Development of a novel dry powder inhalation formulation for the delivery of rivastigmine hydrogen tartrate

Alice Simon[1,2]; Maria Inês Amaro[2]; Lucio Mendes Cabral[1]; Anne Marie Healy[2*]; Valeria Pereira de Sousa[1]

[1] Department of Drugs and Pharmaceutics, Faculty of Pharmacy, Federal University of Rio de Janeiro, Av.CarlosChagas Filho, 373, CCS, Bss,sl15, Rio de Janeiro, RJ, 21941-902, Brazil.

[2] School of Pharmacy and Pharmaceutical Sciences, Trinity College Dublin, Dublin 2, Ireland.

***Corresponding author:**

Anne Marie Healy

School of Pharmacy and Pharmaceutical Sciences, Trinity College, University of Dublin, Dublin 2, Ireland

Tel.: +353 1 8961444; fax: +353 1 8962810

E-mail address: healyam@tcd.ie

ABSTRACT

The purpose of this study was to prepare engineered particles of rivastigmine hydrogen tartrate (RHT) and to characterize the physicochemical and aerodynamic properties, in comparison to a lactose carrier formulation (LCF). Microparticles were prepared from ethanol/water solutions containing RHT with and without the incorporation of L-leucine (Leu), using a spray dryer. Dry powder inhaler formulations prepared were characterized by scanning electron microscopy, powder X-ray diffraction, laser diffraction particle sizing, ATR-FTIR, differential scanning calorimetry, bulk and tapped density, dynamic vapour sorption and *in vitro* aerosol deposition behaviour using a next generation impactor. The smooth-surfaced spherical morphology of the spray dried microparticles was altered by adding Leu, resulting in particles becoming increasingly wrinkled with increasing Leu. Powders presented low densities. The glass transition temperature was sufficiently high (>90ºC) to suggest good stability at room temperature. As Leu content increased, spray dried powders presented lower residual solvent content, lower particle size, higher fine particle fraction (FPF <5μm), and lower mass median aerodynamic diameter (MMAD). The LCF showed a lower FPF and higher MMAD, relative to the spray dried formulations containing more than 10% Leu. Spray dried RHT powders presented better aerodynamic properties, constituting a potential drug delivery system for oral inhalation.

Keywords: Dry powder inhaler, particle engineering, spray drying, L-leucine, rivastigmine hydrogen tartrate

1. INTRODUCTION

There is an increasing interest in systemic drug delivery via the pulmonary route, mainly because the respiratory system represents an attractive non-invasive administration route for new disease therapeutics (Hickey, 2013; Pilcer and Amighi, 2010; Stank and Steckel, 2013), and, in most cases, using a inhalation device, a lower dose of drug is required to achieve a therapeutic effect relative to oral administration (Price et al., 2002). In addition, the pulmonary route can provide substantially higher bioavailability, as the lungs has a large surface area (70 - 140 m^2 in adult human lung) (Groneberg et al., 2003) combined with an extremely thin alveolar epithelial barrier (0.1 - 0.2 μm). The lungs also present a high level of vascularization that allows for rapid drug absorption with relatively low local metabolic activity, and no hepatic first pass effect (Marianecci, et al., 2011; Pilcer and Amighi, 2010; Stank and Steckel, 2013).

Devices for pulmonary drug delivery introduce the drug into the airways in the form of an aerosol. Drug delivery to the lungs requires inhalable particles in the dispersed phase of this aerosol to have an aerodynamic diameter between 1 and 5 μm in order to be deposited in the lower respiratory tract (Stank and Steckel, 2013; Zeng et al., 2001). Most dry powder inhaler (DPI) formulations consist of micronized drug particles blended with larger carrier particles, typically α-lactose monohydrate, which enhance powder flowability, dispersion and reduce particle agglomeration (Healy et al., 2014; Pilcer et al., 2012; Young et al., 2009; Zeng et al., 2001). Drug particles stick to the carrier particle surface by physical forces of interaction and should detach from the carrier on device actuation and powder inhalation (Pilcer et al., 2012; Young et al., 2009). A number of factors influence these interactions, e.g. physicochemical properties such as, particle size, particle shape and morphology, surface area and surface energy, which in turn determine flow, dispersion, and deposition in the respiratory tract (Pilcer et al., 2012; Telko and Hickey, 2005; Zeng et al., 2001).

Nonetheless, the carrier may be omitted and the performance of DPI formulations may be significantly enhanced through particle engineering approaches, by lowering the geometric diameter of the particles and/or particle density (Boraey et al., 2013; Bosquillon et al., 2001, 2004; Nolan et al., 2009; Seville et al., 2007; Steckel and Brandes, 2004), altering particle shape (Bosquillon et al., 2001; Feng et al., 2011; Kaialy et al., 2011; Larhrib et al., 2003; Steckel and Brandes, 2004) and by forming particles with rough surfaces (Seville et al., 2007; Sou et al., 2013; Young et al., 2009). Research in the last decade, has focused on the development of aerodynamically light particles with particle size lower than 5 μm, bulk density less than 0.3 g/cm^3 and mass median aerodynamic diameter (MMAD) between 1 and 5 μm to achieve a higher respirable fraction and, consequently successful drug delivery (Boquillon et al., 2004; Edwards et al., 2005; Healy et al., 2014; Pilcer et al.; 2012; Steckel and Brandes, 2004).

Spray drying has been explored as a promising technique to produce particles with the above mentioned characteristics, often without the need to use coarse carriers, and as a process that can be easily translated to large scale production (Chow et al., 2007; Healy et al., 2014; Pilcer et al., 2012; Vehring, 2008). A number of studies suggest that further improvement in the aerodynamic properties of spray dried particles could be achieved through the inclusion of L-leucine in formulations, since L-leucine is a low-density amino acid with hydrophobic characteristics (Boraey et al., 2013; Chow et al., 2007; Seville et al., 2007; Sou et al., 2013).

Past research has shown that the morphology of spray dried microparticles changed from solid spheres to wrinkled surfaces (Boraey et al., 2013; Feng et al. 2011; Sou et al., 2013) or imparted additional porosity to the particle (Chow et al., 2007) when the L-leucine mass fraction was increased. This change in the particle properties is thought to be due to L-leucine precipitation on the surface of drying droplets, forming a hydrophobic outer shell layer with wrinkled texture (Boraey et al., 2013; Feng et al., 2011; Healy et al., 2014; Sou et al., 2013; Vehring, 2008). The non-displacement of L-leucine into the droplet centre can be considered characteristic of a system where the ratio of time for solute diffusion from the droplet surface to its centre to time for droplet drying is greater than 1 (i.e. Peclet number > 1) (Feng et al.; 2011; Healy et al., 2014; Vehring, 2008). Consequently, the wrinkled or raisin-like morphology that results causes improved dispersion of particles, resulting in efficient drug delivery into the lower regions of the lungs (Boraey et al., 2013; Feng et al., 2011; Healy et al., 2014; Sou et al., 2013; Vehring, 2008). Moreover, spray dried particles containing L-leucine have also demonstrated a low density (Boraey et al., 2013) and an anti-hygroscopic effect, which has been attributed to the enrichment of the excipient on the particle surface (Chang et al., 2014). Improvements in dispersibility, flowability and *in vitro* particle deposition (as demonstrated by increased fine particle fraction (FPF) and decreased MMAD), have all been reported (Boraey et.al, 2013; Chow et al., 2007; Feng et al., 2011; Najafabadi et al., 2004; Seville et al., 2007; Sou et al., 2013; Xu et al., 2014).

Rivastigmine is currently a commonly used drug for the symptomatic treatment of mild to moderately severe dementia in Alzheimer's disease. Rivastigmine hydrogen tartrate (RHT) is a carbamate derivative ((s)-N-ethyl-N-methyl-3-[1-1(dimethylamino)ethyl]-phenyl carbamate hydrogen-(2R,3R)-tartrate) that reversibly and non-competitively inhibits the metabolism of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE), preferentially in the central nervous system (Williams et al., 2003). RHT, as a 3 mg oral dose, presents a pharmacokinetic half-life of 1.5h and a bioavailability of 36%, suggesting a high first pass metabolism, however its pharmacodynamic half-life is approximately 10h due to binding to the esteratic site of the AChE enzyme, from which dissociation is slower than acetylcholine (Agid et al., 1998; Polinsky, 1998; Williams et al., 2003).

Research has previously been conducted in order to develop delivery systems for RHT in the form of controlled release tablets (Ogorka and Kalb, 2005), nanoparticles (Craparo et al., 2008; Fazil et al., 2012; Ismail, 2013; Joshi et al., 2010; Nagpal et al., 2013; Wilson et al., 2008), buccoadhesive films (Kapil et al., 2013) and liposomes (Mutlu et al., 2011; Scialabba et al., 2012; Yang et al., 2013); however no commercial product of any of the above-listed formulations is, as yet, available. RHT is commercially available as capsules and a solution for oral administration and as transdermal patches (Exelon® and Exelon®Patch, respectively) produced by Novartis. Dry powder inhalation represents an administration route that has not been previously explored for RHT. Given the increasing interest in DPIs for inhalation therapy to treat diseases other than lung conditions, there is potential to develop a DPI formulation of RHT with potential for improved bioavailability and consequently increased drug therapeutic effectiveness for the treatment of Alzheimer's disease.

The purpose of the present study was to develop a novel DPI formulation of RHT by particle engineering via spray drying. A lactose carrier based formulation was also developed for comparison purposes. The physicochemical properties of the lactose carrier-free and lactose carrier-containing formulations were investigated and the *in vitro* deposition characteristics of the engineered particles of RHT prepared by spray drying compared to the formulation containing lactose carrier.

2. MATERIAL AND METHODS

2.1 Materials

S-Rivastigmine hydrogen tartrate (RHT) was purchased from Zhejiang Jiuzhou Pharmaceutical (China). L-Leucine (Leu) was purchased from Sigma-Aldrich (Ireland). Inulin DP23 (Inu) was a gift from Sensus (Netherlands). α-Lactose Monohydrate NF Inhalation 40M was kindly supplied by Kerry (Ireland). Acetonitrile and methanol HPLC grade were acquired from Fisher Scientific (Ireland). Ammonium hydroxide (NH_4OH), ammonium phosphate monobasic ($NH_4H_2PO_4$), and fluorescein sodium salt were purchased from Sigma-Aldrich (Ireland).

2.2 Methods

2.2.1 Lactose carrier-free spray dried formulations preparation

Lactose carrier-free formulations were spray dried as 1% (w/v) solutions of RHT, Inu and Leu in ethanol:water comprising 30% (v/v) ethanol. The spray dried solutions were maintained

at 40ºC during the spray drying process in order to solubilize the Inu. Formulation composition is presented in Table 1. In order to allow quantification of the powder deposited in the Next Generator Impactor (NGI) (section 2.2.12) a fluorescent marker, fluorescein sodium salt, was incorporated at a low loading (0.2%, w/w) in all the spray dried formulations. Ní Ógáin et al. (2011) have previously shown that the incorporation of a fluorescent marker at this level has no impact on the morphology of spray dried powders.

All prepared solutions were spray dried, as previously described (Amaro et al., 2011, 2014; Ní Ógáin et al., 2011), using a Büchi B-290 Mini spray dryer, with a standard 2-fluid nozzle with a 0.7 mm tip and 1.5mm cap (Büchi, Switzerland). The spray dryer was operated in the open mode, whereby the drying gas (compressed air) passes through the drying chamber and is then exhausted. A high performance efficiency cyclone, designed to improve the separation rate and collection efficiency of particles, was also used (Amaro et al., 2011, 2014; Ní Ógáin et al., 2011). The air flow rate was 601 L/h, the inlet temperature was set at 160ºC, the pump setting was 30% and the aspirator capacity setting was 100%. Each formulation was prepared in triplicate, and stored in a desiccator at 4°C. Yields were expressed as weight percentage of the final product compared to total amount of the material put into the feed solution.

2.2.2 Lactose carrier formulation (LCF) preparation

Commercially available carrier α-lactose monohydrate for DPIs was used as supplied (median diameter of 40 μm). RHT was micronized by jet milling using a Jet-O-Mizer Model 00 (Fluid Energy Processing and Equipment Company, Telford, PA, USA) to obtain a median diameter of 2 μm (d_{50} measured by laser diffraction as 2.00 ± 0.02 μm). The pusher and both grinder nozzle pressures were set at 6 and 5 bar, respectively, using nitrogen as micronizing gas. RHT (7.5% w/w) and lactose monohydrate (92.5% w/w) were blended in a Turbula® T2F Shaker-Mixer (Glen Mills, UK) for 1 hour at 42 revolutions per min to obtain a homogeneous mixture. The lactose carrier-containing formulation (LCF) was prepared in triplicate and the homogeneity of the powder mixture for each formulation obtained was assessed by sampling 5 locations (in triplicate), followed by HPLC analysis (3 injections per sample; RSD < 5%).

2.2.3 Scanning electron microscopy (SEM)

Particle morphology was investigated using a Zeiss Ultraplus Thermal Field (Germany) microscope operating at a voltage of 5 kV. The samples were fixed on aluminium stubs using double-sided adhesive tape and sputter-coated with gold (Amaro et al., 2014). Photomicrographs were taken at different magnifications.

2.2.4 Particle size distribution

Particle size distributions of raw materials and prepared formulations for inhalation were determined using a Mastersizer 2000 laser diffraction instrument (Malvern Instruments, UK) with a dry powder dispersion accessory (Scirocco 2000) (Amaro et al. 2011, 2014; Healy et al. 2008). The dispersive air pressure was set at 2 bar and a vibration feed rate of 50% was used in order to achieve an obscuration between 0.5% and 6%. Mastersizer 2000 software was used for data evaluation. The particle size was expressed as geometric median diameter based on a volume distribution (d_{50}), and the polydispersity of the powders was expressed by the span values. Span = $[d_{90} - d_{10}]/d_{50}$, where d_{90}, d_{50} and d_{10} indicate the equivalent volume diameters corresponding to the 90, 50 and 10% points of the cumulative distribution curve, respectively (Amaro et al. 2011, 2014; Chew and Chan, 2002; Healy et al. 2008). The values presented are the average of three determinations.

2.2.5 Bulk and tapped density

Bulk and tapped density measurements were performed as previously described (Amaro et al., 2014; Healy et al., 2008; Nolan et al. 2009). Bulk density was measured by weighing the amount of powder required to occupy a 1 mL volume in a graduated glass syringe (Lennox Laboratory supplies, Ireland). Tapped density was then evaluated by tapping the syringe onto a level surface at a height of 5 cm, 100 times. The result was obtained by calculating the ratio of the mass to the tapped volume of the sample. The results given are the average of three determinations.

2.2.6 Powder X-ray diffraction (PXRD)

The solid state nature of powders was evaluated by X-ray powder diffraction measurements using a Rigaku MiniFlex II desktop X-ray diffractometer (Japan) with Haskris (USA) WA1 cooling unit (Amaro et al., 2014; Healy et al., 2008; Ní Ógaín et al. 2011). Measurements were taken from 5° to 40° on the two theta scale at a step size of 0.05 per s. A minimum of two analyses was performed for each sample.

2.2.7 Differential scanning calorimetry (DSC)

Differential scanning calorimetry measurements were carried out under nitrogen purge using a Mettler Toledo DSC 821^e (Mettler Toledo Ltd., U.K.) as previously described (Amaro et al., 2014; Healy et al., 2008; Ní Ógaín et al., 2011). Samples were accurately weighed

(approximately 5 to 8 mg) and placed into 40 µL sealed aluminium pans with three vent holes and scanned over a temperature range of 0–180 °C with a scanning rate of 5 °C/min. The DSC system was controlled by Mettler Toledo STAR^{e}software (version 6.10). The glass transition temperature of RHT as supplied was determinate by the melt quench technique, through fast heating the sample (20 °C/min) to 120 °C, and subsequently fast cooling (to 0ºC); the sample was then scanned over a temperature range of 0 to 150 °C with a scanning rate of 5 °C/min. Glass transition temperature for the formulations F and G (see Table 1) were obtained using a modulated temperature DSC (MTDSC) and scans were taken by means of a DSC Q200 (TA Instruments, United Kingdom) with a RCS90RP refrigerated cooling system (Curtin et al., 2013). Samples were scanned over a temperature range of 0–150 °C with a scanning rate of 0.5 °C/min and 0.8 modulation and a period of 60 s. The glass transition temperature (Tg) was defined as the midpoint of the transition and the crystallisation and melting points are reported as the onsets of the exo/endothermic processes, as reported by Amaro et al. (2014). The results presented are the average of three determinations.

2.2.8 Thermogravimetric analysis (TGA)

TGA measurements were performed using a Mettler TG50 module linked to a Mettler MT5 balance (Mettler Toledo, UK) as previously described (Amaro et al., 2011, 2014; Ní Ógáin et al., 2011). Samples were accurately weighed (approximately 5 to 8 mg) into 40 µL aluminium pans that remained open for the duration of the analysis. Analysis was carried out under nitrogen purge and a heating rate of 10 °C/min was implemented in all measurements. The TGA system was controlled by Mettler Toledo STARe software (version 6.10). The residual solvent content (RSC) was defined as the weight loss in TGA between 25 and 100 °C, and the values presented are the average of three analyses.

2.2.9 Attenuated Total Reflection–FTIR Spectroscopy (ATR-FTIR)

Infrared spectra were collected using a Perkin-Elmer Model Spectrum One FTIR Spectrometer (USA) equipped with a UATR and a diamond/ZnSe crystal accessory as previously described by Grossjohann et al. (2015). Data were evaluated in Spectrum version 5.0.1 software. Each spectrum was acquired over the range from 650 to 4000 cm^{-1} with resolution of 4 cm^{-1} and a minimum of six scans were collected to obtained an average good quality spectra. A minimum of two analyses was performed for each sample.

2.2.10 Dynamic vapour sorption (DVS)

The water vapour sorption-desorption isotherms of lactose carrier-containing and carrier-free systems were obtained by means of an automated gravimetric vapour sorption analyser, DVS Advantage-1 (Surface Measurements Systems Ltd, UK) as previously described (Amaro et al., 2014). Samples were equilibrated at 0% RH until a steady dry reference mass was recorded. The samples were exposed to the following relative humidity (% RH) profile: 0% to 90% in 10% steps and the reverse for desorption at 25.0 ± 0.1°C. At each stage, the sample mass was allowed to reach equilibrium, defined as $dm/dt \leq 0.002$ mg/min over 10 min, before the RH was changed. The amount of water uptake for each RH stage was expressed as a % of the dry sample mass (m_0) (Amaro et al., 2014). A minimum of two analyses was performed for each sample.

2.2.11 High performance liquid chromatography (HPLC) analysis

A Waters Alliance HPLC system was used with a 2695 separation module and a Waters 2487 Dual λ Dual Absorbance UV–VIS detector and Waters 2475 Multi λ fluorescence detector (Waters, USA). The analytical method used was based on the chromatographic conditions for quantification of RHT raw material described in the United States Pharmacopoeia (USP, 2014). Chromatographic separation was achieved isocratically at ambient temperature with a Kromasil 100A C8 column (4.6 x 150 mm; 5 μm). The mobile phase consisted of monobasic ammonium phosphate buffer (8.6 mg/mL; pH 7.0), acetonitrile and methanol in 50:25:25 (v/v/v) ratio, and was run at a flow rate of 1.2 mL/min. The eluent was monitored for 9 min at 215 nm with UV detector for RHT determination, and, when applicable, the eluent was monitored for 4 min with a fluorescence detector (excitation wavelength 490 nm; detection wavelength 530 nm). Retention times were 5 min for RHT and 2 min for fluorescein.

The RHT content of each DPI formulation was quantified by HPLC and expressed as the percentage of the initial amount. Each powder was measured in triplicate.

2.2.12 *In vitro* aerosol deposition studies using the Next Generation Impactor (NGI)

The pulmonary deposition of the spray dried powders and lactose carrier formulation was estimated *in vitro* using a Next Generation Impactor (NGI, Copley Scientific Limited, Nottingham, UK) operated under pharmacopoeial conditions (European Pharmacopeia, 2014). The flow rate was adjusted to achieve a pressure drop of 4 kPa in the powder inhaler (Handihaler®, Boehringher Ingelheim, Ingelheim, Germany) and the time of aspiration was adjusted to obtain 4 L air flow (Amaro et al., 2011, 2014; Tewes et al., 2010). The pre-separator was used in the deposition studies for the LCF formulation to remove coarse particles (lactose-carrier particles). NGI stages (stages 1 to 7 and filter) were coated with 1 mL of water. As previously described by Amaro et al. (2011, 2014), the dry powder inhaler was loaded with a

no. 3 hard gelatin capsule loaded with 20 ± 2 mg of powder for each formulation test. After the deposition on the NGI, the powder was collected from each individual stage (device, throat, stages 1-7 and filter) and dissolved in a suitable volume of water prior to quantification by HPLC using the method described in 2.2.11. The deposition profile of each formulation was carried out in triplicate and the results presented are the average results of the replicated analyses.

The emitted recovered dose (ED) was determined as the percent of total powder mass exiting the capsule. The total amount of particles with aerodynamic diameters smaller than 5 and 3 µm was calculated by interpolation from the inverse of the standard normal cumulative mass distribution less than stated size cut-off against the natural logarithm of the cut-off diameter of the respective stages. This amount was considered as the fine particle fraction (FPF %) below 5 µm and 3 µm, and expressed as a percentage of the ED. The mass median aerodynamic diameter (MMAD) of the particles was determined from the same plot as the particle size corresponding to the 50% point of the cumulative distribution, and the geometric standard deviation (GSD) as$GSD = \sqrt{\frac{SizeX}{SizeY}}$, where X is the particle size corresponding to the 84% point and size Y is the particle size corresponding 16% point of the cumulative distribution (Amaro et al., 2011, 2014; Bosquillon et al., 2001).

2.2.13 Statistical analysis

Microsoft® Excel software was used to determining median and standard deviation. Difference between means was determined through statistical analysis of variance, ANOVA with Tukey's multiple comparison post-hoc test or Student's t-test when applicable, using GraphPad Prism® software (version 5.0), with at least a 95% confidence level.

3. RESULTS

3.1 Characterisation of physicochemical properties of lactose carrier-free spray dried formulations

A total of 7 solutions (Table 1, A to G) were spray dried. The yield of spray dried powders increased with the addition of Leu to the formulation from 55.1% up to 67.9%, with significant difference among samples containing Leu ($p = 0.0038$) (Table 2). Analysis of the RHT content of the spray dried formulations indicated that the drug loading was 7.41 ± 0.07% and 15.27 ± 0.08% (nominal concentrations were 7.5% and 15.0% w/w, respectively) (Table 2), demonstrating that RHT does not undergo degradation upon spray drying.

Representative scanning electron micrographs of the spray dried formulations are shown in Fig. 1. Formulations comprising Leu (both 7.5 % and 15 % RHT) presented wrinkled or raisin-like powders, with apparent increase of rugosity with increasing content of Leu (Fig. 1B to E). The 0% Leu formulation presented smooth spherical particles. SE micrographs (not shown) confirmed that the incorporation of 0.2% (w/w) fluorescein marker has no impact on the morphology of spray dried particles, as was also previously observed by Ní Ógáin et al. (2011).

Laser diffraction particle size analysis was employed to investigate the particle size distribution of the spray dried powders (Table 2). The particle size volume distribution, in all cases, was narrow and monomodal with span values decreasing from 2.59 to 1.76 as the Leu content increased. The median particle size (d_{50}) decreased from 2.12 to 1.40μm as the proportion of Leu increased from 5% to 20% w/w. A correlation between d_{50} and Leu concentration was investigated and found to be significant ($r^2 = 0.75$, $p = 0.02$) - as the amount of Leu increases the particle size decreases.

The bulk and tapped densities of spray dried formulations are presented in Table 2. The tapped density of the spray dried powders ranged between 0.18 and 0.50 g/cm^3, although, only the formulations containing 20% Leu exhibited a tapped density higher than 0.3 g/cm^3. In general, the increase of Leu concentration in the formulations slightly increased the tapped and bulk densities. A linear relationship between density (bulk and tapped) and %Leu was observed ($r^2 = 0.8352$, 0.8529, $p < 0.05$, respectively).

PXRD diffractograms of the RHT as supplied and Inu and Leu after spray drying are shown in Fig. 2. The diffractogram showed that the RHT as supplied was crystalline, the diffraction peaks (9.5, 11.3, 13.2, 14.2, 15.5, 19.1 and 20.0° 2θ) were characteristic of form II polymorph (Benkic et al., 2008). Crystalline peaks of spray dried Leu were evident demonstrating its crystallinity after spray drying - characteristic XRD peaks appeared at 6.12, 12.05, 19.1, 24.35 and 30.61° 2θ, as were also observed by Najafabadi et al. (2004). In contrast, Inu spray dried was amorphous, characterized by an amorphous halo and the absence of diffraction peaks in the diffractogram. This amorphous halo for Inu was also observed for Inu as supplied raw material. The PXRD patterns of the spray dried formulations (A to G) shown in Fig. 2, exhibit evidence of an amorphous form, however with increasing Leu content (> 15% w/w) in the formulation, peaks become visible at 6.1 and 19.1° 2θ; these peaks grow in intensity when the content of Leu increased to 20% w/w.

DSC scans (Fig. 3) showed a broad endotherm, probably due to residual solvent loss, the glass transition, and thermal decomposition for both spray dried formulations and Inu as supplied. A large broad endothermic peak present in the Inu as supplied, over the heating range of 30 – 100°C, was attributed to water loss, as confirmed by thermogravimetric analysis (Table 2). DSC scans were similar for all formulations, with a single glass transition step followed or not by an exothermic peak (recrystallisation of the amorphous phase), that can be observed in

formulations C to G, and finally decomposition of the material. The T_g value of melt quenched RHT was determined to be 38.24 ± 0.27°C and the T_g value of Inu as supplied was 156.02 ± 0.64°C. The T_g values of the spray dried formulations are listed in Table 2, and indicate that the presence of Leu significantly decreased ($p = 0.0013$) the T_g compared with the corresponding spray dried formulation without Leu (formulation A). There was a correlation between the amount of Leu (5 to 20% w/w) in the spray dried formulations (B to E) and the T_g ($r^2 = 0.951$).

An increase in RHT loading in the spray dried formulations from 7.5% to 15% RHT (formulation F and G) did not appear to change the roughness of the particles (as viewed by SEM, Fig. 1F and G compared to 1C and E). The d_{50} was less than 1.5 µm for the formulations with higher RHT loading. However, the increase of RHT loading significantly reduced the T_g of the spray dried formulation (F and G), which was expected due to the low T_g presented by the drug (38.24 ± 0.27ºC). The residual solvent content (RSC) determined by TGA in the range of 25 -100 ºC for RHT, Leu and Inu as supplied was 0.55 ± 0.02%, 0.89 ± 0.07% and 6.18 ± 0.25%, respectively. As expected, Leu showed a low water content due to its hydrophobic properties. The RSC of the spray dried formulations is shown in Table 2. The statistical analysis of RSC values revealed significant differences between formulations ($p = 0.0045$).

The ATR-FTIR spectrum of RHT as supplied was similar to that reported by Benkic et al. (2008) for the polymorph II (3455, 3415, 1699, 1655, 1406, 1338 cm^{-1}), with the carbamate band present at 1695 cm^{-1} (Fig. 4). Likewise, the amino acid Leu exhibited an ATR-FTIR spectrum similar to that recorded by Li et al. (2006), with characteristic bands at 2955 and 1575 cm^{-1}, which correspond to C=O and N-H stretching (Li et al., 2006, Silverstein et al., 2006). For Inu, the ATR-FTIR spectrum showed the spectral region between 1200 and 900 cm^{-1} is dominated by a sequence of intense peaks characteristic in oligo- and polysaccharides. As for all carbohydrates, two overlapped bands at 3000-2800 cm^{-1} region are assigned to C-H stretching bands (Grube et al., 2002; Silverstein et al., 2006). The broad band at 3300 cm^{-1} is assigned to O-H stretching (Tummala and Kumar, 2013). The ATR-FTIR spectra of all spray dried formulations (7.5 and 15 % RHT loading) were characterized by principal absorption bands present in the Inu, with possible masking of RHT and Leu bands. However, an increase in the intensity of the absorption bands in 1700 cm^{-1} may be related to the carbamate band in the RHT, while an increase in the intensity of the absorption bands between 3000-2800 cm^{-1} and 1600-1300 cm^{-1} may be indicative of the presence of RHT and the increase of Leu in the spray dried formulations.

3.2 Characterisation of physicochemical properties of lactose carrier formulation

In this study, a lactose carrier formulation (LCF) was developed and characterized in order to compare the aerosolization properties with lactose carrier-free formulations. The LCF

formulation consisted of a mixture of micronized RHT with carrier-lactose particles (Table 1). Through quantitative HPLC analysis (Table 2) we demonstrated that the LCF formulation presented homogeneity of 98.02% with a RSD of 1.46%, indicating that the mixing time was sufficient to promote the adhesion of RHT particles to the lactose carrier particles, resulting in the formation of a homogeneous ordered mix.

The morphology of the LCF formulation and RHT as supplied after micronization are shown in Fig. 1H and 1I, respectively; it was observed that micronized RHT particles adhered to the coarse lactose particles. Table 2 presents the data obtained from tapped and bulk density measurements of the LCF formulation (0.58 ± 0.005 and 0.45 ± 0.005 g/cm^3, respectively), with no significant differences in the values presented when compared with those presented for lactose monohydrate as supplied (0.58 ± 0.01 and 0.49 ± 0.004 g/cm^3, respectively).

Lactose monohydrate as supplied, RHT after micronization and the LCF formulation were characterised using laser diffraction. RHT micronized particles had a median diameter of 2.00 ± 0.02 µm (d_{50}), while the carrier lactose was coarser, with d_{50} of 31.25 ± 0.80 µm and d_{90} of 75.13 ± 1.31 µm. The LCF formulation exhibited d_{50} of 25.87 ± 0.34 µm and d_{90} of 69.04 ± 1.16 µm (Table 2).

PXRD diffractograms of the LCF formulation, micronized RHT and lactose as supplied are shown in Fig. 5. The diffraction patterns for the α-lactose monohydrate are indicative of a crystalline material and consistent with that previously described (Haque and Roos, 2005; Kirk et al., 2007), while the micronized RHT diffraction pattern was characteristic of the form II polymorph (Benkic et al., 2008), indicating that there was no change to the crystal structure on micronization. The PXRD diffractogram of the LCF formulation showed a pattern characteristic of α-lactose monohydrate with characteristic peaks at 12.5, 16.4, 20.0 and 20.1° 2θ (Haque and Roos, 2005).

DSC scans (not shown) of the lactose as supplied showed two endothermic transitions at ~148°C (ΔH ~ 140.5 Jg^{-1}) and ~205°C, confirming that the lactose sample is lactose monohydrate. According to Larhrib et al. (2003), the first endothermic peak corresponds to the loss of crystallization water and the second transition corresponds to the lactose melting followed by its decomposition. DSC of micronized RHT (Fig. 6) showed two endothermic peaks at ~102°C (ΔH ~ 113.7 Jg^{-1}) and 126°C (ΔH ~ 3.3 Jg^{-1}), representing the polymorph forms II and I, respectively, the melting points of which are characteristic of the polymorphism exhibited by this drug (Benkic et al., 2008). The LCF formulation exhibited three endothermic peaks at 102.85 ± 0.15°C, 125.95 ± 0.10°C and 148.30 ± 0.21°C, similar to the position of the endotherms of the individual formulation components, and the melting enthalpy of endothermic peaks were ΔH ~ 5.5 , 2.3 and 94.3 Jg^{-1}, respectively.

The ATR-FTIR spectrum of the LCF formulation was similar to that of lactose as supplied, however, a change in the absorption band in 1655 and 1699 cm^{-1} for the LCF

formulation was observed, which corresponds to the carbonyl double bond found in the chemical structure of RHT (Silverstein et al., 2006), indicating the presence of the drug in the powder blend (Fig. 7).

3.3 Water vapour sorption and desorption isotherms of powders

Leu (as supplied) showed no vapour sorption (i.e. no increase in mass) with increase in %RH (Fig. 8), and no change in the crystallinity of the sample compared to the material as supplied (Fig. 9), which was expected due to the high hydrophobicity of the material (Gliński et al., 2000).

Hancock et al. (1995) described how the maximum amount of water absorbed by amorphous carbohydrates is usually limited by crystallisation of the sugar at high relative humidity (RH). The Inu sorption isotherm presented an inflection point at ~70%; such an inflection point is generally recognized as an indication of a solid state change in a material. The PXRD pattern of the Inu sample post DVS analysis presented low intensity crystalline peaks. Hence, it is thought that the sample did not fully recrystallize. Ronkart et al. (2009) have reported the same observation upon exposure of Inu to high humidity.

The water vapour sorption and desorption isotherms for RHT were characterized by the presence of an open hysteresis loop. The desorption isotherm showed a high initial mass loss until ~30% RH followed by a constant mass loss and a final moisture uptake of approximately 4.3%. RHT is a very hygroscopic material; as RH increased so did the water uptake by RHT, with solubilisation/deliquescence of the RHT at RH $\geq$ 70 %. The sample collected for PXRD post DVS analysis was a glass-like material, which showed a loss of the original crystalline pattern, to be replaced by what appeared to be an amorphous halo (Fig. 9). However, it is likely that the pattern reflects the deliquesced material and not a solid amorphous state.

Sorption and desorption isotherms for the lactose carrier-free formulations are shown in Fig. 10. It was observed that the Leu content increase in the systems induced a delay in Inu recrystallization (from 70% to 60%) as well as a reduction in water uptake. No water was retained in the systems containing Leu post desorption as % mass change was zero at the end of analysis (Fig. 8). The formulation without Leu (A) presented a final water uptake of approximately 1.8% and was characterised by the presence of an open hysteresis loop, indicating that water was entrapped in the system. The inflection point at 60% RH in all samples was due to Inu crystallization, as evidenced by diffraction peaks in the PXRD, after DVS analysis, that are characteristic of Inu (Fig. 9). However, diffraction peaks related to Leu (6.2º and 19.1º) were also identified. PXRD after DVS analysis revealed that all lactose carrier-free formulations showed evidence of crystallinity.

The water vapour sorption and desorption isotherm for micronized RHT was similar to that of the RHT as supplied (unmicronised material), for which high RH induced a high water uptake (Fig. 11), and PXRD analysis of the sample post DVS analysis again showed what appeared to be an amorphous halo (Fig. 12). Lactose monohydrate exhibits very low hygroscopicity, as can be seen from the dynamic vapour sorption isotherms in Fig. 11. Lactose monohydrate exhibits only a low level of water adsorption (about 0.12% water at 90% RH) indicative of surface adsorption on a crystalline structure, and the final moisture uptake observed was approximately 0.02%. This may be explained by the fact that the crystalline structure of the lactose as supplied already incorporates water molecules (~5% of water content by Karl-Fisher, according to the supplier).

The water vapour sorption and desorption isotherms for the lactose carrier formulation (LCF) are shown in Fig. 11. The apparent solubilization of RHT at 60% RH was observed, however, the LCF formulation retained crystalline characteristics after the DVS run, which is attributed to the large proportion of the lactose monohydrate ingredient (92.5% w/w) present (Fig. 12). The LCF formulation absorbed about 4.2% water at 90% RH, and the final moisture uptake observed (post the desorption phase) was approximately 0.1%.

3.4 *In vitro* aerosol deposition studies using the Next Generation Impactor (NGI)

The NGI deposition profiles and fine particle fractions of the spray dried formulations and lactose carrier formulation are shown in Table 2 and represented in Fig. 13.

DPI formulations presented favorable deposition characteristics that improved with increasing Leu proportion, namely high emitted recovered dose (ED) between 85% and 93% and minimal loss of drug in the capsule and device (Handihaler®) - less than 15% for all formulations evaluated. In general, the formulations containing Leu produced higher FPF (< 5 µm and < 3 µm) compared to their counterpart formulation without Leu. The increase in Leu from 5% to 20% w/w improved the FPF (< 3 µm) of the spray dried powders by almost two-fold. In contrast, the formulation spray dried without Leu (formulation A) and the LCF (formulation H) demonstrated the lowest FPFs, FPF (< 5 µm) of 39% and 41% and FPF (< 3 µm) of 28% and 30%, respectively. A statistically significant difference ($p < 0.05$) was evident between the FPF < 5 µm and FPF < 3 µm values measured for these samples. Likewise, the formulation without Leu (formulation A) showed a profile with less deposition in stages with cut off diameters less than 3 µm (after stage 2), while the spray dried powders combined with different concentrations of Leu (formulation B to E) exhibited improved deposition profiles, due to less deposition in the capsule, inhaler, mouth adapter and throat (MA/IP) and stage 1, in comparison to a relatively higher deposition on stages 4 – 7, with cut-off diameter < 2 µm. The spray-dried formulations F (10% Leu) and G (20% Leu) which had a higher content of RHT

(15.0% w / w) compared to RHT 7.5% (w/w) loading, showed the highest emitted recovered dose (ED > 90%), high FPFs (between 57 - 68%), and exhibited better deposition profile, with relatively higher deposition on stages with cut-off diameters < 2 μm (4 to 7).

Table 2 depicts the MMAD values for the aerosols that were generated from DPI formulations, in which all systems showed an acceptable range for respirable particles (1 – 5 μm). The spray dried formulations containing Leu presented smaller MMAD and GSD. A correlation was found between increasing Leu concentration and decreasing MMAD values (r^2 = 0.78, $p < 0.05$). The GSD values of all spray dried powders was significantly different ($p < 0.0001$), in general, larger GSDs were determined for powders that presented the higher values for MMAD. The lactose carrier formulation showed a MMAD value of 3.44 μm and small value for GSD of 1.61.

4. DISCUSSION

Lactose carrier-free spray dried formulations were produced by spray drying 1% (w/v) solutions of mixtures of RHT, Inu and Leu, with the same processing conditions using a laboratory scale spray dryer. The particle size and morphology have a pronounced effect on all aspects of drug delivery to the lungs (Chow et al., 2007). The microparticle size is controlled by choosing the appropriate process settings in spray drying (Leone-Bay and Baughman, 2010; Vehring, 2008). The spray-drying process developed in the current work has been shown to be effective in preparing dry particles within the respirable size range, in which the median size of the spray dried particles was between 1.3 and 2.1 μm. The use of trileucine as a functional excipient in inhalable powders was investigated by Lechuga-Ballestros et al. (2008), and they observed that the addition of small amounts of trileucine in the formulations resulted in stable dry powders with improved inhalation properties. In the current work, when Leu content was increased in the formulations, a relatively narrow particle size distribution was observed, with a corresponding decrease in span and d_{90} values (Table 2), indicating that the powders were of an appropriate size range to avoid deposition by inertial impaction in the oropharyngeal cavity (Chow et al. 2007; Larhrib et al., 2003; Seville et al., 2007) (in comparison with the powder spray dried without Leu (A) and the lactose carrier formulation (LCF)). The addition of Leu (5 to 20% w/w) and/or an increase in the RHT loading (7.5 to 15% w/w) in the spray dried formulations resulted in a reduction in the median particle size.

The presence of Leu clearly affected the particle morphology, as depicted in Fig. 1. The morphology of particles without Leu were spherical and slightly smooth, while the morphology of microparticles composed of Leu were shown to be dependent on the proportion of amino acid in the formulation, as a result of the relatively hydrophobic nature and surfactant like properties of Leu (Gliński et al., 2009; Seville et al., 2007), that might be related to the relatively strong

hydrophobic alkyl side chain present in the Leu molecule (Wolfenden et al., 1981). Leu molecules show surface-active properties in aqueous solutions and are likely to accumulate at an air–solvent interface (Raula et al., 2007; Seville et al., 2007). Moreover, the low solubility of Leu leads to supersaturation early in the drying process, favoring a Leu precipitation/crystallization on the surface of drying droplets forming a hydrophobic outer shell layer (Boraey et al., 2013; Feng et al., 2011; Healy et al., 2014; Sou et al., 2013; Vehring, 2008), and as suggested earlier, the Leu crust on the droplet surface is unable to flow as rapidly as the shrinking of the particle as the solvent evaporates during drying (Peclet number > 1); thus the Leu surface layer collapses resulting in the wrinkled texture particles (Healy et al., 2014; Raula et al., 2007; Vehring et al., 2007). This change in the surface of the particles can be observed by SE micrographs (Fig. A to G); where, the higher the proportion of Leu in the formulation, the more wrinkled the surface and smaller the particle size became. Sou et al. (2013) suggested that such changes in surface wrinkling/corrugation, resulting from Leu addition, improves the dispersibility by reducing contact points between particles. In the current work an improvement in dispersibility of the spray dried particles was demonstrated by the better aerosolisation properties and deposition performance achieved with formulations B to G that contain Leu, in comparison with the formulations without Leu (formulation A and LCF).

In general, particle bulk and tap density increased with increasing Leu content in the formulation. These physical properties of powders are known to be affected by particle size, particle shape, and interparticle contact. The inclusion of Leu in the formulation resulted in significant reduction in particle size, hence more particles/powder could occupy a particular volume than particles of larger size. In addition, particle shape and interparticle contact were also modified. These particle properties led to particle packing modification: smaller particles tend to pack more tightly with less interparticle voids; a corrugated shape can also pack more tightly than a spherical shape and consequently result in particle mechanical interlocking with less void space between particles. All these events tend to increase powder tap density, as more particles/powder is packed into a smaller volume (Elversson and Fureby, 2005; Pilcer and Amighi, 2010).

Inu is an amorphous oligosaccharide with high viscosity and is considered an inert excipient and non-toxic. Inu was selected as a carrier material to formulate the DPI, in order to increase the volume of powder loaded and delivered from the DPI device (Ní Ógáin et al., 2011). As a saccharide excipient, the Inu also functions to provide stability for amorphous material during spray drying and on storage – due to its high glass transition temperature and ability to replace hydrogen bond interactions as the water is removed during drying, thus reducing molecular mobility and improving stability (Barclay et al., 2010; Hinrichs et al., 2001).

Spray-dried Inu exhibits an amorphous structure by PXRD as seen in Fig. 2, confirmed by the glass transition event shown in thermal analysis (Fig. 3). The Inu was able to promote the

glass stabilization of the API during the spray drying process of the formulations,in which the formulation without the Leu component remained PXRD amorphous, as did the formulations Leu containing, which showed a dominant amorphous signal. However, in the formulations with a Leu mass fraction larger than 15%, two of the original diffraction peaks (6.1º and 19.1º 2θ) corresponding to Leu are evident, when compared to the Leu after spray drying (Fig. 2), which is clearly crystalline. However, changes in the intensity and position of the original diffraction peaks of the spray-dried Leu when compared with the original diffraction peaks of Leu as supplied (6.2°, 12.1°, 24.4°, 30.5° 2θ; not shown) were observed. These changes were explain by Sou et al. (2013), who proposed that Leu after spray drying exhibits a partially ordered molecular arrangement, formed as a result of the drying crust (formed by drying/crystallization process), which has strong two dimensional layered order, however a complete three dimensional order is not fully achieved. In the formulations containing Leu, peak intensity may have been also reduced as a result of dilution with high amorphous material fraction (RHT and Inu).

As expected, the use of Inu (T_g ~156ºC) resulted in spray dried systems with T_g greater than 90 ºC, demonstrating that RHT, an API with a low glass transition temperature (T_g ~38ºC), might be physically stabilized by designing an amorphous microparticle formulation. Higher T_g values are expected to promote the physical stability of amorphous powders during storage, as long as the T_g of the formulation is approximately 50ºC greater than the storage temperature (Hancock et al., 1995; Zhou et al., 2002).

The thermal results (TGA) also showed that high inlet temperature (160 ºC) used in the spray drying process led to an efficient drying of droplets, resulting in dry powders with low residual solvent content (RSC), leading to good yields for all formulations due to reduced powder adherence to the spray drier wall. A decrease in RSC was related to the increase of the proportion of Leu in the formulations, where a significant decrease in RSC was achieved with an increase in Leu content (Table 2; $p = 0.0045$). It is speculated that during the process of particle formation, the non-displacement of Leu into the droplet centre led to the formation of a hydrophobic outer shell layer that reduced the affinity between the particle surface and the water molecules, resulting in a decrease in the RSC, dependent on Leu concentration. The hydrophobic nature of the surface of the particles leads to a low capacity for moisture sorbed due a surface of low polarity, as seen in DVS analysis, that consequently improves the flowability and dispersibility, by reducing particle interactions that promote formation of agglomerates. Chang et al. (2014) also previously observed that the addition of Leu in the spray dried solution was a convenient means of improving flowability of spray-dried powders, reducing agglomeration and maintaining integrity against moisture stress.

The efficient delivery of an API by dry powder inhalation depends on the aerosolization properties of particles. Clearly, there was an improvement in the aerosol particle delivery with

649 the addition of Leu to the spray dried formulations (formulations B to G), with higher
650 deposition on stages with lower cut-off points (≤ 2 μm) in comparison with LCF and
651 formulation A. In particular, the *in vitro* deposition profile of the formulations E and G (20%
652 Leu; 7.5% and 15% RHT loading, respectively) independent of RHT loading, showed greater
653 deposition efficiency, resulting a higher FPF < 3 μm and lower MMAD that may be correlated
654 with lower RSC, lower median particle size (d_{50}) and more wrinkled particles, indicating that
655 the particles can easily reach the alveolar sacs and will be available to exert therapeutic effects.
656 The LCF formulation showed high deposition in the mouthpiece adaptor/induction port and
657 separator, thus, this powder will be expected to deliver a lower dose when compared to spray
658 dried particles containing Leu. The LCF formulations are generally poorly performing (it is not
659 uncommon to have respirable drug fractions < 40% (Smith and Parry-Billings, 2003), as found
660 in our study (FPF < 41%), besides which, these carrier-containing systems can be very sensitive
661 to variation in dose due to a non-homogeneous mixing (El-Sabawi et al., 2006; Young et al.,
662 2011).
663 Besides the incorporation of Leu, the influence of drug loading (7.5 and 15.0%) on
664 aerosol performance was investigated. No negative influence on the characteristics of spray
665 dried particles or on the aerosolisation properties was found when RHT loading was increased
666 in the spray dried solutions. The nominal dose of the formulations A to E and LCF in each
667 aerosolisation test was 1.5 mg RHT (approximately 20 mg powder per capsule, 7.5% w/w
668 nominal drug load) and in the case of formulations F and G the nominal dose was 3 mg RHT
669 (approximately 20 mg powder per capsule, 15.0% w/w nominal drug load). Considering that
670 RHT administered orally (3 mg dose) reaches peak plasma concentration in ~1 hour, with
671 absolute bioavailability of about 36% (Polinsky, 1998), theoretically the bioavailable dose is
672 about 1 mg RHT; therefore, the results presented here suggest that the bioavailability of the
673 RHT could be readily improved by inhalation of respirable particles, since the aerosol particles
674 do not undergo first-pass effect, and after inhalation, the particles containing drug have the
675 potential to become 100% bioavailable in the systemic circulation.
676
677 **5. CONCLUSION**
678
679 In this study the use of a spray drying technique allowed the optimization of RHT
680 particles for pulmonary delivery through the addition of Inu and Leu, which resulted in novel
681 lactose carrier-free spray dried powders containing RHT. The results obtained suggest that the
682 amino acid, Leu, can be used to enhance the aerosolisation performance of spray dried powders
683 containing RHT for pulmonary drug delivery. The lactose carrier-free spray dried formulations
684 present physicochemical characteristics and aerosolisation performance that are superior to the
685 lactose carrier formulation containing RHT (LCF). The lactose carrier-free formulation provides

686 the advantage of being able to deliver a higher dose of active drug with a smaller total amount
687 of inhaled powder.
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689
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691
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LIST OF FIGURES

Figure 1. SE micrographs of lactose carrier-free spray dried formulations containing 7.5% w/w RHT and (A) 0, (B) 5, (C) 10, (D) 15, (E) 20%(w/w) Leu; lactose carrier-free spray dried formulations containing 15%(w/w) RHT and (F) 10 and (G) 20%(w/w) Leu; (H) lactose carrier formulation (LCF) containing 7.5% w/w micronized RHT; (I) micronized RHT.

Figure 2.XRPD diffractogram of lactose carrier-free spray dried formulations containing 7.5% (w/w) RHT and (A) 0, (B) 5, (C) 10, (D) 15 and (E) 20% (w/w) Leu; spray dried formulations containing 15% (w/w) RHT and with (F) 10 and (G) 20% (w/w) Leu; (H) Inu and (I) Leu after spray drying and (J) RHT as supplied.

Figure 3.DSC scans of lactose carrier-free spray dried formulations containing 7.5% (w/w) RHT and (A) 0, (B) 5, (C) 10, (D) 15 and (E) 20% (w/w) Leu; spray dried formulations containing 15% (w/w) RHT and with (F) 10 and (G) 20% (w/w) Leu; (H) Inu as supplied.

Figure 4. FTIR spectras of lactose carrier-free spray dried formulations containing 7.5% (w/w) RHT and (A) 0, (B) 5, (C) 10, (D) 15 and (E) 20% (w/w) Leu; spray dried formulations containing 15% (w/w) RHT and with (F) 10 and (G) 20% (w/w) Leu;(H) Inu, (I) RHT and(J) Leu as supplied.

Figure 5.XRPD diffractogram of (A) LCF, (B) lactose monohydrate as supplied and (C) RHT micronized.

Figure 6. DSC scans of(A) LCF , (B) lactose monohydrate as supplied and (C)RHT micronized.

Figure 7. FTIR spectras of (A)LCF formulation, (B) lactose monohydrate as supplied and (C) RHT micronized.

Figure 8. Water vapour sorption and desorption isotherms of materials as supplied: RHT, Inulin and Leucine.

Figure 9. XRPD diffractograms after DVS analysis of lactose carrier-free spray dried formulations containing 7.5% (w/w) RHT and (A) 0, (B) 5, (C) 10, (D) 15 and (E) 20% (w/w) Leu; lactose carrier-free spray dried formulations containing 15% (w/w) RHT with (F) 10 and (G) 20% (w/w) Leu;(H) Inu, (I) RHT and(J) Leu as supplied.

Figure 10. Water vapour sorption and desorption isotherms of (A) lactose carrier-free spray dried formulations containing 7.5% (w/w) RHT and (a) 0, (b) 5, (c) 10, (d) 15 and (e) 20% (w/w)Leu. (B) lactose carrier-free spray dried formulation containing 15% (w/w) RHT and (f) 10 and (g) 20% (w/w) Leu.

Figure 11. Water vapour sorption and desorption isotherms of lactose carrier formulation (LCF), lactose monohydrate as supplied and RHT micronized.

Figure 12. XRPD profiles after DVS analysis of(A) lactose carrier formulation (LCF), (B) lactose monohydrate as supplied and (C) RHT micronized.

Figure 13. Aerosol *in vitro* deposition of lactose carrier-free spray dried formulations with7.5% (w/w) RHT and (A) 0%, (B) 5%, (C) 10%, (D) 15% and (E) 20% w/w Leu; lactose carrier-free spray dried formulations containing 15% (w/w) RHT and (F) 10 and (G) 20% (w/w) Leu, and (H) lactose carrier formulation with 7.5% (w/w) RHT. MA – mouth adapter; IP – induction port.

LIST OF ABBREVIATIONS

API - Active pharmaceutical ingredients

ATR-FTIR - Attenuated Total Reflection–FTIR Spectroscopy

DPI - Dry powder inhaler

DSC - Differential scanning calorimetry

DVS - Dynamic vapour sorption

ED - Emitted recovered dose

FPF - Fine particle fraction

GSD - Geometric standard deviation

HPLC - High performance liquid chromatography

Inu – Inulin

LCF - Lactose carrier formulation

Leu – L-Leucine

MMAD - Mass median aerodynamic diameter

NGI - Next generator impactor

PXRD - Powder X-ray diffraction

RH - Relative humidity

RHT – Rivastigmine hydrogen tartrate

RSC - Residual solvent content

RSD - Relative standard deviation

SD - Standard deviation

SEM - Scanning electron microscopy

TGA - Thermogravimetric analysis

Table 1.Composition of lactose carrier-free spray driedformulations (A to G) and lactose carrier formulation (LCF).

Formulations	Materials (%, w/w)			
	L-Leucine	Inulin	RHT	Lactose
A - 0% Leu	0.0	92.5	7.5	-
B - 5% Leu	5.0	87.5	7.5	-
C - 10% Leu	10.0	82.5	7.5	-
D - 15% Leu	15.0	77.5	7.5	-
E - 20% Leu	20.0	72.5	7.5	-
F - 10% Leu	10.0	75.0	15.0	-
G - 20% Leu	20.0	65.0	15.0	-
LCF	-	-	7.5	92.5

Table 2. Outlet temperature, yield of spray dried powders, RHT loading, geometric particle size, bulk and tapped density, residual solvent content, glass transition and aerodynamic characteristics of lactose carrier-free spray dried formulations (A to G) and lactose carrier formulation (LCF).

		A - 0%Leu	B - 5%Leu	C - 10%Leu	D - 15%Leu	E - 20%Leu	F - 10%Leu	G - 20%Leu	LCF
	Outlet (°C)	71.33 ± 0.58	75.00 ± 7.00	75.00 ± 3.46	76.33 ± 1.53	78.33 ± 0.58	80.33 ± 2.31	84.00 ± 1.00	n/a
Yield of spray dried powder (%)		55.1 ± 2.2	64.0 ± 4.8	65.3 ± 2.8	65.8 ± 2.9	67.9 ± 1.4	66.9 ± 2.5	66.3 ± 1.9	n/a
RHT loading (%)		7.41 ± 0.33	7.36 ± 0.10	7.52 ± 0.19	7.41 ± 0.15	7.35 ± 0.17	15.32 ± 0.10	15.21 ± 0.20	7.35 ± 0.11
Geometric Particle size	d_{10} (µm)	0.62 ± 0.02	0.67 ± 0.04	0.63 ± 0.06	0.57 ± 0.04	0.56 ± 0.04	0.53 ± 0.03	0.55 ± 0.06	3.07 ± 0.08
	d_{50} (µm)	2.09 ± 0.11	2.12 ± 0.19	1.77 ± 0.12	1.45 ± 0.07	1.40 ± 0.06	1.45 ± 0.06	1.34 ± 0.10	25.87 ± 0.34
	d_{90} (µm)	6.03 ± 0.41	5.73 ± 0.59	4.20 ± 0.32	3.19 ± 0.15	3.00 ± 0.15	3.61 ± 0.34	3.22 ± 0.42	69.04 ± 1.16
	Span	2.59 ± 0.11	2.39 ± 0.15	2.02 ± 0.04	1.82 ± 0.08	1.76 ± 0.13	2.12 ± 0.22	1.99 ± 0.11	2.55 ± 0.01
Bulk density (g/cm^3)		0.14 ± 0.002	0.14 ± 0.003	0.23 ± 0.003	0.24 ± 0.002	0.37 ± 0.001	0.16 ± 0.004	0.36 ± 0.001	0.45 ± 0.005
Tapped density (g/cm^3)		0.18 ± 0.01	0.19 ± 0.05	0.34 ± 0.04	0.35 ± 0.03	0.50 ± 0.01	0.21 ± 0.06	0.45 ± 0.00	0.58 ± 0.00
Thermal analysis	RSC (%)	5.27 ± 0.78	4.60 ± 0.49	5.05 ± 0.27	4.06 ± 0.80	3.48 ± 0.79	3.34 ± 0.41	3.27 ± 0.44	11.77 ± 0.73
	T_g (°C)	137.23 ± 5.61	132.00 ± 6.08	126.58 ± 1.28	120.28 ± 3.05	120.10 ± 0.07	112.05 ± 0.53	97.82 ± 0.29	n/a
Aerodynamic characteristics	ED (%)	85.33 ± 4.22	85.05 ± 9.18	91.52 ± 4.22	89.21 ± 2.82	87.69 ± 1.85	91.58 ± 1.21	92.73 ± 2.17	90.52 ± 1.98
	FPF < 5 µm (%)	39.36 ± 4.06	42.02 ± 6.51	44.87 ± 0.53	54.10 ± 1.99	64.20 ± 0.92	65.22 ± 0.75	67.64 ± 2.26	40.97 ± 3.22
	FPF < 3 µm (%)	27.93 ± 3.77	29.53 ± 5.13	30.76 ± 0.59	42.07 ± 3.29	53.68 ± 1.41	57.20 ± 1.02	58.25 ± 2.61	30.90 ± 6.95
	MMAD (µm)	4.30 ± 0.52	3.71 ± 0.24	3.89 ± 0.16	2.84 ± 0.08	2.46 ± 0.08	2.69 ± 0.08	2.51 ± 0.12	3.44 ± 0.25
	GSD	2.09 ± 0.16	1.93 ± 0.06	1.91 ± 0.03	1.78 ± 0.13	2.22 ± 0.01	1.70 ± 0.06	1.76 ± 0.02	1.61 ± 0.07

RSC – residual solvent content; T_g – glass transition temperature; ED – emitted recovered dose (no capsule and device); FPD – fine particle dose/RHT; FPF – fine particle fraction; MMAD – mass median aerodynamic diameter; GSD - geometric standard deviation.