particles between 0.5 and 1.0 μm charge negative, whereas particles above 1 μm charge positive again. Based on the small size of the error bars (showing standard deviation of $n=3$ actuations), this charging pattern is quite reproducible. The effect is not related to the deposition of fine excipient particles either, which is minimal as

Table V. Effect of Excipient, Excipient Sieve Fraction, Drug Concentration, and Fluidization Conditions on the PSD (Using d_{25}, d_{50}, and d_{75} as Surrogate Markers) and FPF of Deposited Albuterol. In the Model, Created Using SPSS Software, the Default Value Is Given by the Model Intercept and the Contribution for each Factor Is Given in Each Column

Factor/response	d_{25} (um)	d_{50} (um)	d_{75} (um)	FPF
Data average±σ	0.94±0.18	1.47±0.16	2.10±0.25	0.101±0.051
Data range	0.64–1.24	1.29–1.97	1.80–3.12	0.015–0.194
Model intercept	1.05	1.32	1.80	0.193
Excipient	Significant ($p=0.000$)	Significant ($p=0.000$)	Significant ($p=0.000$)	Significant ($p=0.004$)
Lactose, SV94	0	0	0	0
Lactose, SV425	+0.30	+0.29 ($p=0.003$)	−0.08	−0.036
Lactose, ML58	+0.01	+0.03	+0.05	+0.134 ($p=0.014$)
Lactose, ML80	−0.11	+0.05	+0.10	−0.019
Glucose	+0.17	+0.31 ($p=0.033$)	+0.58 ($p=0.006$)	−0.088
Calcium phosphate	+0.49 ($p=0.054$)	+0.46 ($p=0.001$)	+0.65 ($p=0.001$)	+0.051
Excipient size fraction	Not significant	Significant ($p=0.015$)	Significant ($p=0.001$)	Significant ($p=0.006$)
45–75 μm		0	0	0
75–106 μm		−0.02	−0.04	+0.090 ($p=0.005$)
106–150 μm		+0.04	+0.09	+0.025
Drug concentration	Not significant	Not significant	Not significant	Significant ($p=0.003$)
1.00%				0
0.50%				−0.149 ($p=0.001$)
0.25%				−0.041
Fluidization conditions	Not significant	Significant ($p=0.000$)	Significant ($p=0.000$)	Significant ($p=0.015$)
Re=18,440 (SET A)		0	0	0
Re=11,070 (SET C)		+0.18 ($p=0.065$)	+0.35 ($p=0.013$)	−0.041
Re=7,720 (SET D)		+0.46 ($p=0.000$)	+0.57 ($p=0.000$)	−0.109 ($p=0.000$)

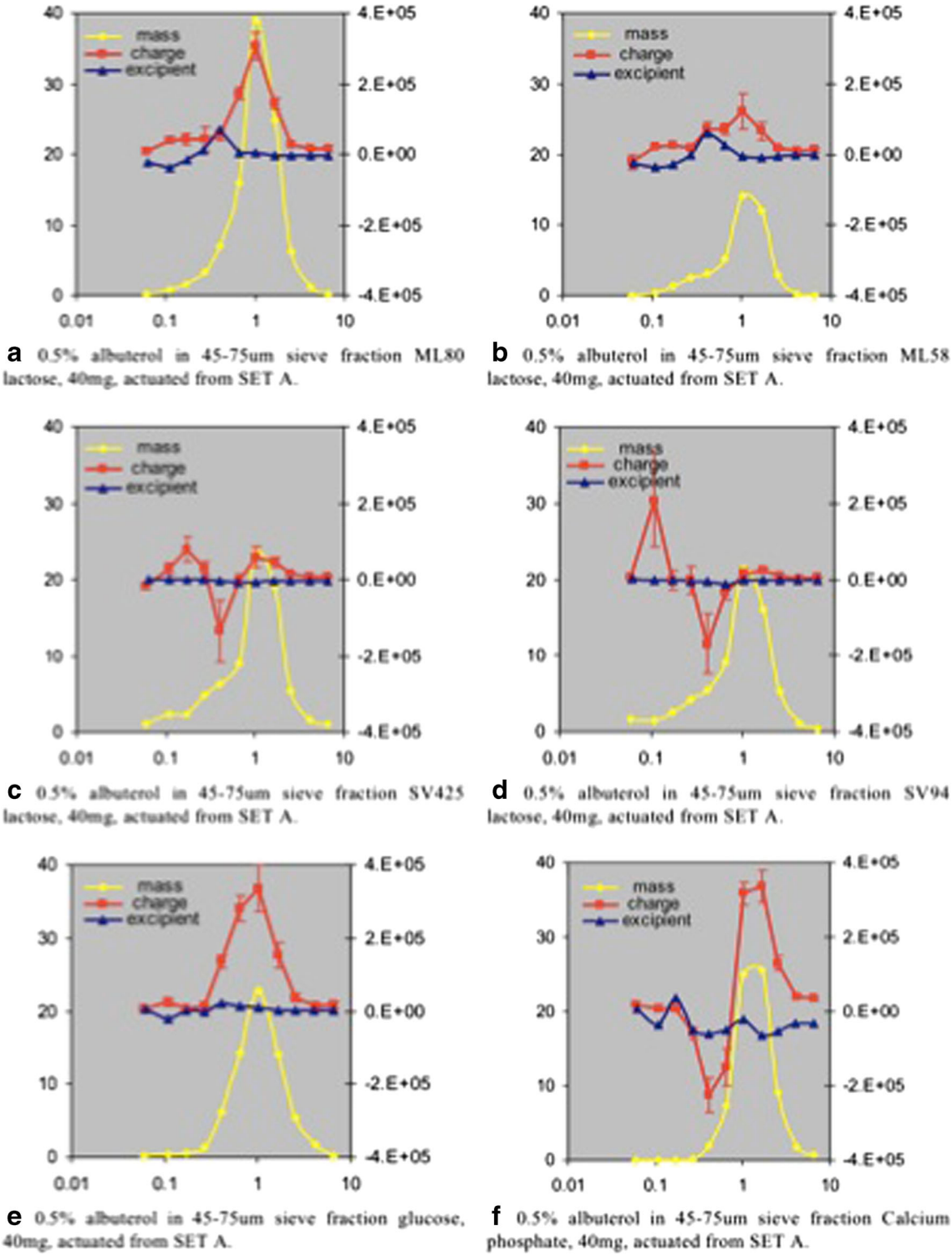
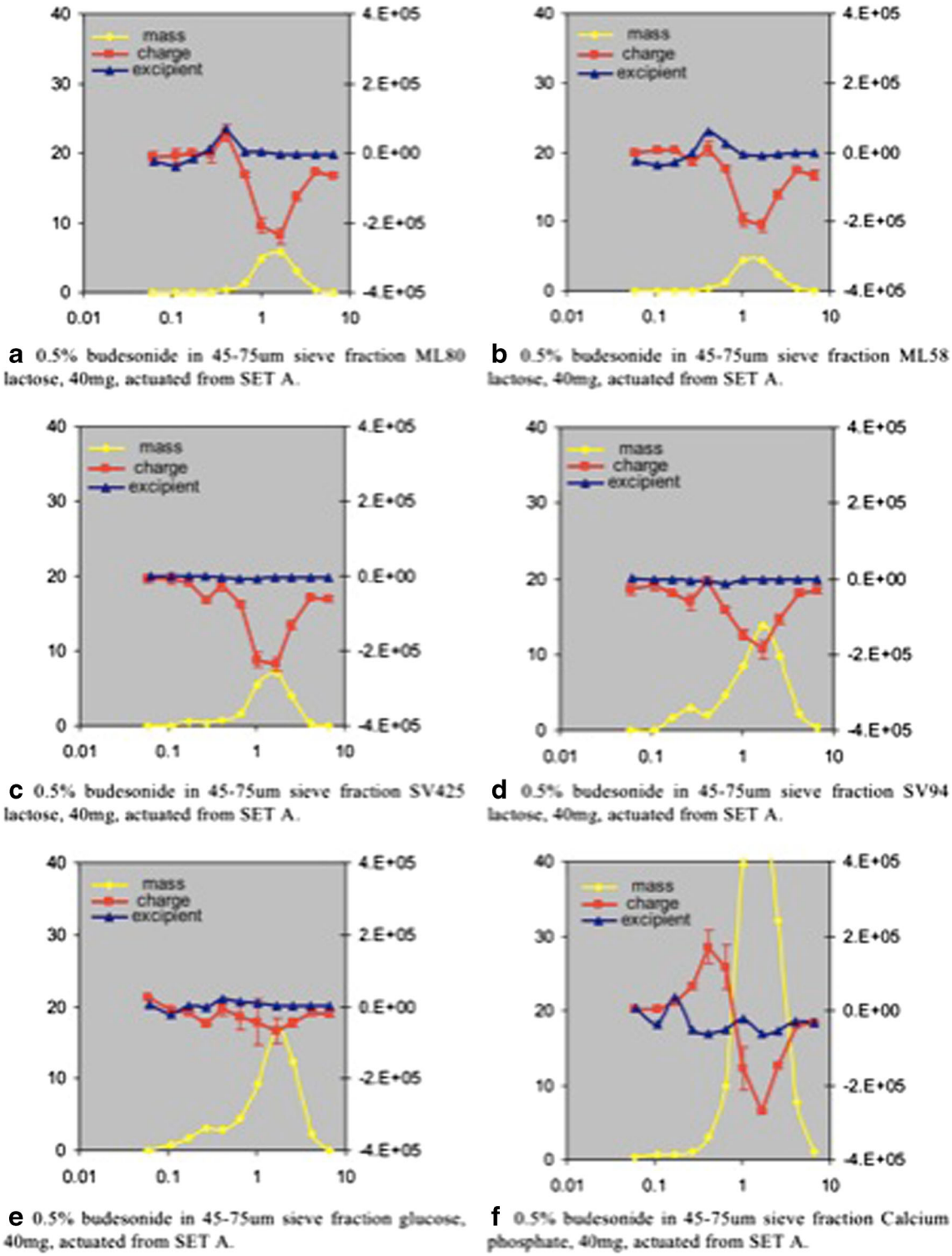


Fig. 10. Effect of formulation excipient on FPM and charge distribution of albuterol. Mass depositions of drug (μg, *yellow diamonds*, *axis on left*), charge distribution of formulation (fC, *red rectangles*, $n=3\pm\sigma$, axis on *right*), and excipient charging (fC, *blue triangles*, *right axis*) are shown. Lines were drawn between points for better visibility

shown by the blue graphs in the figures. Clearly, different drug size fractions have different charging properties.

The phenomenon of charging between particles within a polydisperse mixture of a material having the same chemical composition (and thus presumably the same effective work function) has been observed in other natural and industrial settings (27–29). In polydisperse mixtures with many surface contacts, it was found that smaller particles typically acquire an excess of negative charge while the larger particles tend to charge more positive (28–30). The phenomenon has been



a 0.5% budesonide in 45-75um sieve fraction ML80 lactose, 40mg, actuated from SET A.

b 0.5% budesonide in 45-75um sieve fraction ML58 lactose, 40mg, actuated from SET A.

c 0.5% budesonide in 45-75um sieve fraction SV425 lactose, 40mg, actuated from SET A.

d 0.5% budesonide in 45-75um sieve fraction SV94 lactose, 40mg, actuated from SET A.

e 0.5% budesonide in 45-75um sieve fraction glucose, 40mg, actuated from SET A.

f 0.5% budesonide in 45-75um sieve fraction Calcium phosphate, 40mg, actuated from SET A.

Fig. 11. Effect of formulation excipient on FPM and charge distribution of budesonide. Mass depositions of drug (μg, *yellow diamonds*, *left axis*), charge distribution of formulation (fC, *red rectangles*, $n=3\pm\sigma$, *axis on right*), and excipient charging (fC, *blue triangles*, *right axis*) are shown. Lines were drawn between points for better visibility

explained using a largely statistical argument in conjunction with particle dynamic simulations (31); it was postulated that high energy electrons are initially uniformly distributed and during a mixing process concentrate on the surface of the smaller particle fraction, leaving the smaller particles with an excess of electrons relative to larger particles in the mixture.

However, this explanation does not adequately describe the DPI system, where the larger drug particles are not

necessarily more positively charged. Moreover, in the DPI formulation, the drug particles are primarily in contact with the excipient. Both drug and excipient are somewhat disperse within some defined particle size ranges (in the figures, it is 0.1–8 μm for the drug and ~45–75 μm for the excipient particles). The bimodal charging phenomena observed in this study are more complicated and no explanation is offered. However, given the small drug particle size and the low concentration, the drug particles cannot be controlled directly. Instead, the formulation components and variables offer the better opportunity for drug particle control, so we now turn to the statistical analysis of the contributions of the formulation variables to drug triboelectrification.

Statistical Analysis: General

As was the case for the particle size and mass deposition data, the charge distribution data (consisting of charge distribution data for each ELPI stage over time) were consolidated into a smaller number of descriptive parameters and analyzed using SPSS. As before, the general linear model multivariate procedure was applied. The data in the tables, Tables VI (budesonide) and VII (albuterol), are organized as previously discussed. The data average is just the simple, non-weighted average of all measurements of a given response. This is important to remember because the statistical design is unbalanced as more experiments were conducted around a central set of conditions (*e.g.*, milled lactose excipient) than for the other formulations and conditions; this explains part of the variability.

It is clear that for both budesonide and albuterol formulations, the data variability is very large. Some of this variability is due to the naturally random element in charging phenomena. Some of the extreme data points may be due to experimental error. Nonetheless, to prevent the introduction of bias into the analysis, no data points were removed from the analysis. Since the dataset is extensive, the analysis is not overwhelmed by a few outliers.

Two important response variables were the total charge and the dominant polarity. However, to understand the strength of the charging phenomena, it is important to determine charge effects relative to the quantity of drug that is delivered. Charge deposition was recorded using the ELPI and deposition of drug was determined from chemical assay of the ELPI stages. Having this data, there are a number of different ways in which the two measures could be correlated. Several different response factors were evaluated. Each response variable correlates charge to quantity of drug in a different way using a set of assumptions; the response variables used are normalized for mass or normalized for surface area. The different response factors are described in detail:

1. Polarity of charge is determined by summing all the charges recorded for a given deposition across all the stages and then, depending on the polarity of the charge, assigned a −1 or a +1. This response variable is simple and requires no assumptions. However, the response is nominal (+1 or −1) and thus not scalable.
2. The total of absolute charge (Sum $|Q|$), given in units of nanocoulombs, is the sum of absolute values of charge recorded across the different ELPI stages. This response does not take into account the quantity of material delivered. It is nevertheless considered as a first step, since the charging phenomena could also turn out to be a property of the act*u*ation process, relatively independent of mass.
3. Absolute charge per mass (Sum $|Q|/m$), given in units of microcoulombs per gram, is determined by summing all the absolute values of the charges recorded across all the ELPI stages and then dividing by the total mass that was recovered from all the stages.
4. Charges/area, given in elementary charges ($1e^{-}=1.6\times 10^{-19}$ C) per square micrometer, is the sum of charges divided by the surface area of the deposited drug particles. This response variable assumes that deposited drug on each stage consists of spheres with particle size exactly at the midpoint between the cutoff sizes of the corresponding and the previous stage. This is an oversimplification as the drug particles are prismatic in shape and have a range of sizes. The assumption of sphericity underestimates the true surface area; thus, the true charges/area measures are likely to be smaller. But this simplification should not affect the outcome of the analysis, since it applies the simplifying assumption to all size fractions for each drug.
5. Average charges per particle (stages 7–10) is the estimated per-particle charge (in elementary charges) for drug deposited on stages 7–10, which includes particles from 0.655 μm (cutoff size of stage 7) to 4.09 μm (cutoff size of stage 11). These four stages account for most of total drug deposited inside the ELPI (in most cases, over 85%). Due to the high material depositions on these stages, there is a high level of confidence associated with the mass determinations. Using the material densities (1.3 g/cm^3 for albuterol, 1.25 g/cm^3 for budesonide) and basic geometric assumptions (particles are uniform spheres of average size for a given stage), the mass depositions on each stage are converted into numbers of particles. The charge collected on each stage is given in elementary charges, which are divided by the number of particles. This measure is an oversimplification, though the approach is uniform and does not introduce any bias into the statistical analysis. While the charge density (charge per area, in (4)) is physically the most important response, the "per particle" charge is probably the most clinically relevant measure. After all, the total charge on a particle determines its trajectory and deposition.

The three normalized response variables (3–5) follow the same basic trends in most cases and are not discussed individually in detail.

Statistical Analysis: Budesonide

Table VII summarizes the effects of formulation variables on the charging behavior of budesonide particles. The drug consistently charged negatively and the polarity was not affected by the type of excipient. Every other response was significantly affected by the choice of excipient, the

Table VI. Formulation Variables Contributing to the Charging of Budesonide. Effect of Excipient, Excipient Size Fraction, Drug Concentration, Fluidization Conditions, and Dose on the Charging Behavior of Fine Particulate Albuterol. In the Model, Created Using SPSS Software, the Default Value Is Given by the Model Intercept and the Contribution for Each Factor Is Given in Each Column. Also Shown Is the Significance Level of the Formulation Variable (from ANOVA), as well as the Level of Significance for Each Individual Variable Level (Where $p \leq 0.05$)

Factor/response	1. Polarity of charge	2. Sum $\|Q\|$ (nC)	3. Sum $\|Q\|/m$ (μC/g)	4. Charges/area ($e^-/\mu m^2$)	5. Average charges per particle (stages 7–10)
Data average±σ	−1±0	0.73±0.27	64.8±76.4	1,333±2,003	6,112±7,930
Data range	−1	0.35–1.31	6.9–304.1	70–8,054	363–32,312
Model intercept	−1	1.20	−8.8	−294	−296
Excipient	Not Sign.	Significant (p=0.008)	Significant (p=0.000)	Significant (p=0.000)	Significant (p=0.003)
Lactose, SV94		0	0	0	0
Lactose, SV425		+0.11	+23.3 (p=0.006)	+379 (p=0.005)	+1,640 (p=0.039)
Lactose, ML58		+0.00	+40.7 (p=0.001)	+759 (p=0.000)	+3,210 (p=0.005)
Lactose, ML80		+0.11	+38.6 (p=0.001)	+762 (p=0.000)	+2,690 (p=0.011)
Glucose		−0.31 (p=0.020)	−9.8	−104	−971
Calcium phosphate		+0.24 (p=0.047)	−7.6	−76	−874
Drug concentration	Not significant	Significant (p=0.000)	Significant (p=0.000)	Significant (p=0.000)	Significant (p=0.000)
1.00%		0	0	0	0
0.50%		−0.43 (p=0.010)	+25.4 (p=0.008)	+487 (p=0.003)	+1,690 (p=0.056)
0.25%		−0.90 (p=0.000)	+0.2	−156	+773
Fluidization conditions	Not Sign.	Significant (p=0.003)	Significant (p=0.006)	Significant (p=0.000)	Significant (p=0.000)
Re=18,440 (SET A)		0	0	0	0
Re=11,070 (SET C)		−0.37 (p=0.010)	+10.2	+258 (p=0.022)	+2,040 (p=0.018)
Re=7,720 (SET D)		−0.52 (p=0.004)	+248.8 (p=0.000)	+7,100 (p=0.000)	+2,820 (p=0.000)
Dose (mg)	Not Sign.	Significant (p=0.003)	Significant (p=0.006)	Significant (p=0.010)	Significant (p=0.041)
40		0	0	0	0
20		−0.33 (p=0.028)	+2.0	+38.6	+158

concentration of the drug, the fluidization conditions, and even the dose. The effects of drug concentration, fluidization conditions, and dose on the absolute magnitude of the charge are expected and are related in part to the larger drug depositions. However, as the mass normalized charge measures show, the effects are smaller than might be expected based on mass depositions alone. On a per particle basis, the milled lactose batches result in the strongest (negative) charging of the drug particles, while glucose and CP result in least charge per particle. The lower drug concentrations of 0.25% and 0.50% produce higher particle charges than the higher 1% drug concentration formulations. This may be the result of stronger contact charging due to closer drug–excipient contact in lower concentration drug blends. (The smallest charge contribution of the 0.25% formulation does not support this conclusion, though the effect is variable and lacks statistical significance.) The charge contribution associated with the drug concentration is minor compared with the effects of excipient or fluidization conditions. Fluidization conditions significantly affect the charge. The parameter estimates for this effect are counterintuitive: the analysis reveals higher per particle charges for the less intense fluidization conditions (Re=11,070 and 7,720) than the more turbulent conditions (Re=18,440). A possible explanation is that when fewer particles are dispersed, the likelihood of contact and charge backflow in transit is smaller, *i.e.*, it is less likely that opposite charges cancel each other. This explanation is supported by the fact that the smaller actuation dose also gives rise to more highly charged particles. Additionally, lower Re fluidization conditions result in the release of fewer fine particles shifting the distribution to larger particles, which have larger surface areas and can accommodate more charges.

Statistical Analysis: Albuterol

The effects that formulation variables have on the charging of albuterol are summarized in Table VII. With a larger number of trials conducted, the dataset around albuterol is richer (it includes an analysis of excipient size fractions as well), and as a result, there is more confidence in the individual parameter estimates. The excipient clearly impacts the polarity of charge. Whereas budesonide always charged negatively, albuterol charged either positive or negative, depending on the excipient in the formulation. Formulated with SV94 lactose, albuterol particles were predominantly negatively charged. Formulated with the other sieved lactose batch, SV425, albuterol particles were either negatively or positively charged, whereas in combination with glucose and CP, albuterol primarily charged positively. It can be deduced that albuterol has a pseudo-work function that is somewhere in between that of sieved lactose and glucose. No other variables affected charge polarity significantly. However, all formulation variables, *i.e.*, excipient, excipient size fraction, drug concentration, and fluidization conditions, had effects on absolute and relative charge magnitudes. In the case of drug concentration, fluidization conditions, and dose, larger charges were associated with increased deposition of drug, that is higher concentration, more turbulent charging, and larger dose resulted in higher charge depositions. However, a look at the normalized responses shows that increased mass delivery does not fully account for the

Table VII. Formulation Variables Contributing to the Charging of Albuterol. Effect of Excipient, Excipient Size Fraction, Drug Concentration, Fluidization Conditions, and Dose on the Charging Behavior of Fine Particulate Albuterol. In the Model, Created Using SPSS Software, the Default Value Is Given by the Model Intercept and the Contribution for Each Factor Is Given in Each Column. Also Shown Is the Significance Level of the Formulation Variable (from ANOVA), as well as the Level of Significance for Each Individual Variable Level (Where $p \leq 0.05$)

Factor/response	1. Polarity of charge	2. Sum $\|Q\|$ (nC)	3. Sum $\|Q\|/m$ (μC/g)	4. Charges/area ($e^-/\mu m^2$)	5. Average charges per particle (stages 7–10)
Data average±σ	0.74±0.68	0.77±0.52	6.3±12.9	142±80	542±381
Data range	−1–+1	0.89–2.12	1.5–30.5	16.5–395.3	43–2,092
Model intercept	0	0.72	−6.1	−49.9	−277
Excipient	Significant (p=0.011)	Significant (p=0.013)	Significant (p=0.001)	Significant (p=0.000)	Significant (p=0.000)
Lactose, SV94	−1	0	0	0	0
Lactose, SV425	± 0	+0.26	+10.6 (p=0.008)	+127.2 (p=0.002)	+138
Lactose, ML58	+1 (p=0.000)	+2.10 (p=0.009)	+16.2 (p=0.026)	+207.3 (p=0.005)	+842 (p=0.01)
Lactose, ML80	+1 (p=0.000)	+0.63	+13.2 (p=0.002)	+117.1 (p=0.002)	+597 (p=0.03)
Glucose	+1 (p=0.000)	−0.08	+16.4 (p−0.016)	+191.1 (p=0.005)	+1,236 (p=0.000)
Calcium phosphate	+1 (p=0.000)	+1.47 (p=0.012)	+19.2 (p=0.002)	+316.9 (p=0.000)	+1,599 (p=0.000)
Excipient size fraction	Not Sign.	Significant (p=0.015)	Significant (p=0.000)	Significant (p=0.001)	Significant (p=0.006)
45–75 μm		+0.34	+9.6 (p=0.018)	+96.4 (p=0.013)	+216
75–106 μm		0	0	0	0
106–150 μm		−0.91 (p=0.028)	−5.2	−47.2	−135
Drug concentration	Not Sign.	Significant (p=0.007)	Significant (p=0.001)	Significant (p=0.005)	Significant (p=0.002)
1.00%		0	0	0	0
0.50%		−0.61	+13.8 (p=0.014)	+86.9	+245
0.25%		−0.87 (p=0.035)	+6.1	+72.2 (p=0.048)	+216
Fluidization conditions	Not Sign.	Significant (p=0.002)	Not Sign.	Significant (p=0.010)	Significant (p=0.000)
Re=18,440 (SET A)		0		0	0
Re=11,070 (SET C)		−0.79		−3.7	+267
Re=7,720 (SET D)		−0.78 (p=0.030)		+110.5 (p=0.003)	+65
Dose (mg)	Not significant	Significant (p=0.004)	Significant	Not significant	Not significant
40		0	0		
20		−0.50	+2.59		

additional charge. As seen before with budesonide, smaller drug concentrations and less turbulent actuation conditions result in higher per particle charges. The positive effect of the smaller drug concentration on charge supports the active site hypothesis. The lower Re actuation conditions also result in higher per particle charges, though the effects are variable and the individual parameter contributions lack statistical significance. This result is in agreement with the results for budesonide. As stated previously, one explanation for this is the shift towards larger particle sizes, which can accommodate larger charges. Furthermore, fewer dispersed particles create fewer opportunities for charge neutralization or backflow. Excipient particle size also contributes to particle charging. The charge of the delivered drug decreases as the excipient particles increase in size. While the overall contribution of the effect is modest, the effect is nonetheless consistent with a surface area effect; as the carrier particles become larger, their specific surface area decreases and the drug particles reach a higher degree of surface saturation on the carrier particle surface. The drug particles become less charged because there is less surface area available with which to exchange electrons. This explanation may be seen as an extension of the active site hypothesis, provided the highly energetic surface sites are also more prone to electron exchange.

It is noteworthy that CP and glucose result in the least charged budesonide particles but give rise to the most highly charged albuterol particles. These observations seem at odds but are actually consistent with each other when the polarities of the charges are considered: in both instances, CP and glucose result in the most positive drug charges. This allows CP and glucose to be placed at the very top of the triboelectric series. Construction of a triboelectric series for the materials and the ramifications thereof are discussed in the next section.

Per Particle Charge Levels

One of the primary aims of this research was to determine the role electrostatic charging plays in DPI particle interactions. A number of simulation (32) and *in vivo* (33) studies have suggested that micron-size particles need to have charges in the 100 s of elementary charges range to be affected in their trajectory and their deposition in the respiratory tract. Using Faraday cage data and some basic geometric assumptions, Byron *et al.* (34) estimated that DPI particles could have charges in the 200e per particle range.

The results of this study indicate triboelectric charging in the DPI can give rise to significant charging with charge levels that are much higher than previously estimated. Surveying the data range for budesonide formulations (Table VII) and albuterol formulations (Table VII), the data indicate per particle charge levels of 542±381 and 6,112±7,930 elementary charges

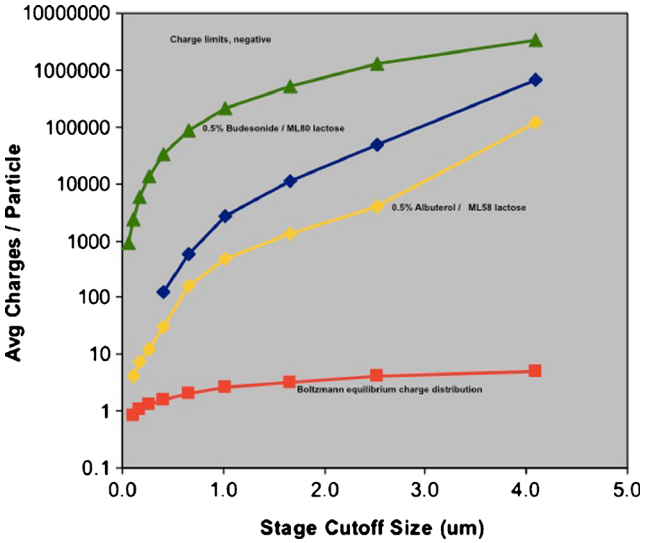


Fig. 12. Charge distribution (average particle charge) for drug deposited in ELPI. Shown are 0.5% budesonide in ML80 lactose (45–75 μm) (*blue diamonds*) and 0.5% albuterol in ML58 lactose (45–75 μm) (*yellow diamonds*), both actuated from SET A (high Re). For comparison, the graph shows the charge limit expected for the particles (*green triangles*) and the Boltzmann charge distribution (*red squares*). In both cases, over 80% of the deposited particles carry >100 charges per particle

per particle (unbalanced, non-weighted means of entire dataset ±standard deviation) on albuterol and budesonide particles in the 0.655-μm (stage 7) to 4.09-μm (stage 10) range, respectively. Particles in this size range account for the bulk of the drug delivered to the lungs. Figure 12 shows the entire charge distribution (absolute magnitude) for two representative formulations, 0.5% budesonide in ML80 lactose (45–75 μm sieve fraction) and 0.5% albuterol in ML58 lactose, as well as the upper and lower charge limits. The lower limit is the Boltzmann equilibrium charge distribution, which is the charge level expected for naturally occurring aerosols. The average number of charges, $\overline{n}$, for aerosol particles can be estimated by Eq. 1:

$$\overline{n} \approx 2.37\sqrt{d_p} \tag{1}$$

where d_p is the particle diameter given in micrometer. Equation 1 is an empirical relationship that is accurate to within ±5% for particles greater than 0.2 μm (26). The Boltzmann distribution predicts charges of less than ±5 (elementary charges) for most particles with d_p<5 μm.

While the Boltzmann charge distribution gives the lower limit of charge for an aerosol, the maximum number of negative charges that a particle can support, n_L, is given by Eq. 2.

$$n_L = \frac{d_p^2 E_L}{4 K_E e} \tag{2}$$

where d_p is the particle diameter, E_L is the surface field strength required for spontaneous electron emission (9.0×10^8 V/m), K_E is the Coulomb's law constant, and e is the charge of an electron, 1.6×10^{-19} C (26). Above this upper limit, spontaneous emission of electrons from a surface is expected. The graphs indicate very high charges, well into the 1,000 s and even 10,000 s of elementary charges per particle range. These charges (not the highest charge levels recorded in these experiments) far exceed the Boltzmann equilibrium charge distribution and clearly implicate an active charging process, specifically contact charging in the formulation. As indicated in Table VII, the formulation variables have significant effects on the charge distribution and can produce much higher particle charge levels.

SUMMARY AND CONCLUSION

The ELPI offers significant advantages for studying electrostatic interaction between the particles in DPI formulations. The most significant advantage, which sets the instrument apart from other scientific instruments or experimental apparatuses, is its ability to measure particle size and charge distributions simultaneously for particles in the single-micron range, the size range required for respiratory drug delivery. An experimental apparatus was developed that allowed different DPI formulations to be tested at the commonly used 60 L/min flow rate and deposition to be measured within the ELPI. An experimental framework was devised to test which formulation factors contribute to triboelectric charging in DPI formulations. The framework included roughly 100 experiments (including replicates and auxiliary experiments), of which a number of replicates had to be excluded due to storage effects. While out of scope of this discussion, the storage effects (highlighted in Fig. 12) are significant and warrant further investigation.

The remaining experiments offer an extensive dataset of charge and mass deposition data that give insight into which formulation factors contribute to triboelectric charging in the DPI. The choice of excipient is clearly important and most profoundly affects the charge characteristics of the deposited drug. Batch-to-batch variability in the behavior of the four lactose batches was clearly indicated; it is clear that some excipients behave differently when combined with albuterol or budesonide, which shows the difficulty in determining certain effects *a priori*.

The particle size of the excipient also appears to have an effect on the charge profile of deposited drug, with smaller particles (with increased surface area) giving rise to stronger charging interactions.

The concentration of drug in the DPI formulation was also shown to affect the particle charge; lowering the drug concentration results in lower fine particle fractions with more highly charged drug particles, a result that adds weight

Table VIII. Using Formulation Techniques to Affect Drug Charging

To minimize drug particle charge	To maximize drug particle charge
• Increase drug coverage (drug concentration) of excipient particle	• Reduce drug concentration
• Increase excipient particle size	• Decrease excipient particle size
• Use excipients with similar effective work function (*i.e.*, close position in triboelectric series)	• Use excipients with different effective work function (*i.e.*, further removed from drug in triboelectric series)

to the often cited "active site hypothesis": due to lower surface coverage, drug particles are concentrated around more energetic carrier particle surface sites where forces are stronger and electron exchange may be more extensive; as a result, fewer particles separate from the carrier.

The actuation conditions also have a strong effect on the particle size and charge distribution of drug. Contrary to expectations, however, more turbulent conditions result in somewhat lower per particle charge levels. This result is consistent with the idea that contact charging is the cause of the particle charge and that fluidization merely affects the particle separation process. A lower delivered dose also slightly raises the particle charge level. An explanation for these results is that a smaller amount of dispersed drug decreases the chance of opposite charges canceling each other during transit or deposition.

While the study provided statistically strong evidence for profound effects of some formulation variables, the study design also had some weaknesses. The inherent variability of the charging phenomena was underestimated; as a result, some of the marginal conditions that were written into the design to test response surface curvature lacked sufficient data and, thus, statistical power to give conclusive results: the very low drug concentration of 0.25% and the very low Re actuation conditions have too few response points and too much variability to provide trends.

The results indicate that particle charging in DPI formulations is highly significant. Charging is at least one order of magnitude higher than had previously been estimated, and greater particle charges are generated during DPI actuation than in many other industrial or laboratory processes. The particle charge levels are indicative of the active contact charging process and provide a scientific basis for the problems with electrostatic charging which have been observed in the clinical use of respiratory delivery devices, MDIs and DPIs.

The charge levels are high enough to have clinical consequences, either due to change in trajectory, adhesion to device, or modified *in vivo* deposition patterns. The study suggests electrostatic interactions should be studied during DPI formulation development. Failure to consider the process is, at best, a lost opportunity to moderate a phenomenon that can be affected through formulation considerations. Table VIII summarizes how charging can be affected.

ACKNOWLEDGMENTS

Dr. Chris Wiesen, Statistical Consultant at the UNC Odum Institute, is gratefully acknowledged for valuable discussions of the experimental design and help with the analysis. M. Telko gratefully acknowledges the financial support from a U.S. Pharmacopeia Fellowship and PhRMA Foundation Predoctoral fellowship during the completion of this work.

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