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Influence of excipients on physical and aerosolization stability of spray dried high-dose powder formulations for inhalation

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Abstract

The aim of this study is to investigate the influence of excipients on physical and aerosolization stability of spray dried Ciprofloxacin dry powder inhaler formulations. The model drug, Ciprofloxacin hydrochloride, was co-spray dried with excipients such as disaccharides (sucrose, lactose, trehalose), mannitol and L-leucine. The spray dried samples were stored at two different relative humidity (RH) conditions of: (1) 20% and (2) 55% RH at 20ºC. Ciprofloxacin co-spray dried with disaccharides and L-leucine in the mass ratio of 1:1 demonstrated an increase in fine particle fraction (FPF) as compared with the spray dried Ciprofloxacin alone when stored at 20% RH. However, deterioration in FPF of Ciprofloxacin co-spray dried with disaccharide and mannitol was observed upon storage at 55% RH as compared to the corresponding formulations stored at 20% RH due to particle agglomeration. Whereas, 10% and 50% w/w L-leucine in the formulation showed no change in aerosol performance (FPF of 71.1 ± 3.5%. and 79.5 ± 3.1%, respectively) when stored at 55% RH for 10 days as compared to 20% RH (FPF of 68.1 ± 0.3% and 73.6 ± 7.1%, respectively). L-leucine demonstrated short-term aerosolization stability by alleviating crystallization of Ciprofloxacin to some extent and preventing significant change in particle morphology. L-leucine is well-recognized as aerosolization enhancer; our study has shown L-leucine is also a physical and aerosolization stabilizer for spray dried Ciprofloxacin DPI formulations. Such stability enhancing activities were attributed to the enrichment of L-leucine on the particle surface as confirmed by XPS data, and intermolecular interactions between L-leucine and Ciprofloxacin as measured by FT-IR.

Graphical abstract

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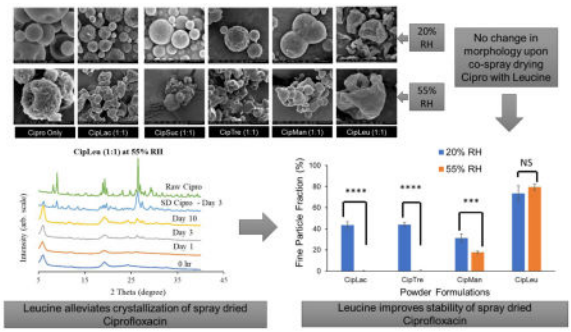

Keywords

dry powder inhaler (DPI); fine particle fraction (FPF); spray drying; excipients; antibiotics

1 Introduction

Dry powder inhalers (DPIs) are popular for pulmonary drug administrations as they are portable, easy to operate and cost effective (Williams et al., 2007; Zhou et al., 2015). DPIs have been used for treating asthma and chronic obstructive pulmonary disease (COPD) for decades, and more recently they have been developed for treating lung infections (Hoppentocht et al., 2014; Zhou et al., 2014b). Drug powder is usually micronized and aerosolized into fine particles through an inhaler device. Direct delivery of antibiotics into the lungs through DPIs will lead to substantially higher drug concentrations at the site of infection, which can maximize therapeutic efficacy and minimize systemic toxicity against lung infections (Muttil et al., 2009; Velkov et al., 2015).

DPI powder formulation essentially consist of micronized drug either with or without a carrier in the dry powder form. No propellants are used in case of DPIs (Jones et al., 2008) but for passive inhalers the entrainment of powders from the device and aerosolization may depend on patients inspiration capability, which could result in inconsistent flow rate and variable dose delivery for patients with compromised lung functions (Sumby et al., 1997). Deposition of these fine powders in the lower respiratory tracts depends upon the interactions between particles, lung deposition mechanisms and powder properties such as particle size distribution, surface morphology, crystallinity, hygroscopicity and surface energy etc. (Zhou et al., 2014b). The physicochemical properties of powders can be altered by particle-engineering technologies such as spray drying. Very recently, spray drying has been intensively explored to engineer drug particles because micronized drug particles produced by the milling process are extremely cohesive and prone to agglomeration (Chow et al., 2007; Malcolmson and Embleton, 1998; Ticehurst et al., 2000).

However, using spray drying technique to develop a DPI formulation with satisfactory aerosol performance while maintaining physical stability could be challenging (Chen et al., 2016; Hoppentocht et al., 2014). For example, spray drying technology has been widely used to produce DPI formulations (Vehring, 2008) but many spray dried compounds are amorphous in nature and physically unstable (Chan et al., 2004; Chen et al., 2016). Results

from our earlier study have shown that the spray dried amorphous Ciprofloxacin powder crystallized when stored at 55% relative humidity (RH), which led to increased surface roughness and enhanced aerosolization (Shetty et al., 2018). Furthermore, these amorphous powders often can absorb significant amounts of moisture under humid conditions (Zhou et al., 2014a), which likely result in deterioration in aerosolization due to increased inter-particulate capillary forces (Young et al., 2007; Zhou et al., 2013). There is a need to formulate DPIs which are physically stable and have consistent aerosol performance when they are stored at varying conditions (Sumby et al., 1997).

Excipients such as lactose monohydrate have been used as a coarse carrier for low-dose DPIs (de Boer et al., 2012); other excipients such as mannitol, trehalose, and L-leucine have been added in the spray dried DPI formulation with a main purpose to improve aerosolization performance or act as fillers. The mostly used carrier for DPIs is lactose as it is nontoxic, stable, and is compatible with most of the active drugs (Kou et al., 2012; Pilcer et al., 2012); use of lactose as a carrier in DPI formulations is well established (Marriott and Frijlink, 2012; Zhou and Morton, 2012). But for high-dose antibiotics, use of coarse carriers is limited to minimize the powder volume (Zhou et al., 2014b). Therefore, here we are focusing on excipients for spray dried DPI formulations, not as coarse carriers.

Mannitol has been frequently examined as a spray drying component for DPI formulations (Rahimpour et al., 2014). Mannitol was found to provide satisfactory aerosol performance and physical stability when added at a concentration of 50% in the co-spray dried Ciprofloxacin-mannitol formulation (Adi et al., 2010). Further, inhaled mannitol may help to remove mucous in patients with cystic fibrosis thereby improving penetration into the mucus and therapeutic efficiency of antibiotics (Yang et al., 2011). Mannitol in its amorphous form was shown to maintain stability of proteins such as salmon calcitonin due to hydrogen bond formation and thus can be used as a stabilizing agent with protein DPI formulations at low relative humidity (Chan et al., 2004).

Trehalose is a disaccharide similar to lactose but it differs from lactose as it is a non-reducing sugar. Unlike lactose, trehalose does not have Maillard reactions with polypeptides or protein-type drugs. Trehalose has been used as a carrier for DPI formulations such as albuterol sulfate, ipratropium bromide monohydrate, disodium cromoglycate and fluticasone propionate etc. (Cline and Dalby, 2002; Mansour et al., 2010). Inhalable trehalose powders were produced by spray drying their methanolic solution or by using other more innovative drying techniques such as spray freeze drying and supercritical fluid drying. (Hamishehkar et al., 2012; Jovanović et al., 2006). Cline et al. reported better aerosol performance of drugs such as albuterol and ipratropium when co-spray dried with trehalose as compared to lactose or mannitol (Cline and Dalby, 2002).

Some amino acids like L-leucine and tri-leucine are also known to enhance the aerosol performance of spray dried DPIs (Arora et al., 2016; Chew et al., 2005; Lechuga-Ballesteros et al., 2008; Rabbani and Seville, 2005); Its capability to protect spray dried inhalation powders against moisture is also reported (Li et al., 2017; Li et al., 2016). It has been demonstrated that co-spray drying active ingredients with leucine will result in enrichment of leucine on particle surface and changes in surface morphology of spray dried particles

(Boraey et al., 2013; Li et al., 2016; Sou et al., 2013), which lead to reduced surface energy and improved aerosol performance (Nokhodchi and Martin, 2015). However, the effects of excipients, particularly L-leucine on the physical and aerosolization stability of spray dried amorphous DPI formulations have not been examined.

The present study aimed to evaluate the impact of excipients such as lactose, sucrose, trehalose, mannitol and L-leucine on physical and aerosolization stability of spray dried Ciprofloxacin hydrochloride on storage at different humidity conditions. Ciprofloxacin was specifically chosen as a model drug as it is a potent antibacterial drug, effective against a range of bacteria that are associated with respiratory tract infections such as *Pseudomonas aeruginosa*. Secondly based on our preliminary findings, spray dried amorphous Ciprofloxacin powder tended to crystallize on storage at RH > 55%, which led to a significant change in aerosol performance (Shetty et al., 2018). It is important to examine the effects of excipients on physical stability and aerosolization of spray dried DPI formulations with a purpose to ensure quality of the DPI formulations.

2 Materials and method

2.1 Chemicals

Ciprofloxacin hydrochloride monohydrate was purchased from Betapharma (Shanghai) Co. Ltd. (Wujiang, China). Lactose was supplied from Avantor Performance Materials, Inc. (Center Valley, PA, USA). D-Mannitol was supplied by Dot Scientific Inc. (Burton, MI, USA). Trehalose was purchased from Spectrum Chemical Mfg. Corp. (Gardena, CA, USA). L-leucine was supplied by Sigma-Aldrich (St. Louis, Missouri, USA). High performance liquid chromatography (HPLC) grade acetonitrile and analytical reagent grade sucrose was supplied by Fischer Scientific (Fair Lawn, NJ, USA).

2.2 Spray drying

A BUCHI spray dryer (B-290, BUCHI Labortechnik AG, Flawil, Switzerland) was used to prepare formulations of Ciprofloxacin with each of the following excipients (i.e. sucrose, lactose, trehalose, mannitol and L-leucine) in the mass ratio (1:1) using water as the solvent. Additionally, Ciprofloxacin-Leucine in the mass ratio (9:1) was spray dried using water as the solvent. Feed solution (total solute concentration of 16 mg/ml) was pumped at the rate of 2 mL/min. Spray drying parameters were kept constant for each formulation: inlet air temperature (T_{in}) 120 ± 2ºC, aspirator at 35 m^3/h and airflow of 700 L/h, which lead to an outlet temperature (T_{out}) of approximately 60 ± 2 °C. The results with a label of “0 hr” represent data for the samples immediately after spray drying at room temperature of approximately 20º C and 40% RH. The spray-dried powders were divided into 2 equal parts and placed in open scintillation vials of 20 mL with powders spread as much as possible to ensure uniformity; one group of vials was stored in desiccators containing silica gel maintaining RH of 20 ± 2% and the other group of vials in a humidity chamber maintaining RH of 55 ± 2% RH using the saturated magnesium nitrate solution, which were selected based on the previous work (Shetty et al., 2018).

2.3 X-ray powder diffraction (PXRD)

X-ray diffractometer (Rigaku Americas, Texas, USA) was used to determine powder crystallinity. Powders were scanned from 5 to 40° 2θ at a step size of 0.02° with a rate of 4°/min, with a Cu-Kα radiation source and a highly sensitive D/tex ultra-detector. A voltage of 40kV and current of 44 mA was used for the experimental operation.

2.4 Scanning electron microscopy (SEM)

Particle morphology was assessed by scanning electron microscopy (NOVA nanoSEM, FEI Company, Hillsboro, Oregon, USA). Samples were smeared on a rectangular carbon tape and coated with platinum (208 HR, Cressington Sputter Coater, England, UK) at 40 mA for 60 seconds, corresponding to approximately 10 – 30 nm coating thickness The platinum coated samples were observed under SEM at 5 kV. Images with varying magnifications were taken depending on the size of the subjects.

2.5 Particle size

Particle sizes were measured using an Image J software based on SEM images (Rasband, 2007) with a magnification of 20,000. The Feret diameters at 10% (D_{10}), 50% (D_{50}) and 90% (D_{90}) undersize were calculated for approximately 100 particles, which were sufficient for size analysis (Shetty et al., 2018).

2.6 Dynamic vapor sorption (DVS)

Dynamic vapor sorption (DVS-Intrinsic, Surface Measurement Systems Ltd., London, UK) technique was employed to evaluate moisture sorption behavior for the spray dried formulations. Each formulation was equilibrated at 0% RH at the beginning to provide a baseline and then exposed to different RH. RH ranging from 0 – 90% at 10 % RH increments at 25ºC was used to measure the sorption mass change; desorption mass change was measured at RH ranging from 90 – 0%. At each testing RH, moisture content was determined by a dm/dt of 0.002% per minute.

2.7 Solid state Fourier transform infrared spectroscopy (FTIR)

A Cary 600 series IR spectrophotometer (Agilent Technologies, Santa Clara, California, USA) equipped with an attenuated total reflectance (ATR) sample stage was used to analyze potential solid-state properties for co-spray dried formulations of Ciprofloxacin-L-leucine in the mass ratios of 1:1 and 9:1. Samples were analyzed at a resolution of 4 cm^{-1} in the range on 400 – 4000 cm^{-1}. To minimize the interferences of water and CO_2 signals, background scans were collected before collecting the sample spectra (Nie et al., 2016).

2.8 X-ray photoelectron spectroscopy (XPS)

Surface composition was quantified using X-ray photoelectron spectroscopy (XPS) (AXIS Ultra DLD spectrometer, Kratos Analytical Inc., Manchester, UK) with monochromic Al Kα radiation (1486.6 eV) at pass energy (PE) of 20 for high-resolution and 160 eV for survey spectra. A commercial Kratos charge neutralizer was used to avoid non-homogeneous electric charge of non-conducting powder (in this case, the powders were conducting) and to achieve better resolution. Typical instrument resolution for PE of 20 eV

is ~0.35 eV. Binding energy (BE) values were calibrated using Au 4f7/2 at 84.0 eV and Cu 2p3/2 at 932.67 eV. Powder samples were placed on a stainless-steel bar using a Cu tape. XPS data were analyzed with CasaXPS software version 2313 Dev64. Prior to data analysis, the C-C component of the C 1s peak was set to a binding energy of 284.8 eV to correct for charge. Curve-fitting was performed following a Shirley background subtraction using reference peaks obtained from pure compounds. The atomic concentrations of the surface elements were estimated after a Shirley background subtraction taking into account the corresponding Scofield atomic sensitivity factors and inelastic mean free path of photoelectrons using the CasaXPS software, assuming homogeneous mixture of the elements within the information depths (~10 nm).

2.9 Drug quantification

Concentration of Ciprofloxacin hydrochloride was measured by high performance liquid chromatography (HPLC) at a wavelength of 215 nm, and no excipient peak was detected in this wavelength. Solution of sodium sulfate (76% v/v of 30mM, adjusted to pH 2.5 with H_3PO_4) and 24% v/v acetonitrile was used as the mobile phase. Isocratic elution of the sample was carried out for 3 min at a flow rate of 1.0 mL/min with a retention time of 2.2 mins for Ciprofloxacin. The calibration curve for Ciprofloxacin hydrochloride was linear ($r^2 > 0.99$) over the concentration range of approximately 0.006 to 0.201 mg/mL. Briefly, the HPLC system consisted of G1311C (1260 Quat Pump VL) pump, G1330B (1290 Thermostate) thermostate, G1329B (1260 ALS) autosampler, G1316A (1260 TCC) thermostated column compartment, G1314F (1260 VWD) variable wavelength detector (Agilant, Waldbronn, Germany), and an Agilant Eclipse Plus, 5 μm C18 150 × 4.60 mm column (Agilant, Waldbronn, Germany).

2.10 In-vitro aerosol performance

A Multi-Stage Liquid Impinger (MSLI) (Copley Scientific Limited, Nottingham, UK) with a USP induction port (USP throat) was used to determine aerosolization performance of the powder formulations at an ambient condition of approximately 20°C and 40% RH. Twenty milliliters of water was added in each of the stages (1–4), and a 0.2 μm glass microfiber filter was placed at the bottom of the device base. The formulation (10 ± 1 mg) was weighed into a size 3 hydroxypropyl methylcellulose capsule (Qualicaps, Whitsett, NC, USA). Four liter of air was passed through the inhaler at an airflow of 100 L/min for 2.4 s, with a pressure drop of approximately 4 kPa across a RS01 monodose DPI inhaler (with a similar design to Osmohaler, Plastiape S.p.A., Osnago, Italy). Stages 1 – 4 of the liquid impinger at 100 L/min had cutoff diameters of 10.4, 4.9, 2.4, and 1.2 μm, respectively (Zhou et al., 2016). For the powder formulations stored at 20% and 55% relative humidity conditions, four replicated dispersions were carried out and each experiment comprised sequential dispersion of two filled capsules. MilliQ water was used to collect the drug particles remaining in the capsule, inhaler device, USP throat, Stage 1–4 and the filter paper in the impactor base. The emitted dose was defined as the drug particles out of the capsule and device relative to the total recovered drug; the fine particle fraction was defined as drug particles with aerodynamic diameters < 4.9 μm relative to the total recovered drug.

2.11 Statistical analysis

One-way analysis of variance (ANOVA) with Tukey-Kramer post hoc tests were used for statistical analysis for the comparison of three and more groups using a GraphPad Prism Software (GraphPad Software, Inc., La Jolla, CA). For the comparison of two groups, unpaired t-test was used. Samples were considered to be significantly different if probability value (p) was < 0.05 and not significant (NS) if p value was > 0.05.

3 Results

3.1 Effects of excipients on physical stability and aerosol performance upon storage at 20% RH

3.1.1 PXRD—Co-spray dried formulations of Ciprofloxacin with sucrose, lactose and trehalose were amorphous at 20% RH for 1 day; the formulation with mannitol was crystalline. The crystalline peaks of mannitol formulation corresponded to the spray dried mannitol, not to Ciprofloxacin. Also, the co-spray dried Ciprofloxacin-L-leucine formulation was found to be crystalline and peaks corresponded to the spray dried L-leucine (Figure 1). Thus, in all formulations stored at 20% RH, Ciprofloxacin was in the amorphous form.

3.1.2 SEM—Figure 2 depicts morphological characteristics for the spray dried Ciprofloxacin alone and the co-spray dried formulations of Ciprofloxacin with different excipients as stored at 20% RH for 1 day. Spray dried Ciprofloxacin alone powders have a spherical shape with a dimpled surface (Figure 2a). Co-spray dried formulations of Ciprofloxacin with disaccharides (i.e. lactose, sucrose and trehalose) had a spherical shape with a rough surface (Figure 2b–d). However, co-spray dried formulation of Ciprofloxacin with mannitol some of the particles had fused (Figure 2e). Co-spray dried Ciprofloxacin-L-leucine powders had irregular and corrugated shape with a scaly surface (Figure 2f).

3.1.3 Physical particle size—Table I shows the physical particle sizes of the co-spray dried Ciprofloxacin formulations with sucrose, lactose, trehalose, Mannitol and L-leucine in the mass ratio of 1:1 as stored at 20% RH for 1 day. All drug particles from the 5 different formulations were shown to have D_{90} smaller than 3 μm.

3.1.4 In-vitro Aerosol Performance—No notable change (p >0.05) was observed between FPF of the spray dried Ciprofloxacin alone and co-spray dried Ciprofloxacin-mannitol formulations (Figure 3). However, a significant increase was observed in ED of the co-spray dried Ciprofloxacin-mannitol formulation compared to the spray dried Ciprofloxacin formulation alone (p < 0.0001). The significant increase in ED could be attributed to lower deposition of the Ciprofloxacin-mannitol co-spray dried formulation in the capsule and device as compared to the spray dried Ciprofloxacin formulation (Appendix A1). Thus, mannitol improves ED of Ciprofloxacin but not dispersibility due to partial particle agglomeration/fusion as observed from SEM images (Figure 2e). On the other hand, significant increase in FPF was observed for the co-spray dried formulation of Ciprofloxacin with lactose (43.5 ± 3.3%), sucrose (44.0 ± 4.3%), trehalose (44.0 ± 1.9%) and L-leucine (73.5 ± 7.1%) as compared to the spray dried Ciprofloxacin alone formulation (28.0

± 3.2%). However, the ED for Ciprofloxacin co-spray dried with sucrose and trehalose did not differ significantly in comparison to the spray dried Ciprofloxacin alone formulation ($p > 0.05$) (Figure 3).

Co-spray drying Ciprofloxacin with disaccharides caused more drug deposition in the capsule as compared to spray dried Ciprofloxacin formulation (Appendix A1) (Bhandari et al., 1997). Also, higher deposition was observed in the throat and Stage 1 for the spray dried Ciprofloxacin alone powders in comparison to co-spray dried Ciprofloxacin-disaccharide formulation (Appendix A1). Co-spray drying Ciprofloxacin with disaccharides helps to improve deposition of the powders in Stage 3, Stage 4 and filter as compared to spray dried Ciprofloxacin alone (Appendix A1). The increase in FPF could be attributed to formation of rougher particles upon co-spray drying Ciprofloxacin with disaccharides (Figure 2). Both FPF and ED for the co-spray dried Ciprofloxacin-L-leucine formulation (ED 93.2 ± 0.9, FPF 73.6 ± 7.1%) were found to be significantly higher as compared to the spray dried Ciprofloxacin alone formulation (ED 69.9 ± 1.1, FPF 28.0 ± 3.2%) ($p < 0.0001$). L-leucine formulation was shown to have the highest ED and FPF of Ciprofloxacin among all tested formulations.

3.2 Effects of excipients on physical stability and aerosol performance upon storage at 55% RH

3.2.1 PXRD—Figure 4 represents PXRD patterns of the raw Ciprofloxacin, spray dried Ciprofloxacin formulation stored at 55% RH for 3 days and co-spray dried formulations of Ciprofloxacin with various excipients stored at 55% RH for up to 10 days. The results with a label of “0 hr” represent samples immediately after spray drying. Since Cipro-Sucrose formulation caked upon storage at 55% RH, PXRD was not performed for this sample. The co-spray dried formulation of Ciprofloxacin with sucrose were found to cake within one day of storage at 55% RH. On the other hand, caking was observed eventually for the co-spray dried formulation of Ciprofloxacin with lactose and trehalose after 10 days. Both the co-spray dried lactose and trehalose formulations remained amorphous up to one day and the drug Ciprofloxacin began to crystallize after 3 days of storage at 55% RH (Figure 4a & 4b). Ciprofloxacin co-spray dried with mannitol crystallized within one day of storage at 55% RH while Ciprofloxacin-L-leucine co-spray dried powder formulation began to crystallize after Day 3 (Figure 4c & 4d respectively). From our previous study we have observed that spray dried Ciprofloxacin stored at 55% RH crystallizes within one hour. Qualitatively, the degree of crystallization for the co-spray dried Ciprofloxacin-L-leucine formulation after 10 days of storage at 55% RH was much lesser as compared to the spray dried Ciprofloxacin alone formulation stored at 55% RH for 3 days. Thus, it is evident that 50% w/w L-leucine alleviates the crystallization of spray dried Ciprofloxacin.

3.2.2 DVS—Moisture sorption/desorption isotherms are shown in Figure 5. Moisture-induced crystallization of the Ciprofloxacin-sucrose formulation began at around 60% RH as indicated by a decrease in mass and completed at around 80% RH (Yu et al., 2008). These powders resumed their sorption behavior above 80% RH as indicated by increase in mass which could be attributed to dissolution of the sucrose crystals in the water being absorbed and formation of a saturated solution (Makower and Dye, 1956; Tzannis and Prestrelski,

1999). An irrevesible change in the Ciprofloxacin-sucrose powder formulation occurred during the sorption phase since the desorption isotherm did not return back to its original value (Figure 5A). In case of the co-spray dried Ciprofloxacin-lactose formulation a remarkable increase in moisture uptake was noted from 0 % to 50% RH beyond which the samples crystallize as indicated by loss in mass due to expulsion of water (Buckton and Darcy, 1995; Price and Young, 2004) (Figure 5B). The sorption isotherm of the Ciprofloxacin-trehalose formualtion shows a rapid increase in moisture uptake from 0% to 40% RH. Crystallization event is not very prominent but a slight decrease in mass was observed between 40% and 55% RH beyond which the samples absorbed more moisture. Excess water is retained in the sample as the desoprtion isotherm does not return to its initial value (0% w/w) (Figure 5C).

The co-spray dried Ciprofloxacin-mannitol formulation absorbed 4.48% (w/w) water between 0% and 60% RH; however beyond 60% RH, a decrease in mass was observed indicative of crystallization. The desorption isotherm contained hysterisis and did not return to 0% w/w value due to retention of water in the crystalline powder (Adi et al., 2010) (Figure 5D). The co-spray dried Ciprofloxacin-L-leucine formulation absorbed 6.09% (w/w) water up to 70% RH. A decrese in mass from 6.09% w/w to 3.50% w/w was observed from 70% to 90% RH due to loss of water and crystallization. The water was retained in the crystal lattice and hence the desoprtion isotherm did not return to 0% w/w (Figure 5E). Unlike spray dried Ciprofloxacin alone, the event of crystalization was delayed in the co-spray dried Ciprofloxacin-L-leucine formulation.

3.2.3 SEM—Figure 6 depicts morphological characteristics for the Ciprofloxacin powder and the co-spray dried formulations of Ciprofloxacin with different excipients stored at 55% RH. The spray dried Ciprofloxacin formulation showed increased surface roughness upon storage at 55% RH (Figure 6a). Co-spray dried formulation of Ciprofloxacin with lactose, sucrose, trehalose and mannitol was fused upon storage at 55% RH (Figure 6b–d). Presence of sugars in the spray dried formulation has a tendency to increase powder stickiness. The sticky behaviour is associated with low glass transition temperature of those sugars such as sucrose, lactose etc. Sucrose has a Tg of 62ºC and lactose has a Tg of 101ºC (Langrish and Wang, 2009; Roos, 1993). Thus, if the spray drying temperature is ≥20ºC higher than their Tg, it would result in sticking powders because the sugar molecules tend to have high molecular mobility (Bhandari et al., 1997; Muzaffar et al., 2015). Inlet temperature of < 120°C may not be sufficient for drying; therefore lower inlet temperature was not tried. However, no change was observed in the surface morphology of the co-spray dried Ciprofloxacin-L-leucine formulation upon storage at 55% RH (Figure 6f) for 10 days as compared to the powders stored at 20% RH (Figure 2f).

3.2.4 Physical particle size—Physical particle size determination could not be performed for Ciprofloxacin co-spray dried with lactose, sucrose, trehalose and mannitol stored at 55% RH due to caking of the powders. However, CiproLeu formulation had fine physical particle sizes (D_{10} 0.89 μm, D_{50} 1.56 μm, and D_{90} 2.66 μm) after storage at RH of 55% for 10 days.

3.2.5 In-vitro aerosol performance—Figure 7 shows the changes in ED and FPF after storage of the co-spray dried formulations at 55% RH for 10 days. A significant decrease ($p<0.0001$) was observed with the FPF when the co-spray dried formulation with lactose was stored at 55% RH (Figure 7A). This was due to significant deposition of the co-spray dried Ciprofloxacin-lactose powders stored at 55%RH in Stage 1 compared to the formulation stored at 20% RH (Appendix A2). No significant difference was observed in the emitted dose for the co-spray dried Ciprofloxacin-lactose formulation stored at 20% RH and 55% RH (Figure 7A). Similarly, a statistically significant difference was observed with the FPF when co-spray dried formulation of Ciprofloxacin with trehalose was stored at 55% RH (Figure 7B). This was attributed to higher deposition of the formulation stored at 55% RH in Stage 1 compared to the powders stored at 20% RH (Appendix A2). Dispersion for co-spray dried formulation of Ciprofloxacin-sucrose stored at 55% RH could not be determined as the powders had caked within 1 day.

For the co-spray dried Ciprofloxacin-mannitol formulation, no change in emitted dose was measured upon storage at 55% RH. However, a significant decrease in FPF was noted for the co-spray dried formulation of Ciprofloxacin with mannitol upon storage at 55% RH (Figure 7C). The FPF for the co-spray dried Ciprofloxacin-mannitol formulation decreased from 31.4 ± 3.9 % when stored at 20% RH to 17.7 ± 1.2 % upon storage at 55% RH.

However, L-leucine as an excipient did not show any significant difference ($p>0.05$) in FPF between the co-spray dried formulation of Ciprofloxacin-L-leucine in the mass ratio (1:1) stored at 20% RH and 55%RH (Figure 7D). The FPF for the co-spray dried Ciprofloxacin-L-leucine formulation was 73.6 ± 7.1% when stored at 20% RH and was 79.5 ± 3.1% upon storage at 55% RH. Higher deposition of the co-spray dried Ciprofloxacin-L-leucine powders were found in Stage 3, Stage 4 and filter at both 20% RH and 55% RH (Appendix A2). Unlike the spray dried Ciprofloxacin alone, the co-spray dried Ciprofloxacin-L-leucine formulation showed no change in particle morphology (Figure 6f) and thus no change in aerosol performance leading to more stable formulation. In the following studies, we further investigated the effects of lower concentration of L-leucine (10%) on stability and aerosol performance of spray dried Ciprofloxacin, with a purpose to reduce the total powder mass for this high-dose DPIs.

3.3 Effects of low concentration (10% w/w) of L-leucine on physical stability and aerosol performance

3.3.1 PXRD—Figure 8 represents PXRD patterns of raw Ciprofloxacin, spray dried Ciprofloxacin alone formulation stored at 55% RH for 3 days and the co-spray dried formulation of Ciprofloxacin with 10% w/w L-leucine stored at 55% RH for up to 10 days. PXRD data suggest that co-spray drying Ciprofloxacin with 10% L-leucine resulted in crystallization of the drug on Day 1 (Figure 8). However, it is evident that the degree of crystallization for the co-spray dried Ciprofloxacin-L-leucine formulation in the mass ratio (9:1) at day 10 was lower as compared to crystallization of spray dried Ciprofloxacin alone at Day 3 upon storage at 55% RH. Thus, L-leucine even at a low concentration of 10% w/w was found to alleviate the degree of crystallization for Ciprofloxacin.

3.3.2 DVS—Co-spray dried Ciprofloxacin-L-leucine formulation in the mass ratio (9:1) was found to absorb 7.5% moisture up to 55% RH. Beyond 55% RH a significant decrease in mass was observed as indicative of crystallization (Figure 9).

3.3.3 SEM—Co-spray dried Ciprofloxacin-L-leucine powders in the mass ratio (9:1) appeared to be spherical in shape with a rough surface. Upon storage at 55% RH for 10 days, no major change in surface morphology was observed for the co-spray dried Ciprofloxacin-L-leucine formulation in the mass ratio (9:1) (Figure 10).

3.3.4 In-vitro aerosol performance—10% L-leucine in the co-spray dried Ciprofloxacin-L-leucine formulation showed no significant difference ($p>0.05$) in the ED and FPF between powders stored at 20% RH and 55% RH (Figure 11). The FPF for the co-spray dried Ciprofloxacin-L-leucine formulation in the mass ratio (9:1) was 68.1 ± 0.3% upon storage at 20% RH for 1 day. At 55% RH for 10 days, FPF for the co-spray dried Ciprofloxacin-L-leucine formulation in the mass ratio (9:1) was 71.1 ± 3.5%. Thus, even at a low L-leucine concentration of 10%, storage at 55% RH led to no change in morphology and aerosol performance for the co-spray dried Ciprofloxacin-L-leucine formulation.

3.3.5 FTIR—FTIR was used to determine any potential interactions between L-leucine and Ciprofloxacin at the molecular level in the co-spray dried Ciprofloxacin-L-leucine formulation. In Figure 12, the raw L-leucine demonstrates two distinct peaks at 1510 and 1577 cm^{-1}, which can be assigned to the symmetric deformation of NH_3^+ and asymmetric stretching of COO^- (Adhikari and Kar, 2012). It was reported that the carbonyl oxygen contribute to the intermolecular hydrogen bonding with one of the hydrogen atoms of the NH_3^+ moiety. Shifting of these peaks towards higher wavenumbers could suggest breaking of such intermolecular hydrogen bonding (Rajkumar and Ramakrishnan, 2000) There is a shift in the NH_3^+ peak from 1510 cm^{-1} for raw L-leucine to the higher wavenumber of 1514 cm^{-1} for the SD L-leucine which is likely indicative of the decreased crystalline content due to lack of regular molecular arrangement caused by hydrogen bonding.

There are likely new intermolecular interactions formed during the process of co-spray drying Ciprofloxacin with L-leucine since there is a slight shift in the COO^- and NH_3^+ peaks to the higher wavenumbers (Joseph and Jemmis, 2007) (Figure 12). By breaking the intermolecular interactions between L-leucine discussed above, the carbonyl oxygen of the carboxylic acid group or hydrogen of amine group can work as a strong hydrogen bond acceptor or donor, respectively, to interact with the hydrogen bond donating or accepting group of Ciprofloxacin (Mangal et al., 2018). However, it is very challenging to confirm the interaction center on Ciprofloxacin due to its complex chemical structure and the limited spectral information. Further investigation of the molecular interactions in the Ciprofloxacin-L-leucine complex will be warranted.

3.3.6 XPS—The XPS data demonstrated that the measured concentration of L-leucine on the particle surface was significantly higher than the theoretical value (Table II). The theoretical concentration of L-leucine was 10% w/w in the co-spray dried Ciprofloxacin-L-leucine formulation in the mass ratio of 9:1; while the measured surface mass concentration was 42% (calculated using C1s curve-fits and mass ratio based on number of carbon atoms).

Likewise, theoretical mass concentration of L-leucine was 50% in the co-spray dried Ciprofloxacin-L-leucine formulation in the mass ratio of 1:1, whereas calculated surface composition was 70% indicating surface enrichment by L-leucine in the co-spray dried Ciprofloxacin-L-leucine formulation.

3 Discussion

For inhalation drug delivery systems such as dry powder inhalation, amorphous powders obtained by spray drying process may have poor physical stability affecting their aerosol performance (Chen et al., 2016; Shetty et al., 2018). Excipients such as sugars or amino acids can be added as stabilizers to improve the physical and aerosol stability. In the present study effects of various excipients on moisture induced-crystallization of spray dried Ciprofloxacin were studied. At 20% RH, the formulations of Ciprofloxacin spray dried with the sucrose, lactose and trehalose were amorphous and showed a significant increase in aerosol performance as compared to the spray dried Ciprofloxacin alone. The increase in aerosol performance by addition of disaccharides could be attributed to increase in surface roughness of these particles in comparison to spray dried Ciprofloxacin alone (Chan et al., 2003; Heng et al., 2000). Ciprofloxacin co-spray dried with mannitol did not show significant change in aerosol performance and the powders appeared to agglomerate at 20% RH. It is interesting to note that literature showed co-spray drying of ciprofloxacin with 50% w/w mannitol resulted in an increase of FPF from 25.5 ± 1.6% for the ciprofloxacin alone spray dried power to 43.5 ± 1.5% (Adi et al., 2010). Such different effects are likely due to the different spray drying conditions used. Nevertheless, additional of only 10% w/w L-leucine resulted in a high FPF of 68.1 ± 0.3%, showing the superior capability of L-leucine in improving aerosolization than other tested excipients at a much lower excipient concentrations, which is beneficial for high-dose DPIs. At 20% RH, the drug Ciprofloxacin was in its amorphous form when co-spray dried with different excipients. However, at 55% RH despite the presence of various excipients, the drug crystallized over time in the different formulations.

Ciprofloxacin co-spray dried with mannitol crystallized within one day of storage at 55% RH while caking was observed eventually for the co-spray dried formulation of Ciprofloxacin with sucrose, lactose and trehalose after 10 days. Ciprofloxacin-mannitol formulation retained unchanged ED and did not cake at 55% RH; however, a decrease in FPF was observed compared to that formulation stored at 20% RH. Co-spray dried formulations of Ciprofloxacin with lactose, sucrose, and trehalose fused upon storage at 55% RH, making it a concern in physical stability. Similar observations were made by Naini et al. (1998) who compared the stability and moisture uptake of crystalline fractions for spray dried powders of lactose, sucrose, mannitol and trehalose on storage at different RH% for 30 days. These sugars recrystallized as sintered masses and became undispersible at > 52% RH (Naini et al., 1998).

Co-spray drying with L-leucine significantly increased the aerosol performance of Ciprofloxacin and enhanced physical and aerosol stability for the DPI formulation with no change in FPF upon storage at 55% RH. It is well recognized that co-spray drying drugs with amino acids such as L-leucine can enhance aerosol performance, and such

improvements depend on the enrichment of L-leucine on the particle surface (Mangal et al., 2018). The XPS data have proved such enrichment of L-leucine on the particle surface, which led to the corrugated particle shape and improved aersol performance compared with the spray dried Ciprofloxacin alone. It is further interesting to note that unlike the spray dried Ciprofloxacin alone powders, the co-spray dried Ciprofloxacin-L-leucine 1:1 formulation showed no major change in particle morphology and aerosolization, and also alleviated crystallization of Ciprofloxacin when stored at 55% RH for 45 days (Appendix A4). These effects of L-leucine on physical stability were measured even at a low L-leucine concentration of 10% w/w. To understand the underlying mechanisms of enhanced physical stability by co-spray drying with L-leucine, we have conducted FT-IR study to examine the molecular interactions between Ciprofloxacin and L-leucine. FT-IR data suggested that intermolecular interactions including hydrogen bonding between L-leucine and Ciprofloxacin may led to the inhibition on the crystallization of amrouphous ciprofloaxicin, which deserve further investigations. Future studies are warranted to use other solid-state technologies such as solid-state NMR to investigate such interactions.

4 Conclusion

The present study has shown various excipients had different effects on the physical and aerosolization stability of spray dried amorphous DPI formulations. It is an interesting finding that among all tested excipients, co-spray drying with L-leucine not only enhanced the aerosolization performance of Ciprofloxacin, but also improved the physical and aerosolization stability upon storage at a mild condition of RH 55%. It is well-recognized that co-spray drying with surface-active L-leucine will reduce surface energy and form corrugated particles, which lead to enhanced aerosolization. Here we have proved enrichment of L-leucine on the spray dried particle surface by XPS data. FT-IR results indicated that inhibitory effects of L-leucine on crystallization of amorphous Ciprofloxacin are attributed to the intermolecular interactions between L-leucine and Ciprofloxacin. This study provides some insights in physical and aerosolization stability of spray dried DPI formulations for high-dose medications, which are critical for the product quality.

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Abbreviations

Cipro	Ciprofloxacin hydrochloride monohydrate
DPI	Dry powder inhaler
DVS	Dynamic vapor sorption
ED	Emitted dose

FPF	Fine particle fraction
FTIR	Fourier transform infrared spectroscopy
Lac	Lactose
Leu	L-leucine
Man	Mannitol
MSLI	Multi-stage liquid impinger
PXRD	Powder X-ray diffractometer
RH	Relative humidity
SEM	Scanning electron microscopy
Suc	Sucrose
Tre	Trehalose
XPS	X-ray photoelectron spectroscopy

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Appendix

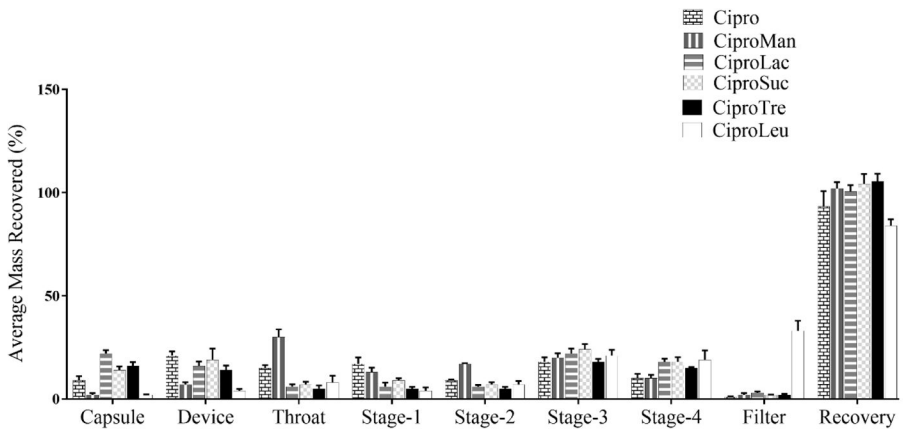
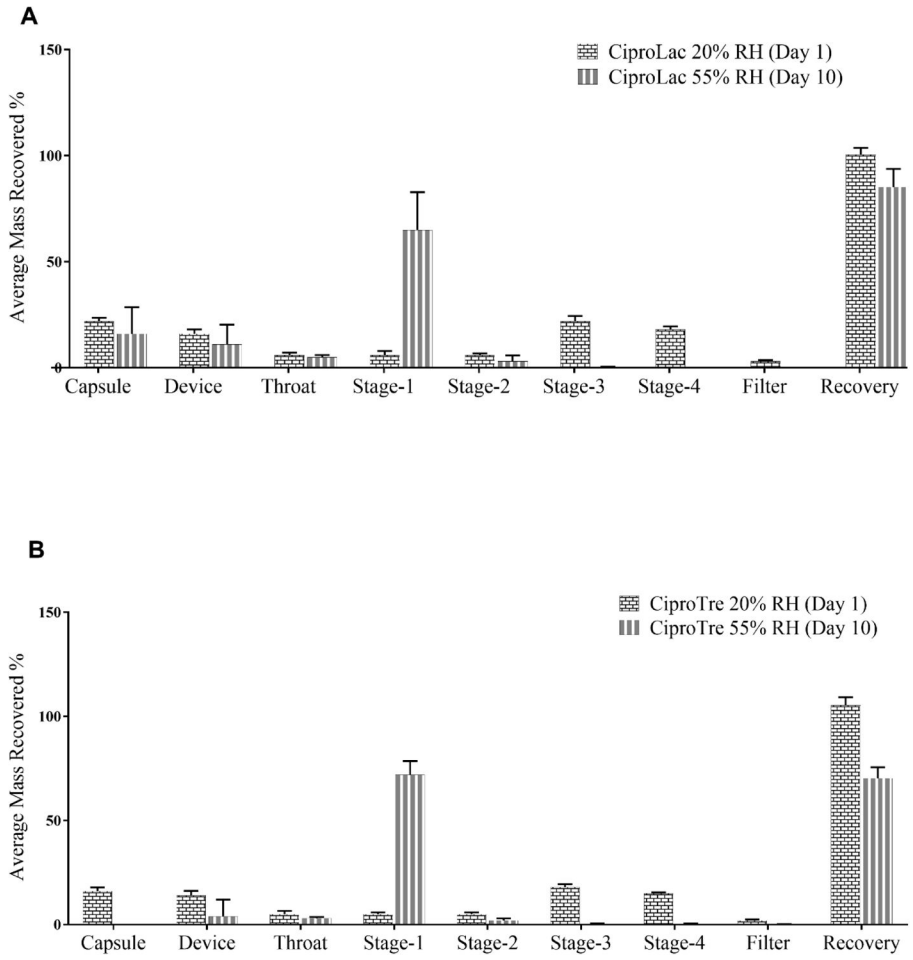


Figure A1.

Deposition profiles of the co-spray dried Ciprofloxacin-excipient stored at 20% RH for 1 day (mean ± SD, n=4)



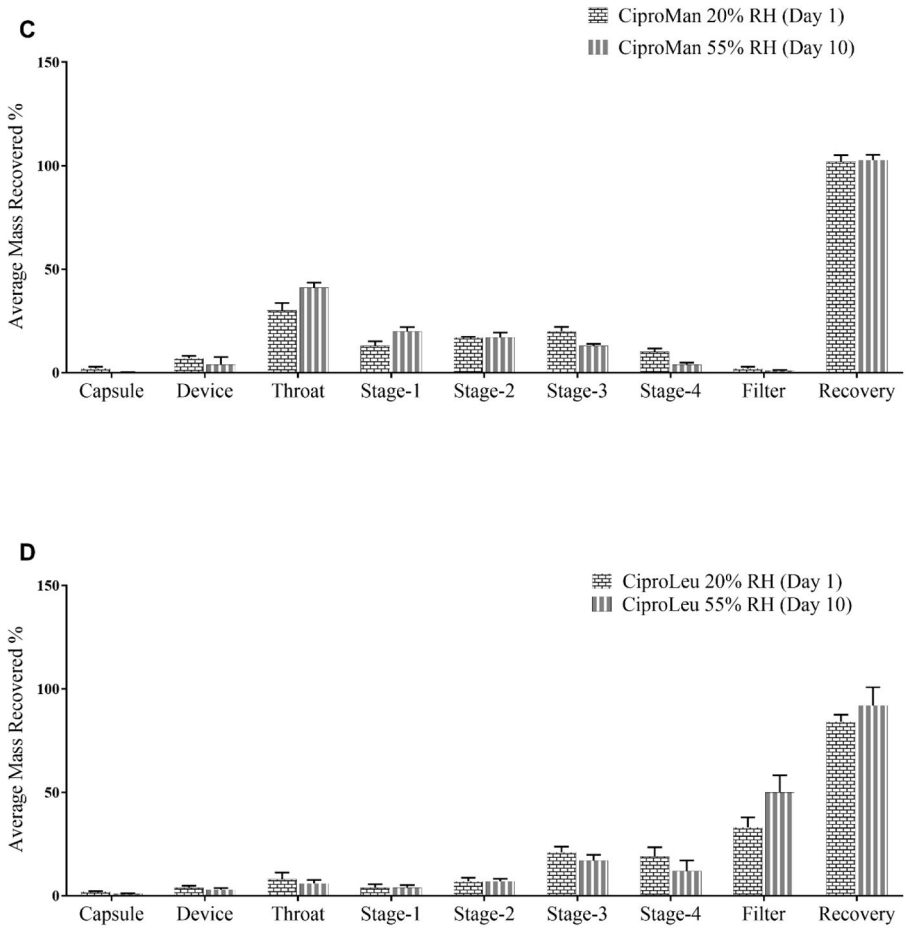


Figure A2.

Deposition profiles of the co-spray dried (A) Ciprofloxacin-lactose (B) Ciprofloxacin-trehalose (C) Ciprofloxacin-mannitol and (D) Ciprofloxacin-L-leucine formulations stored at 20% for 1 day and 55% storage humidity for 10 days (mean ± SD, n=4)

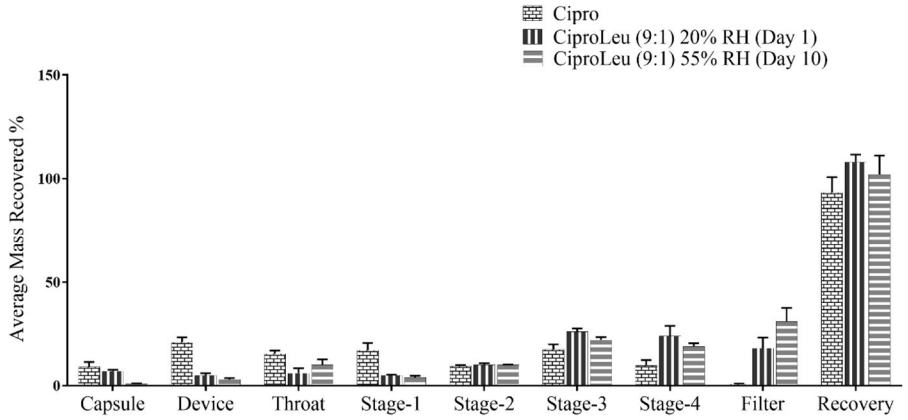


Figure A3.

Deposition profiles of the co-spray dried Ciprofloxacin-L-leucine formulations in the mass ratio (9:1) stored at 20% for 1 day and 55% storage humidity for 10 days (mean ± SD, n=3)

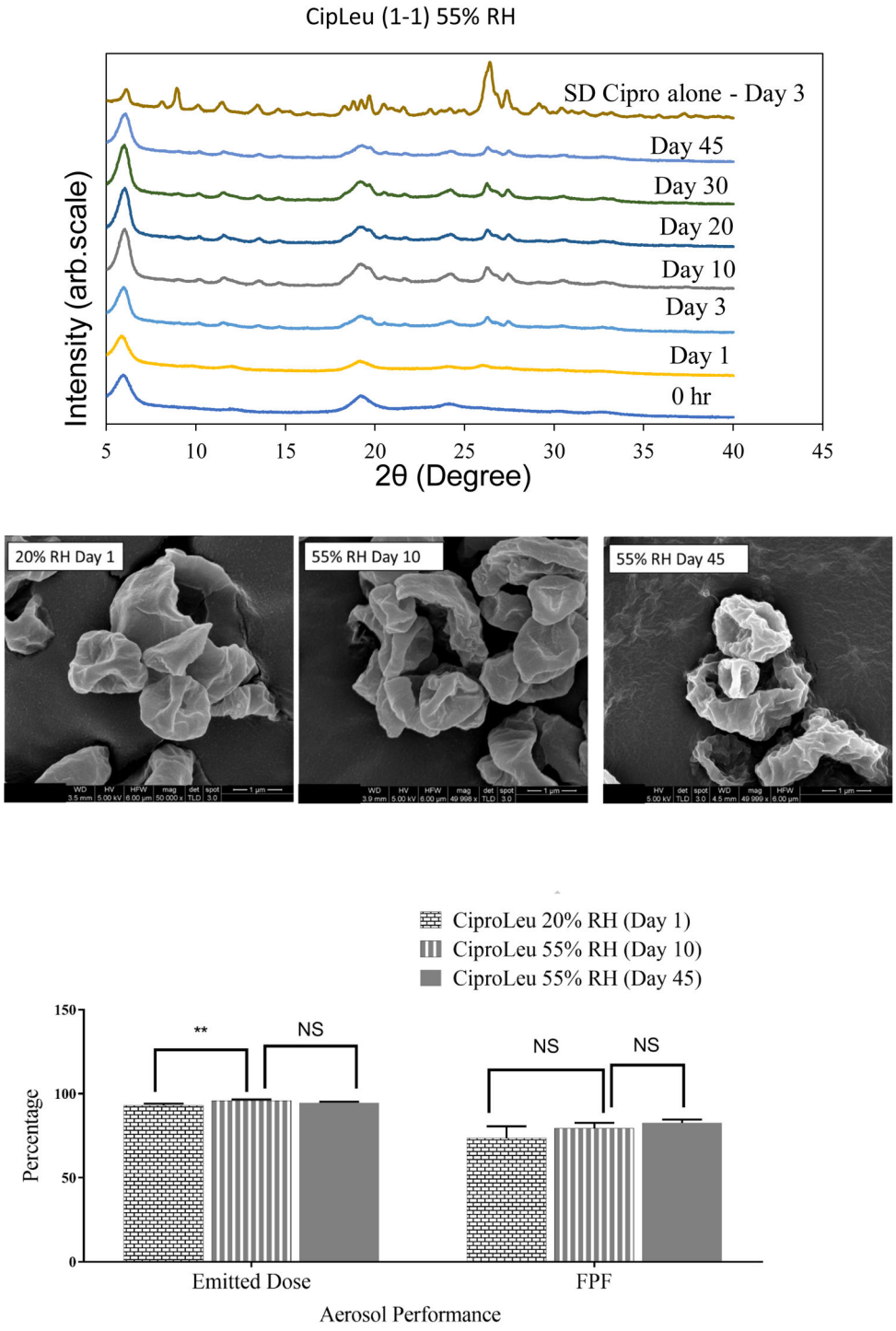


Figure A4.

PXRD, SEM and dispersion for CipLeu (1:1) stored at 20% RH for 1 day and 55% RH for 10 and 45 days respectively (mean ± SD, n=4)

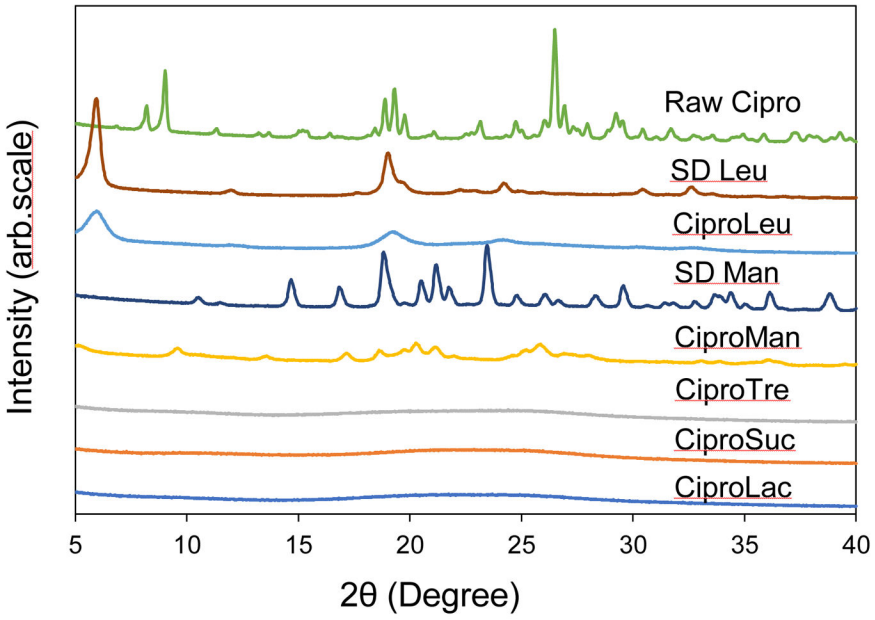


Figure 1.

PXRD patterns for the co-spray dried formulations of Ciprofloxacin with excipents as stored at 20% RH for 1 day and the raw Ciprofloxacin.

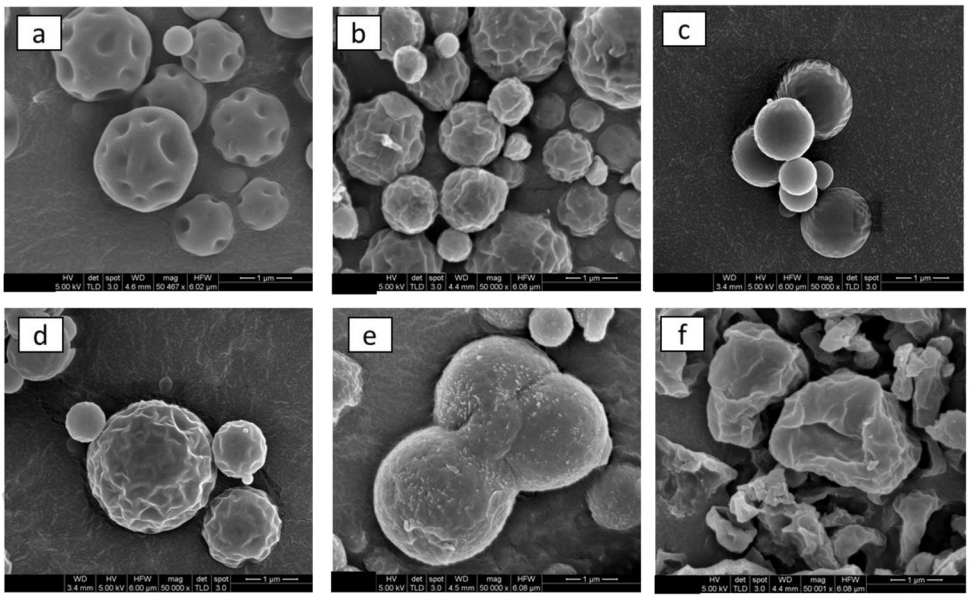


Figure 2.

SEM micrographs of (a) the spray dried Ciprofloxacin powder and the co-spray dried formulations of Ciprofloxacin with (b) lactose, (c) sucrose, (d) trehalose, (e) mannitol and (f) L-leucine stored at 20% RH for 1 day.

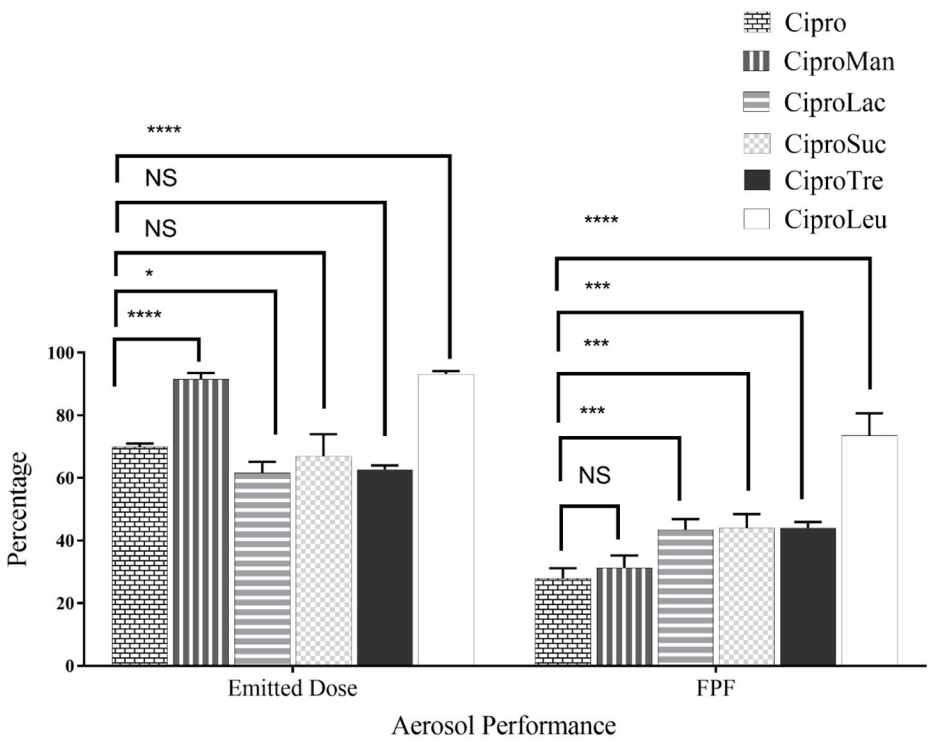
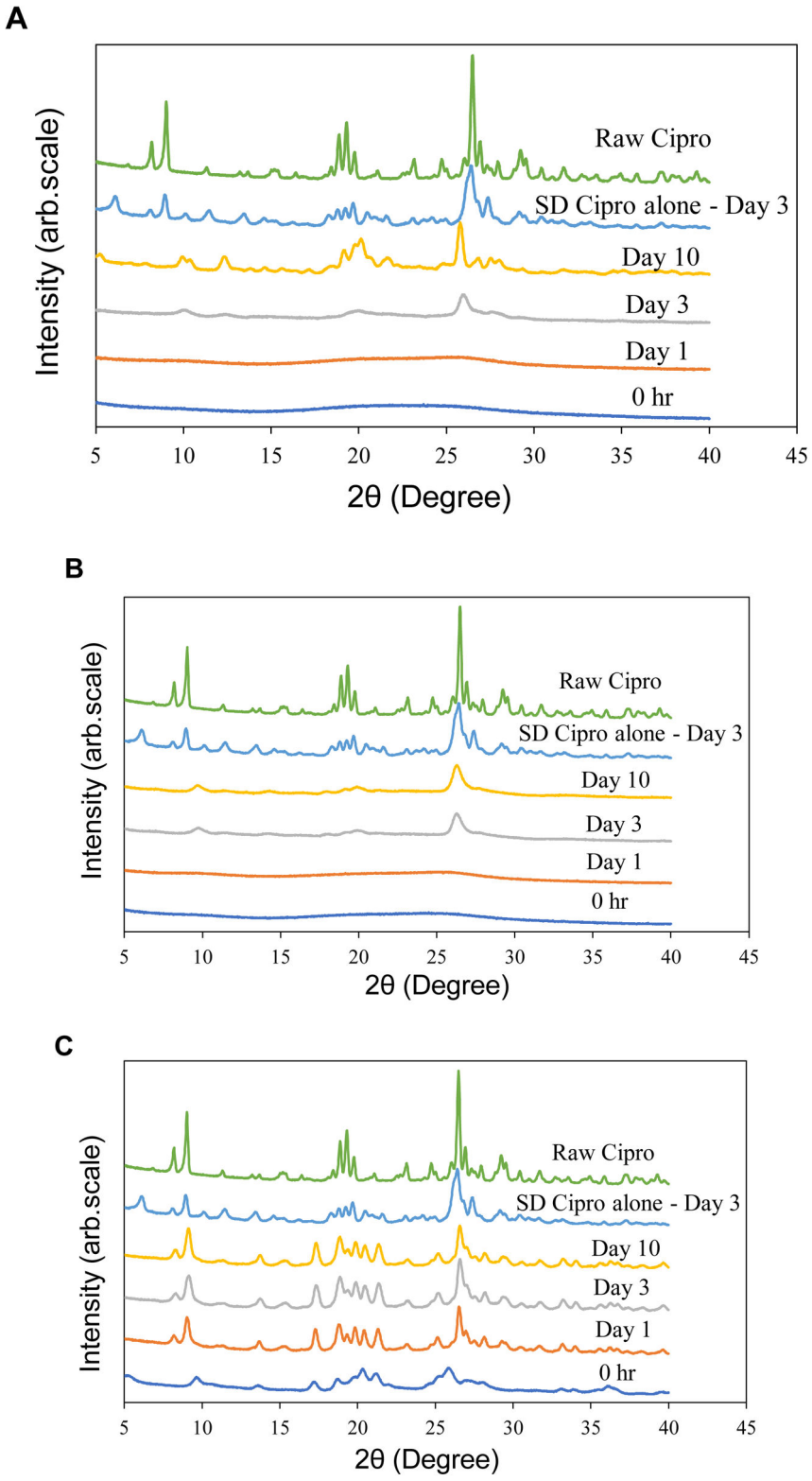


Figure 3.

Aerosol performance of the co-spray dried Ciprofloxacin-excipient formulations stored at 20% storage humidity for 1 day (mean ± SD, n=4; *, p <0.05; **, p <0.01; ***, p < 0.001; **** p <0.0001; NS, no significant difference).



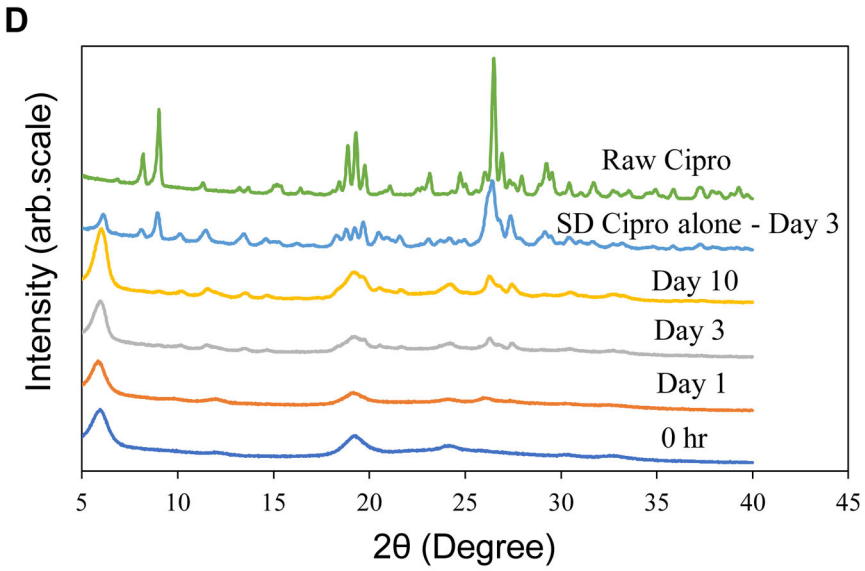


Figure 4.

PXRD patterns for the co-spray dried formulations of (A) Ciprofloxacin-lactose, (B) Ciprofloxacin-trehalose, (C) Ciprofloxacin-mannitol, and (D) Ciprofloxacin-L-leucine stored at 55% RH for 10 days.

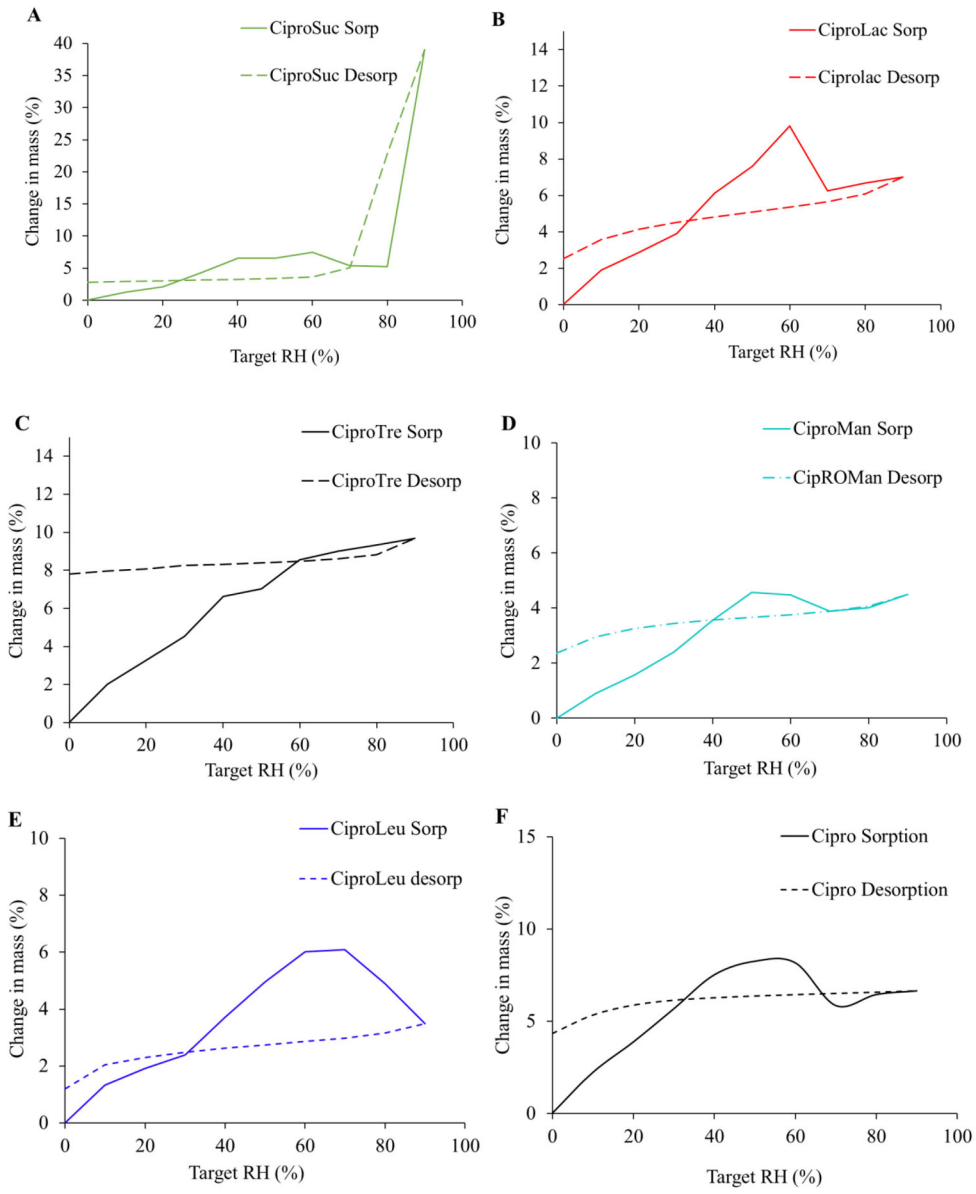


Figure 5.

Moisture sorption behavior for the co-spray dried formulations of (A) Ciprofloxacin-sucrose, (B) Ciprofloxacin-lactose, (C) Ciprofloxacin-trehalose, (D) Ciprofloxacin-mannitol, (E) Ciprofloxacin-L-leucine and (F) SD Ciprofloxacin alone.

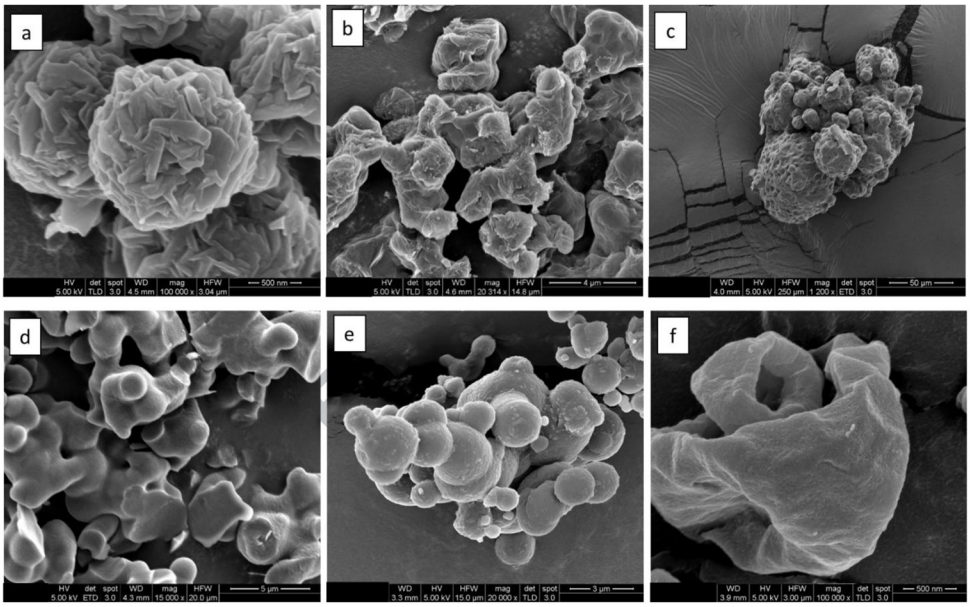
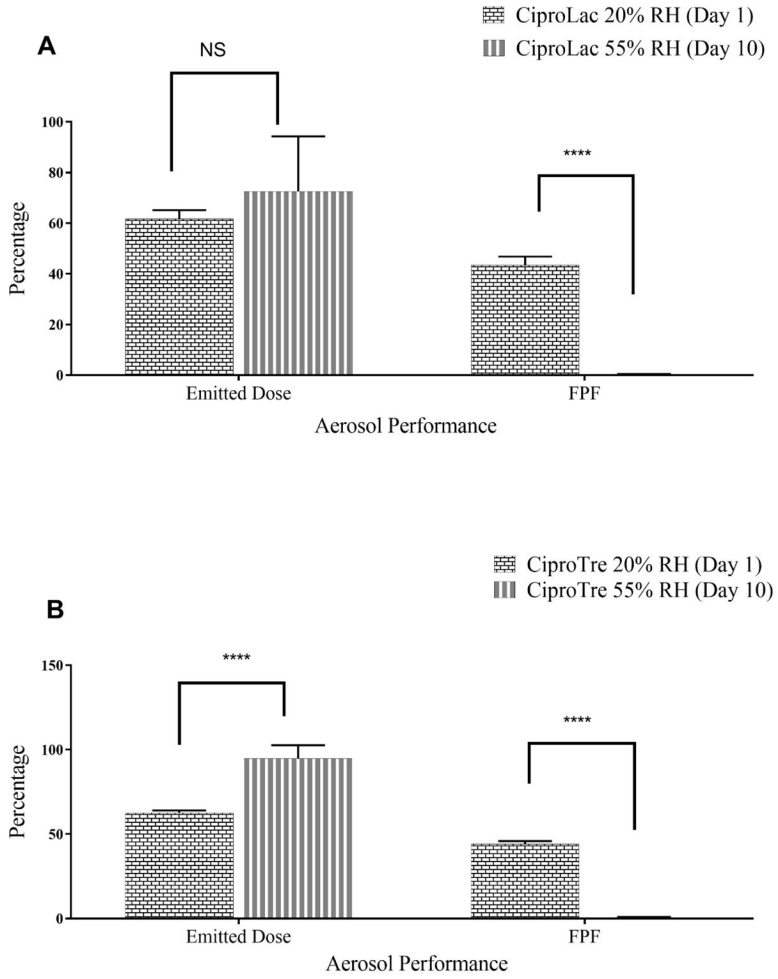


Figure 6.

SEM micrographs of the (a) spray dried Ciprofloxacin powder and the co-spray dried formulations of Ciprofloxacin with (b) lactose, (c) sucrose, (d) trehalose, (e) mannitol and (f) L-leucine stored at 55% RH for 10 days. (*Ciprofloxacin-sucrose was stored for only 1 day and began to cake/fused).



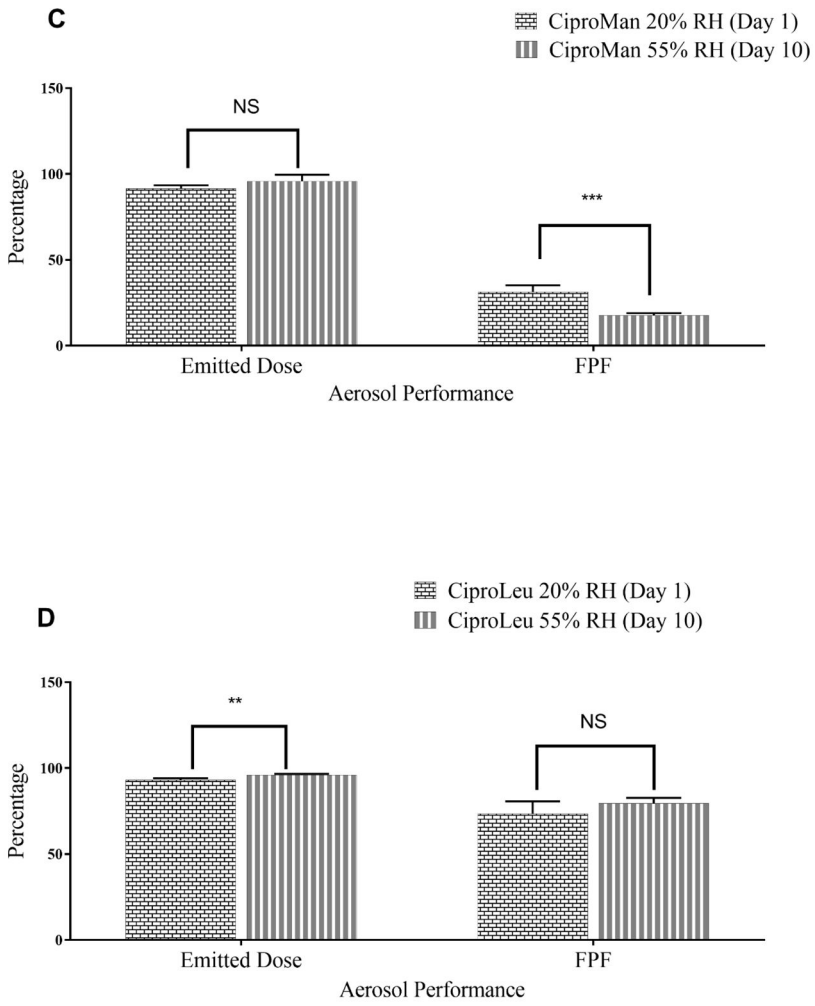


Figure 7.

Aerosol performance of the co-spray dried (A) Ciprofloxacin-lactose, (B) Ciprofloxacin-trehalose, (C) Ciprofloxacin-mannitol and (D) Ciprofloxacin-L-leucine formulations as reflected by ED and FPF at 20% and 55% storage humidity (mean ± SD, n=4; *, p <0.05; **, p <0.01; ***, p < 0.001; **** p <0.0001; NS, no significant difference)

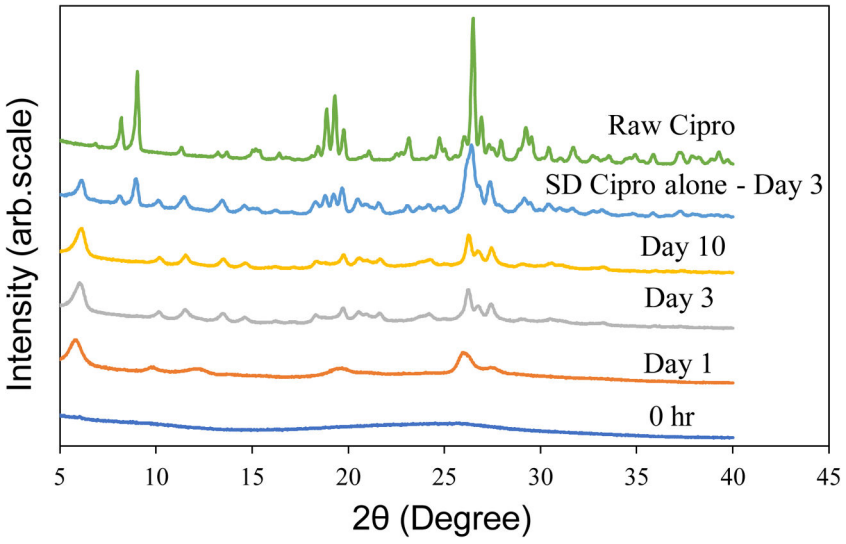


Figure 8.

PXRD patterns for the co-spray dried formulation of Ciprofloxacin-L-leucine in the mass ratio (9:1) stored at 55% RH for 10 days and Ciprofloxacin only formulation at 55% RH for 3 days.

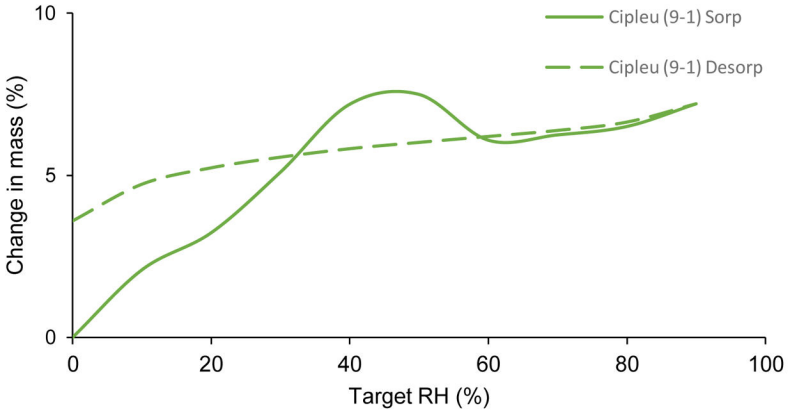


Figure 9.

Moisture sorption behavior for the co-spray dried formulation of Ciprofloxacin-L-leucine in the mass ratio (9:1).

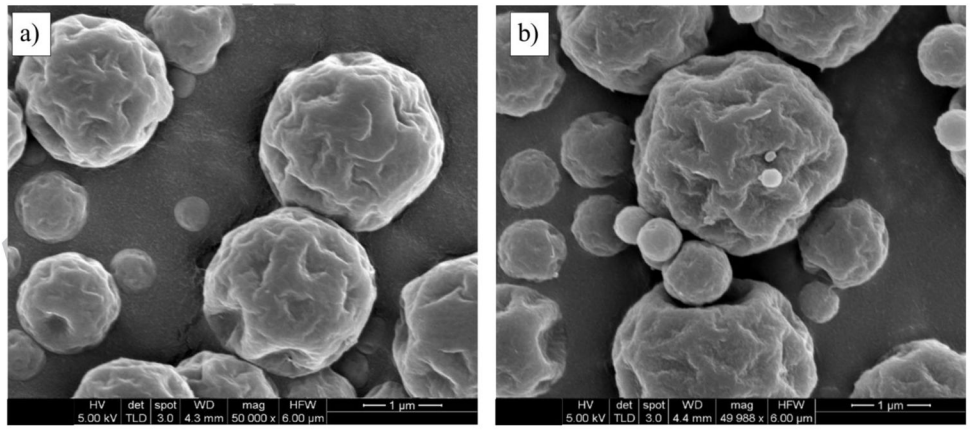


Figure 10.

SEM micrographs of the co-spray dried Ciprofloxacin-L-leucine formulation in the mass ratio (9:1) stored at (a) 20% RH for 1 day and (b) 55% RH for 10 days.

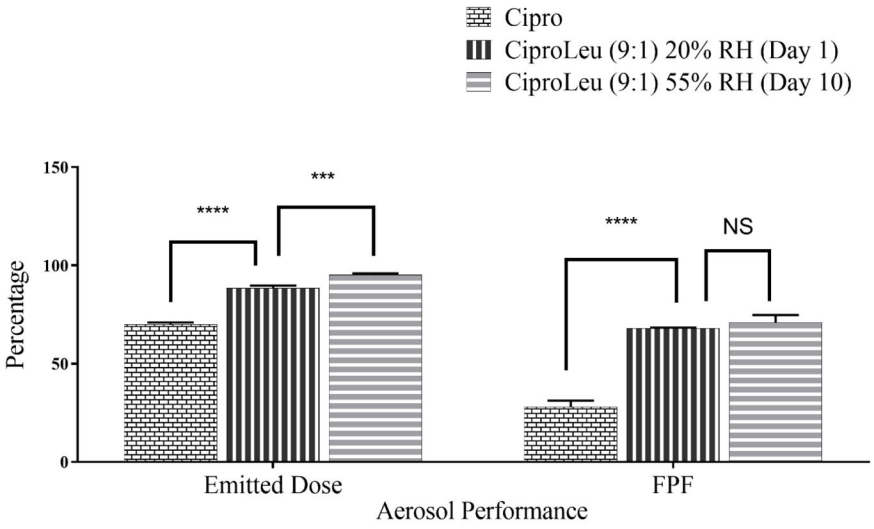


Figure 11.

Aerosol performance of the co-spray dried Ciprofloxacin-L-leucine (9:1) formulations as reflected by ED and FPF at 20% for 1 day and 55% storage humidity for 10 days (mean ± SD, n=3; *, p <0.05; **, p <0.01; ***, p < 0.001; **** p <0.0001; NS, no significant difference)

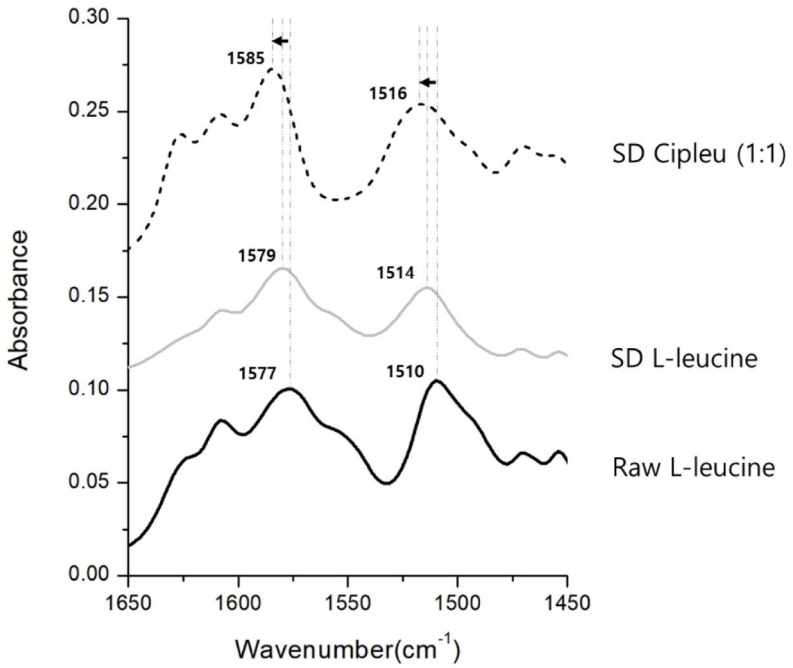


Figure 12.
FTIR spectra for the raw L-leucine, spray dried leucine alone and Cipleu formulations.

Table I

Particle sizes for the co-spray dried formulations in the mass ratio of 1:1 as stored at 20 % RH for 1 day.

Formulation	D_{10} (μm)	D_{50} (μm)	D_{90} (μm)
CiproLac	0.52	0.91	1.84
CiproSuc	0.50	0.84	1.71
CiproTre	0.51	0.92	1.76
CiproMan	0.41	0.83	1.66
CiproLeu	0.83	1.41	2.50

Table II

Theoretical and measured surface mass compositions by XPS for the co-spray dried Ciprofloxacin-L-leucine formulations in different mass ratios.

	% Surface Composition (Theoretical)		% Surface Composition (Measured)	
Formulations	**L-leucine**	**Ciprofloxacin**	**L-leucine**	**Ciprofloxacin**
CiproLeu_9:1	10	90	42	58
CiproLeu_1:1	50	50	70	30