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Novel Spherical Lactose Produced by Solid State Crystallisation as a Carrier for Aerosolised Salbutamol Sulphate, Beclomethasone Dipropionate and Fluticasone Propionate

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Abbreviations: SEM, Scanning electron microscopy; ESDL, Engineered Spray Dried Lactose; SSC, Solid State Crystallisation; DPIs, dry powder inhalers; TED, total emitted dose; FPD, fine particle dose; HPLC, high performance liquid chromatography; PIF, peak inspiratory flow; Vin, inhaled volume; MMAD, mass median aerodynamic diameter; TRD, total recovered dose; TRA, total residual amount.

Abstract

The purpose of the present work was to engineer lactose carrier particles for inhalation using a solid-state crystallisation of amorphous spray dried lactose approach. A suspension of spray dried lactose was contacted with hot ethanol for 10 and 30 seconds to produce spherical particles ($ESDL_{10}$) and ($ESDL_{30}$) with different degrees of crystallinity, particle size, and controlled surface rugosity. Lactohale® (control) and engineered spray dried lactose (ESDL) particles were characterised by Scanning Electron Microscopy, X-ray Powder Diffraction and Tribo-electrification. Lactohale® and engineered lactose particles were mixed separately with salbutamol sulphate (SS), beclomethasone dipropionate (BDP) and fluticasone propionate (FP) and each formulation was assessed for drug content uniformity, drug segregation after tribo-electrification and drug deposition using Andersen Cascade Impactor (ACI). Lactohale® showed the highest but opposite affinity for electrical surface charges compared to engineered lactose. Lactohale® showed the greatest variation in drug content uniformity with SS but to a lesser extent with BDP and FP, whereas the ESDL carriers produced an acceptable uniform mix with all drugs. SS-Lactohale® formulation showed the highest segregation after tribo-electrification up to 119-fold in comparison to that observed with SS-engineered lactose. $ESDL_{10}$ carrier promoted a better drug deposition for both BDP and FP and showed the least variation in both content uniformity and FPD with all three drugs. Therefore, production of crystalline spherical lactose carrier with controlled surface texture, size and crystallinity is achievable using solid state crystallisation for DPIs, whilst providing less variation in drug content uniformity and consistent fine particle dose to the lungs in-vitro for both hydrophilic and hydrophobic drugs.

Key words: *Solid state crystallisation, Tribo-electrification, Salbutamol Sulphate, Beclomethasone Dipropionate, Fluticasone Propionate*

1. Introduction

Lactose, 4-(13-D-galactosido-)-D-glucose, is commonly used excipient in oral solid dosage forms. The reasons for its popularity are safety, cost-effectiveness, availability and compatibility with most drugs and excipients (Steckel et al., 2004; Pilcer et al., 2010). In a solid state, lactose can either be in crystalline or amorphous state. The most well-known crystalline forms are α-lactose monohydrate and β-lactose. The anhydrous form also exists as α-lactose and β-lactose (Koo, 2016). A mixed crystalline form of α and β lactose was also reported under specific crystallisation conditions (Larhrib et al., 2003a). When crystallisation is performed at low temperatures below 93.5 ºC from a supersaturated lactose solution, a so-called α-lactose monohydrate is exclusively formed, whereas β-lactose is obtained at temperatures over 93.5 ºC (Nickerson, 1974). During crystallisation of β-lactose, no water is incorporated in the crystal lattice. Therefore, the crystals of β-lactose exist in a non-hygroscopic, anhydrous form only in contrast with α-lactose, which can occur both as the monohydrate and as anhydrous α-lactose. Lactose is known to crystallise in an elongated shape such as tomahawk or needle shape (Larhrib et al., 2003a). The tomahawk shape is obtained when the crystallisation of a supersaturated α-lactose monohydrate solution is carried out slowly at ambient temperature. Elongated, needle-shaped lactose crystals were also observed at high supersaturation, forcing the crystals to form rapidly by precipitation, for example in the presence of a non-solvent such as acetone (Larhrib et al.,2003a).

The aerosolisation efficiency of a powder for inhalation is highly dependent on the carrier characteristics, such as particle size distribution (Kinnunen et al., 2014), particle shape (Zeng et al., 2000a; Larhrib et al., 2003b) and surface properties (De Boer et al., 2012). The main objective in the inhalation field is to achieve a reproducible, high pulmonary deposition. This can be achieved by successful carrier selection and careful process optimisation (Pilcer et al., 2012). It is known that the attractive forces between drug and carrier particles can be shape dependent (Mullins et al., 1992; Crowder et al., 2001). In vitro inhalation studies have indicated that elongated (Larhrib et al., 2003b; Zeng et al., 2000a), needle-like (Ikegami et al., 2002), porous and wrinkled particles (Chew et al., 2005) have improved lung deposition properties of various formulations. Larhrib et al., (2003b)

found that as the elongation ratio of lactose carrier was increased, the flow of the carrier was reduced and consequently impacted on content uniformity of salbutamol sulphate and as the result the drug emission from the inhaler device was also affected. A spherical shaped lactose carrier can be more preferred compared to other carrier shapes, because its good flowability and contact area between the spherical particles and adhered particles is less than non-spherical particles (Cooley, et al., 2018). It was reported that spherical pollen-shaped hydroxyapatite carrier increased dispersibility of budesonide particles due to a reduction in particle interactions (Hassan et al., 2010).

Spherical particles are desirable to produce solid dosage forms due to their good flow properties and consistent drug loading capacity in comparison to other shapes. Unfortunately, spherical shaped lactose is challenging to produce by crystallisation from solution in comparison to other shapes. Some techniques, such as spray drying, are known to produce hollow and spherical particles but a drawback is the formation of amorphous content. Therefore, the first aim of the present work was to produce novel hollow engineered spherical crystalline lactose carrier particles with controlled size, surface properties and crystallinity using solid state crystallisation (SSC). The Engineered Spray Dried Lactose (ESDL) and Lactohale® (control) were then characterised using Scanning Electron Microscopy (SEM), X-ray Powder Diffraction (XRD) and Triboelectrification. The second aim was to investigate the suitability of this novel lactose as a carrier for DPIs with hydrophilic and hydrophobic model drugs, namely, salbutamol sulphate (SS), beclomethasone dipropionate (BDP) and fluticasone propionate (FP)

2. Materials and Methods

2.1 Materials

Micronized salbutamol sulphate (VMD= 2.4 µm), beclomethasone dipropionate (VMD= 2.3 µm) and fluticasone propionate (VMD= 4.3 µm) (GSK, UK) were used as model drugs. Lactose α-monohydrate (Lactohale® 200) was purchased from DFE Pharma, UK. Ethanol, Absolute was purchased from Fisher Scientific, UK. Ultrapure water system was purchased from Thermo Fisher scientific, UK.

2.2 Methods

- *Preparation of Lactohale® Carrier*

Lactohale® was sieved to obtain a relatively narrow size distribution (63-90μm) to match approximately the particle size of Engineered Spray Dried Lactose (ESDL). Lactose is brittle, and as a result, the powder was sieved manually and slowly for about 30 minutes to limit the particle abrasion, breakage and tribo-charging effect which can occur during a conventional mechanical sieving. The powder was then collected in a sealed glass jar and stored in a desiccator over silica gel until required for further investigation.

- *Production of Spray-Dried Lactose Particles*

A pre-determined amount of Lactohale® was dissolved in ultra-pure water at room temperature to obtain 10% w/v lactose solution. The resulting lactose solution was spray-dried using a laboratory scale spray dryer (SD-06 spray-dryer, Labplant, UK). The spray drying conditions were inlet temperature 180°C; outlet temperature of 102°C; solution feed rate 4rpm (2ml/min); air pressure: (3 bars); 0.5mm spray nozzle. The spray-dried lactose was recovered from the collecting jar and transferred into a glass vial before storing in a desiccator over silica-gel until required.

- *Solid State Crystallisation of Spray Dried Lactose.*

A 100 ml of absolute ethanol (Fisher, UK) was poured into a 600mL glass beaker and transferred to the fume hood, where ethanol was allowed to boil using a hot plate (Cole-Parmer®, UK). A pre-determined quantity of spray-dried lactose particles (about 10 g) was introduced into the hot solvent for 10 seconds while stirring the lactose suspension at 250 rpm. The beaker was then removed from the hot plate and the lactose suspension was filtered using a 500μm sieve into a collecting pan to remove any aggregates. The recovered crystallised spray-dried lactose suspension was dispersed using cool air generated by a hair drier to avoid polymorphic transformation which can be caused by the application of heat. This step was

followed by drying the ESDL in a ventilated oven at 45°C for 48 hours (Memmert, Germany). The particles were collected from the collecting pan as a free-flowing powder and allowed to cool to room temperature for 1 hour before being transferred to a clean sealed glass jar and stored in a desiccator over silica gel until required. The particles resulting from this batch were named Engineered Spray Dried Lactose ($ESDL_{10}$).

The same process was repeated for a second spray dried lactose batch however, the spray-dried particles were left in contact with hot ethanol for a longer period of time of 30 seconds. The particles resulting from this batch were named Engineered Spray Dried Lactose ($ESDL_{30}$).

- *Preparation of Powder Blends:*

Each drug, SS, FP and BDP (Glaxo Smith Kline, Ware, UK) 40 ± 1.45 mg was mixed separately with 2.7 g Lactohale® and ESDL in a ratio of 1:67.5 w/w, in accordance with the ratio employed in the commercial Ventolin® formulation so that each capsule contained 400 ±14.5 µg of drug and 27 mg lactose. Thus, each drug was weighed into a 20ml glass vial to which was added approximately an equivalent amount of lactose carrier, either Lactohale® or ESDL and blended manually using a microspatula. Then more lactose carrier was added similar to the amount of the blend contained in the glass vial and mixed manually using the same microspatula. This process was repeated until all the lactose carrier (2.70 g) was added into the drug/lactose blend to obtain a ratio of drug to carrier of 1:67.5 w/w. The same process was applied to all formulations irrespective of the drug or carrier. The stoppered vials were then placed in a Turbula mixer (Turbula®, UK) and mixed at 72 min^{-1} for 5 min, 10 min, 15 min and 30 min. Finally, the samples were stored at room temperature in a vacuum desiccator over silica gel until required for further investigation. Hard gelatine capsules (size 3) were filled with exactly 27.4 ± 0.5 mg of the powder mixture so that each capsule contained 400 ±14.5µg of the drug. The filling of the capsules was completed manually.

- *Measurement of the Homogeneity of the Mixtures*

The drug content uniformity of SS, FP and BDP and lactose mixes were determined by taking randomly 10 aliquots of approximately 27.4 mg each (3 from the top, 3 from the middle, 3 from the bottom and one randomly from the blend). Each aliquot was poured into a 100 mL volumetric flask and made up to volume with acetonitrile: water (75:25 v/v) for FP and BDP and 30:70 % v/v methanol and 10 mM hexane sulfonic acid adjusted to pH 2.5 with glacial acetic acid for SS. Each solution was assayed for drug content using validated HPLC methods (Thevarajah, 2019). The average mean recovery as % of the nominal dose was calculated and the percentage coefficient of variation (% CV) was the metric used to access the content uniformity of each powder blend.

- *Characterisation of Particle Shape and Size using Scanning Electron Microscopy (SEM)*

SEM was used to assess morphological features: particle size, shape, and surface appearance, for Lactohale® and ESDL. The particles were then coated with approximately 15 to 20 nm gold for one minute using an ion sputter coater (Quorum Technologies Ltd., UK) under vacuum of 0.09 mbar and a current of 40mA. Micrograph images were produced by scanning fields, selected randomly at several magnifications with a Jeol 6060LV SM scanning electron microscope (JEOL, Japan).

- *Solid State Characterisation of Lactohale® and ESDL using X-Ray Powder Diffraction (XRPD)*

X-Ray powder diffraction was used to assess the crystallinity of lactose particles. Powder X-ray diffraction patterns were recorded after samples were spread uniformly over the sample holder using a D8 Advance powder X-Ray diffractometer with Cu Kαl radiation of $\lambda = 1.54$Å (Bruker AXS). The voltage and current applied were 40 kV and 40 mA, respectively. The sample powder was packed into the rotation sample holder and scanned in the 2θ range 5° to

60°. Crystallinity was identified by comparing the characteristic 2θ peaks ("fingerprints") of the XRD pattern.

- *Measurement of Triboelectric Charges*

The charge-to-mass ratio (*Q*/*M*) of the DPI formulations was assessed using a tribo-electric device based on a shaking concept consisting of a Faraday cup, an electrometer, and a shaking machine (Retsch MM400), previously described (Šupuk et. al., 2009). The Faraday cup consists of two concentric cups made of a conducting material. The concentring cups differ in size: the outer cup is larger and acts as a shield to prevent impact of external electric fields. The inner cup was connected to an electrometer (Keithley, model 6514, UK) as per the method described by Secker and Chubb, (1984). The powders used for this study already possessed a certain level of residual charge, referred to as the initial charge, which was measured first, without shaking, by placing a 0.1g of each powder into the Faraday cup connected to an electrometer. This charge was the result of powder being in contact with surfaces before the experiments were conducted. In this work, the initial charge was relatively small compared to the final charge. The final charge was obtained by weighing DPI powder (0.1 g for each run, n=3) and loading the powder inside a cylindrical stainless steel container (10 ml) before being shaken in a horizontal direction using the shaking machine. The powder sample was then poured into the Faraday cup and the net charge (C) present on the powder particles was measured on the electrometer. The charge values were presented in nano-coulombs per gram (nC/g) as the mean charge-to-mass ratio (*Q*/*M*). This was calculated by dividing the final charge with the final mass of the respective powder. The maximum charge-to-mass level was attained to ensure the effect of the initial charge was negligible. The shaking was carried out at a vibration frequency of 20 Hertz in order to induce tribo-electrification inside the container. The maximum charge-to-mass ratio acquired for Lactohale® and ESDL and their respective

formulation blends was 2 minutes after shaking. Measurements were repeated three times to ensure reliable data was produced.

The same procedure was followed for all DPI formulations. The Retsch shaking cylindrical container was cleaned thoroughly after each measurement with isopropyl alcohol to ensure the removal of any residual particles, surface charge from a previous test and impurities which could invalidate the results. Isopropyl alcohol was allowed to evaporate before further tests were carried out. The experiment was carried out in a controlled environment with an ambient temperature of 20-23 °C and relative humidity (RH) of 32-39 %.

- *Aerodynamic Dose Emission Characteristics of SS, BDP And FP Formulations using Andersen Cascade Impactor (ACI)*

The formulations blend of SS, BDP and FP with Lactohale, $ESDL_{10}$ and $ESDL_{30}$ aerodynamic dose emission characteristics was assessed *in-vitro* using compendial dose emission testing for DPIs. A vacuum pump was used to generate an inhalation flow with a constant peak inspiratory flow (PIF) that corresponds to a 4 kPa pressure drop across the inhaler device with an inhaled volume of 4 L (USP, 2014). Breezhaler® is a low resistance device and a PIF exceeding 100 L/min is required to achieve a pressure drop inside device equivalent to 4kPa when using the device (Abadelah, 2017). The ACI (Copley Scientific, UK) was calibrated at three PIFs 28.3 , 60 and 90 L/min, therefore, in the present study the inspiratory parameters used were PIF of 90 L/min and inhaled volume (Vin) of 4 L to be in accordance with the calibrated range of ACI. The ACI was connected to a vacuum pump (HCP5, Copley Scientific Ltd, UK) via the critical flow controller (model TPK; Copley Scientific Ltd, UK). The ACI stages were assembled with 10 mL of 75 % acetonitrile: 25 % ultra-purified water (% v/v) in the pre-separator and a glass fibre GF50 (Whatman; UK) filter was placed in the final stage. For each determination, one dose was prepared and aerosolised according to the manufacturer's recommendations in patient information leaflet (PIL). Three separate determinations were

made for each formulation blend at a set PIF and Vin. Once the dose aerosolised into the ACI, a washing procedure took place to recover the API (SS, BDP and FP) from the mouthpiece, induction port, pre-separator, ACI stages, filter, capsule, and device. A validated HPLC method was used to quantify the amount of the API (SS, BDP and FP) deposited in each part in the ACI as well as the residual amount left in the device and capsule.

- *Drug Quantification using HPLC Method*

The Shimadzu HPLC system comprised a liquid chromatograph (LC-20AT), an auto sampler (SIL-20A), a column oven (CTO-10ASVP), a UV-VIS detector (SPD-20A).

The HPLC method for the detection of SS was a mobile phase of 30:70 % v/v methanol and 10 mM hexane sulfonic acid adjusted to pH 2.5 with glacial acetic acid, SS was detected using a detection wavelength of λ = 276nm. The mobile phase for BDP and FP was 75:25 % v/v Acetonitrile: water using a detection wavelength of λ = 230 nm. For both methods, the stationary phase was Luna® column C18 100A (250mm x 4.6 mm) with a pore size of 5µm (Phenomenex, UK), the flow rate was 1mL/min, and the injection volume of the sample was 20 µl. The retention time for SS, FP and BDP was 10.5, 2.8 and 6.5 mins, respectively. The LOD for SS, BDP and FP were 0.66µg/mL, 0.62µg/mL and 0.23 µg/mL, while the LOQ was 2 µg/mL, 1.89 µg/mL, and 0.75 µg/mL

- *Data Analysis*

The Copley Inhaler Testing Data Analysis Software (CITDAS version 2.0, Copley Scientific Ltd, UK)) was used to calculate the aerodynamic dose emission parameters. The total emitted dose (TED) was obtained from the cumulative amounts of API (SS, BDP and FP) deposited in the mouthpiece (MP), induction port (IP), using the USP throat, the pre-separator (PS) and all the stages of the ACI. The fine particle dose (FPD) was the mass associated with particles < 5 µm. Large particle mass (LPM) was sum of the drug amount deposited in the upper part of the

ACI (MP + IP + PS). Total residual amount (TRA) was the sum of the dose left in the device and capsule after the inhalation manoeuvre. The total recovered dose (TRD) was the sum of the total emitted dose (TED) and the total residual amount (TRA). A one way and two way ANOVA, as well as Tuckey test, were carried out. The statistical analysis comprised of a one way and two-way factorial analysis of variance (ANOVA) which was carried out using the statistical analysis software, SPSS Statistics (SPSS Inc., Chicago, USA) and Excel Microsoft® data analysis.

3. Results and Discussion

3.1 Characterization of Carrier Particle Size, Shape and Surface Texture

Solid state crystallisation (SSC) of the spray dried Lactohale® lactose particles resulted in the formation of spherical particles with a modified surface texture as opposed to spray dried Lactohale® lactose and tomahawk Lactohale® particles with a smoother surface (Figures. 1). The ESDL particles can be produced with different surface rugosity without altering the shape of the particles. The time of exposure of spray-dried particles to hot ethanol was found to be a critical factor in altering surface rugosity as shown in SE micrographs (Figure 1 C and D). The spray dried particles were engineered using two exposure times to hot ethanol, 10 seconds, and 30 seconds, to produce $ESDL_{10}$ and $ESDL_{30}$, respectively. The longer the exposure time to ethanol the rougher, larger, and more porous the particles. Therefore, $ESDL_{30}$ was larger, rougher, and more porous than $ESDL_{10}$. The performance of DPIs is greatly influenced by the physical properties of the carrier, particularly their particle size, morphology/shape, and surface roughness. As these factors are interdependent, it is difficult to completely understand how they individually influence DPI performance (Peng et al., 2016). SSC is predictable in maintaining the original shape of the spray dried particles. Furthermore, the surface roughness

286 can be adjusted using different exposure times to hot ethanol to produce particles with desired
287 surface rugosity so as to provide enough adhesion of drug to the carrier, which is necessary in
288 the production of a stable, homogeneous powder blend with acceptable drug content
289 uniformity, yet allowing easy drug detachment from the surface of the carrier during inhalation
290 manoeuvre.
291 Increasing the temperature in the crystallisation medium is expected to enhance the solubility
292 of lactose, especially very fine lactose particles and amorphous regions within lactose, thus
293 reducing the supersaturation of lactose in the crystallisation medium. Initially, an excess of
294 spray dried particles (10 g) were introduced into the crystallisation medium with a solvent in
295 which the solubility of lactose is not substantially affected, in order to maintain the
296 supersaturation of lactose in the crystallisation medium. Undissolved lactose would act as a
297 seed for crystal growth. It is important to note that increasing temperature reduces the viscosity
298 of the crystallisation medium, thus facilitating the transfer of the dissolved lactose solution
299 onto the lactose seeds for crystal growth. Increasing the time from 10 seconds to 30 seconds
300 affected surface texture of the particles forming rough, porous, spherical particles. The initial
301 spherical shape of lactose is maintained but the particles have increased in size as shown from
302 scanning electron micrographs of spray dried lactose (Figure 1b) in comparison to $ESDL_{10}$
303 (Figure 1d) and $ESDL_{30}$ (Figure 1c). Thus, $ESDL_{30}$ are larger than $ESDL_{10}$ which are
304 significantly larger than spray dried lactose. The hollow volume is significantly larger for
305 Engineered lactose suggesting that the particles expanded in radial direction and both the
306 temperature and time of exposure to the solvent acted as an inflating agent to expand the size
307 of the particles.
308 Pharmaceutical powders are generally divided into three categories depending on their
309 deformation behaviour: plastic, elastic, and brittle. Lactose is known to be brittle as suggested
310 from its high mean yield pressure derived from Heckel plot (Heckel, 1961; Roberts and Rowe,

311 1985). During crystal growth, the hollow volume of ESDL increases and reaches a maximum
312 volume after which the particles burst. The increase in the hollow volume is dictated by the
313 plasto-elasticity of the material. As the growth of the particles progresses, the porous structure
314 of the particles facilitates permeation of the solvent inside the hollow space, thus causing an
315 increase in the vapour pressure inside the particles caused by the evaporation of the solvent.
316 The vapour pressure built inside the hollow space of the particle exerts a radial stress on the
317 shell. If the radial stress exceeds a certain limit, the particle will either expand by plastic or
318 elastic deformation or burst if the shell resists deformation as is the case with a fragmenting
319 powder. Lactose is a fragmenting material and increasing the exposure time of the particles to
320 the solvents beyond 30 seconds caused them to burst to form needle shape particles as they
321 could not withstand the radial stress applied from inside the particles. The vapour pressure
322 increases in a radial direction and equally so that the spherical shape of the particles is
323 maintained. Thus, SSC allows a great control of particle size, surface properties and
324 crystallinity of particles.
325 *Assessment of Crystallinity*
326 Crystalline solids lead to the diffraction of X-rays at a unique combination of angles, enabling
327 identification of the material. Lactohale® is crystalline with diffraction peaks at 12°; 16° and
328 19° (Figure 2a) representative of the crystalline form of α-lactose monohydrate (Gombas et
329 al., 2002)
330 In the spray drying process, the liquid feed is atomised into very small droplets through a
331 narrow nozzle within a hot drying gas. The rapid evaporation of the droplets results in solid
332 amorphous particles. Spray drying is highly scalable and offers high precision control of
333 particle size and bulk density, but the major drawback is the formation of amorphous material
334 which is unstable and can revert back to crystalline material with aging or when exposed to
335 humidity (Wu, et al., 2014).

Spray dried lactose exhibits a broad "halo" effect with no noticeable diffraction, allowing it to be clearly distinguished from crystalline Lactohale (Figure 2b). Spray-drying is known to produce predominantly amorphous material because the transition between the liquid and solid phase is instantaneous (Santos et al., 2018) in other words, the rapid drying of the lactose solute droplet did not allow sufficient time for lactose to form a crystalline structure. Amorphous material tends to be highly cohesive (Young et al., 2007), have a poor flow and is thermodynamically unstable (Shen et al., 2010). The hollow spray dried particles have a significant advantage over solid non-hollow particles, the low density of hollow drug particles have attracted interest in the inhalation field. They provide significant improvements in lung targeting and dose consistency, relative to current marketed inhalers (Weers and Tarara, 2014). The spray dried lactose particles produced in this work are hollow, but too small to be used as a carrier in DPI formulations. Engineered spray dried lactose particles in the presence of hot ethanol increased their particle size, volume (Figure 1) and restored the crystallinity to the particles (Figure 2). The hollow nature of lactose particles may impart low density to lactose carrier particles to provide them with a long time of flight so that they can travel longer distance in the airstream before impaction, thus giving opportunity for drug particles to detach from their surface to maximise drug deposition. The relative degree of crystallinity of different samples of the same crystal form is usually proportional to the ratio of the peak intensity (Zeng et al., 2000). It is interesting to note that higher peak intensities were observed on $ESDL_{30}$ diffractogram (Figure 2c) suggesting that $ESDL_{30}$ was more crystalline than $ESDL_{10}$ (Figure 2d). It is clear that prolonging the exposure time to ethanol has an impact on the crystallinity of the ESDL particles, since it has been reported that an increase in temperature has a linear relationship with the degree of crystallinity of lactose particles (Chiou et al., 2007).

3.2. Drug-Carrier Formulation Assessment:

3.2.1 Drug Content Uniformity

Content uniformity is a critical determinant that helps ensure the strength of the drug in the formulation remains within the specified acceptance limits and to assess the quality of a batch (Williams, Adams, Poochikian, & Hauck, 2002).

Blending uniformity of the binary mixtures varied significantly ($p < 0.05$) with mixing times, drug types and the type of carrier (Tables 1, 2 and 3). The mean (SD) drug weight uniformity varied between 359.54 (15.83) μg and 416.92 (8.36) μg for all batches, which are within the acceptable limit of 90-110% of the target weight [FDA, 1988]. However, mixing time can have a significant effect on the % CV. Of the three drugs investigated, SS was found to have the greatest deviation in the % CV with increasing the mixing time from 5 mins to 30 mins mainly with Lactohale® (Table 1) with a corresponding % CV of 1.04 and 9.69 at 5mins and 30 mins respectively. A short mixing time of 5 minutes provided a better repartition of SS particles on the surface of Lactohale® as shown from the low % CV of 1.04 %. The morphological features of particles are known to affect the blend uniformity (Venables & Wells, 2001). Carrier particles with high elongation ratio are disadvantageous in DPI dose metering and processing at handling scale due to their poor flowability (Larhrib et al., 2003a; Kaialy et al., 2011). Spherical agglomerates were shown to facilitate drug loading, improve the flowability of the powder and improved the blend uniformity (Zellnitz et al., 2021).

Furthermore, smooth carrier particles have a low loading capacity which can promote drug segregation especially for high dose drugs. Lactohale® has an elongated shape with a smooth surface (Figure 1a), these morphological features may have affected both the adhesion of drug to the carrier and the flow of powder inside the Turbula mixer, facilitating segregation between drug and carrier particles by prolonging the mixing time.

Both BDP and FP achieved a smaller %CV with prolonged mixing time with Lactohale® in comparison to SS (Table 1). BDP and FP are both hydrophobic and their extent of adhesion to the carrier may be different to hydrophilic drugs such as SS. It is clear that drug content

uniformity does not depend only on the nature of the carrier but also on the drug adherence to the carrier. SS mixes and de-mixes rapidly when mixed with Lactohale®. Hydrophobic drugs such as BDP and FP are highly cohesive and require longer time of mixing and high shear forces to break up drug aggregates before distributing uniformly on the lactose carrier. Thus, hydrophobic drugs can benefit from prolonged mixing times to provide good content uniformity than hydrophilic drugs such as SS with Lactohale®. Generally, ESDL particles were spherical in shape with a rough surface and gave the highest homogeneity with a low %CV compared with tomahawk shaped smooth Lactohale®, whether with SS, FP or BDP (Table 2 and 3).

3.2.2 Tribo-Charging Behaviours of Carriers Lactohale®, $ESDL_{10}$ And $ESDL_{30}$; Drugs SS, FP, BDP and their Respective Formulations

The carriers, drugs and their blend with carriers were assessed for their triboelectrification using a Faraday cup coupled to an electrometer and the results are summarised in Table 4. The results from the Tribo-charging showed that API materials charge to a higher extent with much greater variability than is seen with excipients which agrees with previous studies using the same technique (Supuk et. al., 2012). Table 4 indicates that different carriers have different charging behaviours. The results show that Lactohale® was negatively charged with a specific charge of -15.38 ±17.89 (nC/g), whereas $ESDL_{30}$ and $ESDL_{10}$ were positively charged with a specific charge of 5.39 ± 1.23 and 1.06 ± 2.43 (nC/g), respectively.

Murtomaa et al. (2002) noticed that the specific charge of lactose decreases as a function of the amorphous content. This is in agreement with the finding of the present work, and there is direct correlation between the specific charge of lactose carrier and XRD data (Figure 2). Lactohale® was most crystalline and exhibited the highest specific charge, whereas $ESDL_{10}$ was the least crystalline and exhibited the lowest specific charge (Table 4). This shows that the charge distribution on carrier surface is also influenced by its crystallinity. $ESDL_{10}$ and $ESDL_{30}$

411 are spherical whereas Lactohale® is an elongated tomahawk shape, therefore, electrical charges
412 may distribute homogeneously on the surface of spherical particles, whereas most charges may
413 concentrate on the tips and edges of tomahawk elongated carrier particles. Since charging
414 behaviour is a surface phenomenon, this will eventually influence the interactions between
415 drugs and carrier particles and could inherently influence adhesion and agglomeration of
416 particles as well as *in-vitro* drug deposition (Bennett et al., 1999).
417 Lactohale® alone or in the formulation with SS, FP or BDP always exhibited negative charge.
418 The electronegative charge on Lactohale® increased even more when formulated with SS
419 particles, the charge increased from -15.38±17.89 nC/g for Lactohale® alone to -25.68 ±2.60
420 nC/g when formulated with SS. Mixing SS to Lactohale® carrier particles charged the
421 formulation more negatively, this could be due to segregation of the binary blend, which was
422 supported by drug content uniformity data (Tables 1) showing an increase in the % CV with
423 increasing mixing time. The particle motion inside the shaking container represents a unique
424 system in that it describes triboelectrification in every aspect, i.e., friction through sliding,
425 impact on the walls and between particles, and collisions by particles rolling. In order to assess
426 the largest contributor to charge generation by tribo-electrification in a given application, the
427 method of charging should yield the total saturated charge. A shaking container considers the
428 whole of powder sample to avoid bias sampling and a rate process is involved to confirm that
429 saturation level has been reached. The use of electrometer and a Faraday cup set up means that
430 a high sensitivity of charge measurements is obtained. Following tribo-electric charging tests
431 to determine the saturation level, it was found that particles adhered to the inner surface of the
432 shaking container. The adhesion of powders to the contact surface may cause changes in the
433 composition of the powders during tribo-electric charging and ultimately affect the
434 homogeneity of the sample. The particles which adhere to the container surfaces may also
435 cause variations in the interactions of free moving particles and therefore prolong powders

reaching their saturated value early in the tribo-electrification process. The saturated charge-to-mass values and adhesion data obtained using the shaking concept may provide important information when new inhaler devices and formulations are designed in order to improve the drug deposition. The exact effect of such particle charge and adhesion on inhalation performance needs to be further investigated.

3.3.3 Assessment of Deaggregation of Drug Particles by Measuring Drug Amounts Recovered from the Wall of the Shaker

Table 5 showed that the amount of drug recovered from the walls of the shaking container after tribo-electrification for all DPI formulations was found to be dependent on the nature of the carrier and the drug used. For example, SS-Lactohale® formulation showed the highest amount of drug adhesion to the stainless steel container with an average value of 1051.3 ± 13.86 µg corresponding to approximately 72% of SS recovered from the wall of the shaker. However, the amount of SS was significantly reduced to 135.21± 1.27µg for SS-ESDL$_{30}$ and 28.56± 0.43µg for SS-ESDL$_{10}$ corresponding to 88 and 119 fold reduction in SS for SS-ESDL$_{30}$ and SS-ESDL$_{10,}$ respectively (Table 5). SS-Lactohale® formulation showed the highest drug recovered from the stainless steel container and also showed the highest variation in drug content uniformity with increasing mixing time (Table 1). SS-ESDL$_{30}$ and SS-ESDL$_{10}$ showed less drug adhesion to the wall of shaking container (Table 5) but also less % CV in drug content uniformity in comparison to SS-Lactohale® (Tables 2 and 3). Therefore, the tribo-electric charging device based on the shaking concept may provide a rapid mean for screening DPI formulations less prone to segregation that provide a stable mix for good drug content uniformity. Drug adhesion to the tribo-electrification stainless steel shaker was dependent not only on the carrier but also on the drug. BDP and FP are both hydrophobic drugs and when mixed with Lactohale® showed a substantial reduction in the amount of drug adhered to the

cell shaker (Table 5). The degree of adhesion to Lactohale® carrier is stronger compared to SS providing a stable mix and as confirmed by the drug content uniformity study (Table 1) where both BDP and FP showed generally smaller % CV when compared to SS-Lactohale®. The tribo-electrification drug de-aggregation assessment study (Table 5) corroborates with drug content uniformity study (Tables 1,2 and 3) demonstrating that drug adhesion to the wall of the shaker could be mainly caused by segregation of drug weakly adhering to the carrier.

3.3.4 *In-Vitro* Aerodynamic Dose Emission Characteristics Study of SS, BDP And FP with Lactohale® and Engineered Carriers ($ESDL_{10}$ And $ESDL_{30}$).

It is widely accepted that breath activated DPIs are often associated with flow rate dependent changes in the emitted dose and also the aerodynamic characteristics such as the fine particle dose (FPD) and mass median aerodynamic diameter (MMAD) (Abadelah et al., 2017; Abadelah et al., 2018).

In the present study, we investigated the deposition profiles of SS, BDP and FP after formulating each drug separately with Lactohale® and engineered carriers $ESDL_{10}$ and $ESDL_{30}$. The three model drugs (SS, BDP, FP) showed different charging behaviour in tribo-electrification study (Figure 3) and different degrees of adhesion to the wall of the stainless steel shaker (Table 5). The carriers: Lactohale®, $ESDL_{10}$ and $ESDL_{30}$ showed differences in their morphological features (crystallinity, surface charges, surface roughness and shape) all of which may influence drug adhesion and detachment during aerosolisation. The carrier is a major component in DPI formulations, and it is critical to design a carrier with desired morphological features to provide sufficient adhesion with drug particles to form a stable mix with acceptable drug content uniformity, yet to allow drug detachment from its surface during the inhalation manoeuvre.

SS behaved differently in its deposition profile with the three carriers, providing the highest FPD (156.78± 2.62) with Lactohale® in comparison to $ESDL_{10}$ FPD (79.48± 1.40) and $ESDL_{30}$ FPD (100.21± 1.61) (Figure 4). SS–Lactohale® formulation showed the greatest variation in drug content uniformity (Table 1) and the highest amount of drug adhering to the wall of the stainless steel shaker in the Tribo-electrification study (Table 5). The high FPD for SS-Lactohale® formulation (Figure 4) are in line with the triboelectrification study (Table 5), suggesting weak adhesion of SS to Lactohale® promoting drug detachment during inhalation manoeuvre. The weak adhesion between SS and Lactohale® is also reflected in the amount of SS adhered to capsule and device (total residual amount, TRA) which was significantly higher ($p < 0.05$) (52.31 ± 4.26 µg) for Lactohale® in comparison to (44.52 ± 3.18 µg) and (35.00 ± 2.57 µg) for $ESDL_{10}$ and $ESDL_{30,}$ respectively.

The rank order of FPD for BDP and FP was $ESDL_{10}$ > Lactohale® > $ESDL_{30}$ (Table 6). Despite Lactohale® performing better with SS formulation in terms of FPD, its performance was worse than $ESDL_{10}$ with BDP and FP, this shows that there is no universal carrier that performs very well and equally with all drugs, i.e., a carrier may perform well with one drug but not necessarily perform well with other drugs. However, for all three drugs tested, $ESDL_{10}$ showed the least variation in the FPD when compared with Lactohale® and $ESDL_{30}$.

BDP and FP results showed that nearly 50% of the nominal dose 400µg deposited as large particle mass (LPM) in the upper part of the impactor (Table 6), this is in accordance with previous studies (Mohammed et al., 2012, Abadelah et al., 2017). MMAD values ranged between 1.6 and 2.6 µm (Table 6). An MMAD < 5 µm is considered to be necessary for sufficient airway deposition (Mitchell et al., 1987).

Geometric standard deviation (GSD) values were > 1.2 µm (Tables 6) suggesting polydispersity of the aerosol, this common for drug particles generated by micronisation, which is the case for all the model drugs used in this work. Drug retention inside the inhaler continues

510 to be a factor plaguing the performance of novel inhalers (Tajber et al., 2009). Drug retention
511 varies between inhaler devices in that some studies have reported between 30-50% of the
512 nominal dose being retained within the device (Heng et al., 2013). It is important that the
513 complete dose is released from the inhaler to maximise the therapeutic effect, minimising drug
514 wastage and avoiding potential dosage errors during the next inhalation. The lowest TRA for
515 both BDP and FP was observed with $ESDL_{10}$, suggesting lower amount of drug retained in the
516 capsule and device.

517 ## 4. Conclusion

518 A novel hollow, crystalline, spherical lactose carrier was produced using a solid-state
519 crystallisation technique from a spray-dried lactose suspension in hot ethanol. This novel
520 crystallisation technique is more predictable in forming spherical shaped particles with desired
521 size, crystallinity, surface charge, hollow and surface rugosity. Engineered spray dried Lactose
522 (ESDL) particles were formed with different sizes, hollow volume, crystallinity, and surface
523 rugosity. The longer the time where particles are exposed to hot ethanol, the larger the size,
524 hollow, crystallinity, and surface rugosity. The plasto-elasticity of the outer shell of the
525 particles can dictate the final inner hollow volume of a particle and hence particle size.
526 Engineered lactose $ESDL_{10}$ and $ESDL_{30}$ showed less variation in drug content uniformity
527 compared to Lactohale® when formulated with SS, BDP and FP. The results from the tribo-
528 electric charging device show all formulations formed with $ESDL_{10}$ and APIs produce the most
529 stable blends with lowest charge-to-mass ratio. The triboelectrification device may provide a
530 rapid means for screening DPI formulations less prone to segregation that provide a stable mix
531 for good drug content uniformity. Lactohale® was the most suitable carrier for SS in providing
532 high FPD, but care must be taken in optimising the mixing procedure to ensure an acceptable
533 drug content uniformity. $ESDL_{10}$ carrier promoted a better drug deposition for both BDP and
534 FP and showed the least variation in both content uniformity and FPD irrespective of the model

drug when compared to Lactohale and $ESDL_{30}$. Therefore, production of crystalline spherical lactose carrier is achievable using solid state crystallisation. The surface texture, size and crystallinity can be easily controlled to achieve the optimal spherical carrier for DPIs providing less variation in drug content uniformity and consistent fine particle dose to the lungs in-vitro for both hydrophilic and hydrophobic drugs.

Conflict of interest

All authors declare no conflicts of interest in this work.

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List of Figure Captions:

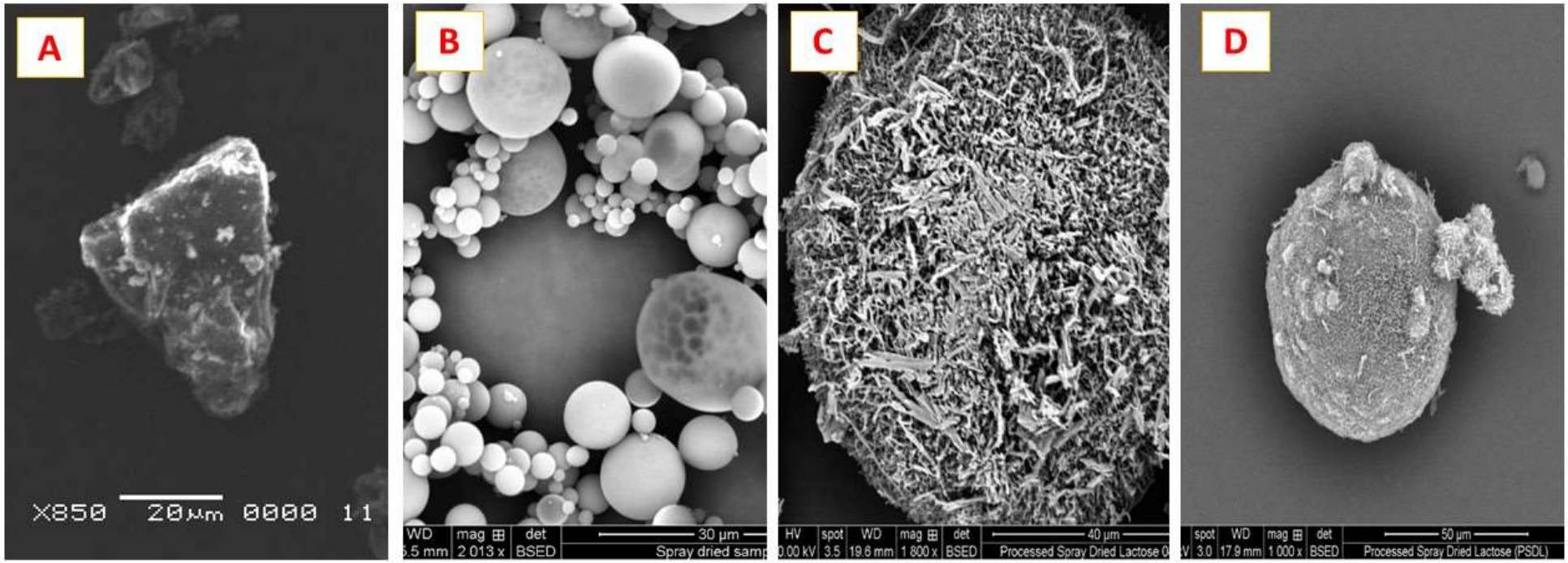
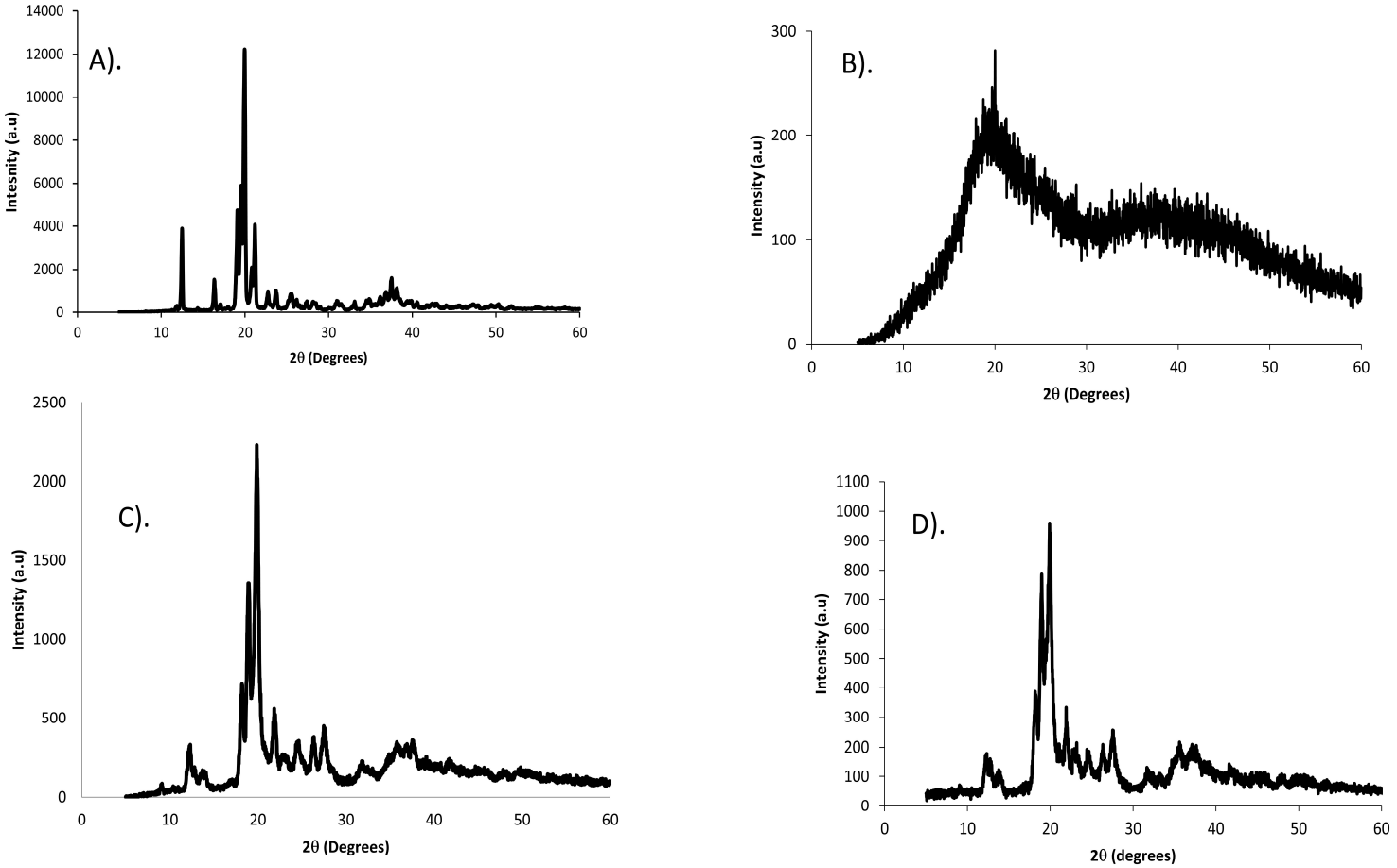


Figure 1: Scanning electron micrographs of A) Lactohale®, B) spray dried Lactohale®, C) Engineered spray dried lactose ($ESDL_{30}$), D) Engineered spray dried lactose ($ESDL_{10}$).



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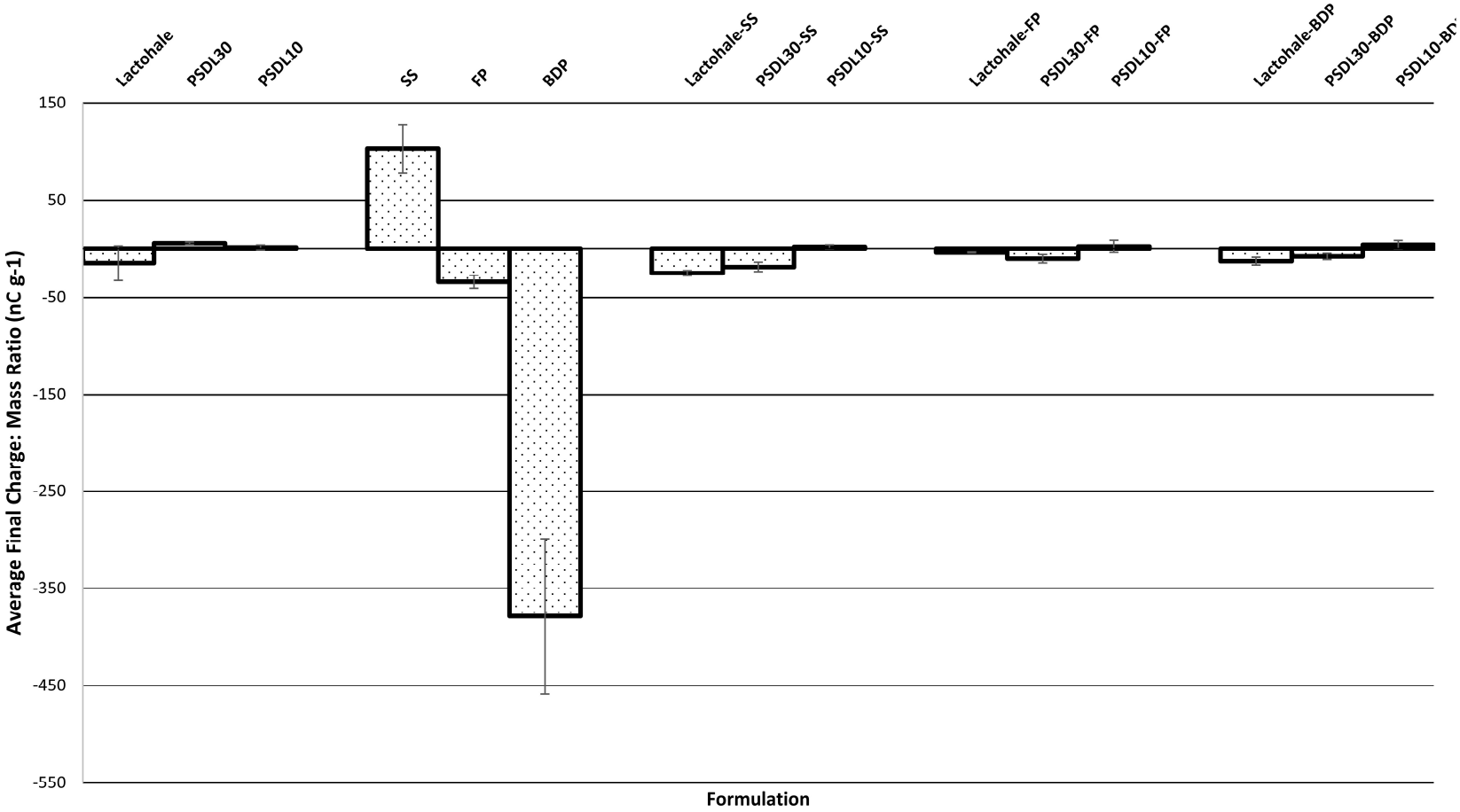
731 **Figure 2: X-ray diffraction pattern of [A) Lactohale® (63-90 µm) particles, B) spray dried lactose, C) $ESDL_{30}$ and D) $ESDL_{10}$ [n=3].**



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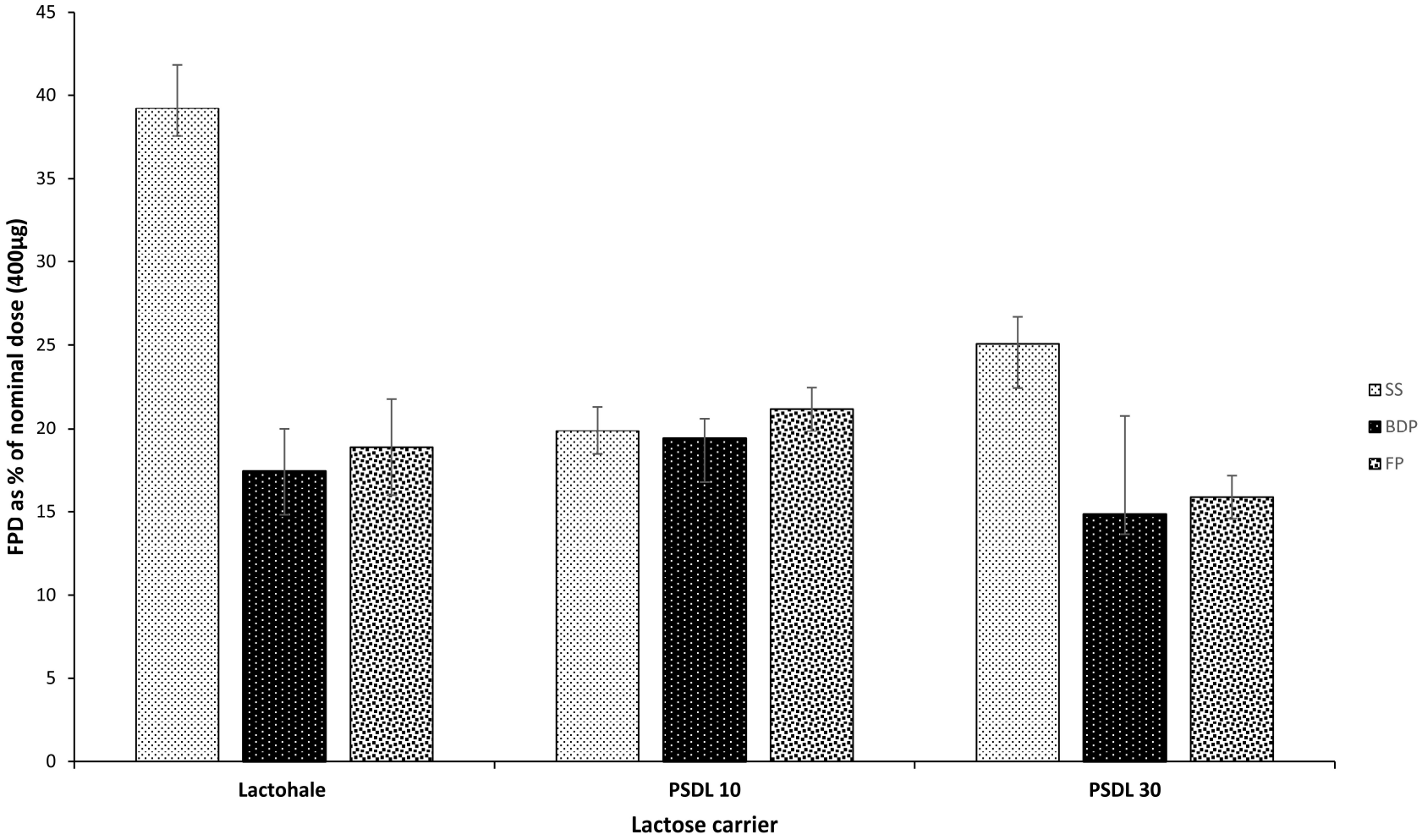
733 **Figure 3: The average final charge: mass ratios (nC g^{-1}) of carriers, drugs, and formulations after tribo-electrification [n=3].**



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735 **Figure 4: Mean (SD) of Fine particle dose (FPD) of SS, BDP and FP with Lactohale®, $ESDL_{10}$ and $ESDL_{30}$.[n=3].**

736

Table 1: Content uniformity of SS, FP and BDP in µg with Lactohale® at different mixing times: 5, 10, 15 and 30 mins [n=10].

Lactohale®						
Time	**BDP**		**FP**		**SS**	
	Amount in µg Mean (SD)	% CV	Amount in µg Mean (SD)	% CV	Amount in µg Mean (SD)	% CV
5 Min	396.78(24.73)	6.23	412.86(6.57)	1.59	404.48(4.23)	1.04
10 Min	359.54(15.83)	4.40	406.67(5.79)	1.42	409.85(25.53)	6.23
15 Min	399.00(13.65)	3.42	414.27(7.31)	1.76	408.66(37.95)	9.29
30 Min	405.85(16.55)	4.08	416.78(15.53)	3.72	410.33(1.05)	9.69

Table 2: Content uniformity of SS, FP and BDP in µg with $ESDL_{10}$ at different mixing times: 5, 10, 15 and 30 mins[n=10].

$ESDL_{10}$						
Time	**BDP**		**FP**		**SS**	
	Amount in µg Mean (SD)	% CV	Amount in µg Mean (SD)	% CV	Amount in µg Mean (SD)	% CV
5 Min	404.76(11.56)	2.85	409.67(3.40)	0.83	408.81(14.56)	3.32
10 Min	405.21(8.63)	2.13	398.16(6.11)	1.53	413.64(11.91)	2.88
15 Min	416.29(15.43)	3.71	398.79(6.24)	1.56	403.42(11.12)	2.75
30 Min	406.71(14.73)	3.62	406.06(14.64)	3.60	398.81(9.68)	2.28

Table 3: Content uniformity of SS, FP and BDP in µg with $ESDL_{30}$ at different mixing times: 5, 10, 15 and 30 mins [n=10].

$ESDL_{30}$						
Time	**BDP**		**FP**		**SS**	
	Amount in µg Mean (SD)	% CV	Amount in µg Mean (SD)	% CV	Amount in µg Mean (SD)	% CV
5 Min	425.10(15.36)	3.61	407.51(5.20)	1.28	414.64(14.79)	3.57
10 Min	412.37(10.32)	2.50	416.92(8.36)	2.00	404.80(9.57)	2.36
15 Min	404.45(9.87)	2.44	412.32(9.13)	2.21	408.31(20.61)	5.05
30 Min	402.93(10.45)	2.59	406.23(17.84)	4.39	392.23(19.41)	4.95

Table 4: The average final charge-to-mass ratios of the formulations after tribo-electrification. [n=3]

Formulation	Average final charge: mass ratio (nC g^{-1})
Lactohale®	-15.38 ± 17.89
$ESDL_{30}$	5.39 ± 1.23
$ESDL_{10}$	1.06 ± 2.43
SS	102.79 ±24.67
FP	-34.52 ±6.59
BDP	-378.77 ±80.25
SS-Lactohale®	-25.68 ±2.60
SS-$ESDL_{30}$	-19.39 ±4.88
SS-$ESDL_{10}$	1.75 ±2.02
FP-Lactohale®	-4.51 ±0.53
FP-$ESDL_{30}$	-10.93 ±4.29
FP-$ESDL_{10}$	2.01 ±6.40
BDP-Lactohale®	-13.35 ±3.96
BDP-$ESDL_{30}$	-8.49 ±3.14
BDP-$ESDL_{10}$	3.91 ±4.27

Table 5: The average drug content of SS, FP and BDP recovered from the wall of the stainless-steel shaker after tribo-charging measurements (n=3)

Formulation	amount (µg)	SD	% Recovery ± SD
SS-L	1051.31	13.86	71.9 ±0.95
SS-ESDL$_{30}$	135.21	1.27	9.26 ±0.09
SS-ESDL$_{10}$	28.56	0.43	1.96 ± 0.03
BDP-L	11.9	2.06	0.8± 0.14
BDP-ESDL$_{30}$	1.95	0.26	0.13±0.01
BDP-ESDL$_{10}$	24.93	0.42	1.71 ±0.02
FP-L	8.83	2.94	0.6± 0.2
FP-ESDL$_{30}$	0.81	0.06	0.05 ±0.0
FP-ESDL$_{10}$	57.08	0.1	3.9 ±0.0

792 **Table 6**: Mean (SD) of aerodynamic dose emission characteristics of SS, BDP and FP with

793 Lactohale®, $ESDL_{10}$ and $ESDL_{30}$ at a PIF of 90 L/min and Vin of 4 L using ACI [n=3].

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	Lactohale®	**$ESDL_{10}$**	**$ESDL_{30}$**
SS deposition			
MP	2.51(0.36)	16.49(2.56)	5.22(0.29)
IP	41.64(2.71)	94.77(2.69)	44.29(2.90)
PS	95.29(6.44)	136.35(5.91)	165.96(3.24)
LPM	139.77(3.29)	247.61(2.89)	215.47(5.16)
FPD	156.78(2.62)	79.48(1.40)	100.21(1.61)
TED	309.22(2.97)	337.04(3.50)	328.52(3.27)
TRA	52.31(3.72)	44.52(2.21)	35.00(2.61)
TRD	361.53(3.09)	382.31(3.30)	363.51(2.67)
MMAD (µm)	1.60(0.00)	2.00(0.10)	1.90(0.00)
GSD	2.27(0.06)	2.40(0.00)	2.47(0.06)
BDP deposition			
MP	12.90(2.61)	17.74(0.52)	6.49(0.41)
IP	53.41(6.04)	96.53(2.64)	100.85(0.26)
PS	132.78(7.06)	133.26(3.92)	132.65(5.51)
LPM	199.09(4.39)	247.53(7.88)	239.90(12.06)
FPD	69.66(2.60)	77.58(1.20)	59.38(5.90)
TED	277.27(1.56)	340.72(2.70)	307.69(2.29)
TRA	51.63(5.08)	23.98(1.19)	80.32(9.61)
TRD	328.89(2.37)	364.70(2.10)	388.02(2.45)
MMAD (µm)	1.93(0.06)	2.30(0.10)	2.67(0.06)
GSD	2.27(0.25)	2.70(0.90)	1.90(0.02)
FP deposition			
MP	6.75(0.78)	17.02(0.48)	17.87(0.97)
IP	90.24(3.49)	84.49(2.28)	120.89(4.74)
PS	146.89(5.77)	125.12(3.62)	109.19(6.98)
LPM	243.87(8.41)	226.63(6.01)	247.95(7.49)
FPD	75.49(2.90)	84.61(1.30)	63.47(1.82)
TED	332.43(2.52)	323.57(2.30)	324.36(1.76)
TRA	59.78(2.37)	26.38(3.30)	58.85(2.09)
TRD	392.21(3.79)	349.95(2.30)	383.22(2.17)
MMAD (µm)	2.67(0.06)	2.30(0.12)	1.80(0.00)
GSD	2.17(0.06)	2.5(0.00)	2.93(0.06)

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