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Research Article

Use of leucine to improve aerodynamic properties of ciprofloxacin-loaded maltose microparticles for inhalation

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Abstract

Ciprofloxacin (CIP) apparent permeability and absorption rate across the pulmonary epithelium can be controlled by its complexation with copper (II) ion. The aim of the current study was to formulate CIP-Cu-loaded microparticles comprising three main excipients, calcium carbonate, maltose and L-leucine, and to process by spray drying so as to generate particles with suitable aerodynamic properties for pulmonary delivery using a dry powder inhaler. Different maltose:calcium carbonate ratios were used to prepare microparticles, and the role of the excipients on the particles' physicochemical properties, stability, and aerosolization characteristics were investigated. All the formulations without L-leucine were fully X-ray amorphous. In the presence of L-leucine, diffraction peaks of low intensity were observed, which were attributed to the crystallization of the L-leucine at the particle surfaces. The addition of L-leucine modified the particle morphology and reduced the median geometric and aerodynamic diameters to 3.2 and 3.4 μm , respectively. The fine particle fraction of powder emitted from a Handihaler® device was increased up to 65.4%, predicting high total lung deposition. Stability studies showed that the powder X-ray diffraction pattern did not change over 21 months of storage in desiccated conditions, suggesting a good physical stability of the optimized formulation comprised of CIP-Cu, maltose and L-Leucine.

Keywords:

Ciprofloxacin metal complex; Microparticles; Aerosol; Controlled permeability; Pulmonary delivery; L-leucine.

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1. Introduction

Antibiotics (ATBs) with low epithelium permeability are suitable for pulmonary administration as aerosols. In fact, this low epithelium permeability reduces their elimination rate from the lung

and allows for better control of their concentration in this tissue. Accordingly, pulmonary delivery improves ATB efficacy and decreases their systemic exposure (d'Angelo *et al.*, 2014; Dudley, Loutit, & Griffith, 2008; Palmer, 2011; Velkov, Abdul Rahim, Zhou, Chan, & Li, 2015). Ciprofloxacin (CIP) is an ATB that is currently

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administered *per os* for the control of chronic *P. aeruginosa* lung infections (FDA, 2017; Follath *et al.*, 1986; Hoiby, 2011). Recent studies demonstrated that CIP powder for inhalation was well tolerated and resulted in significant reductions in total bacterial load (Stass, Delesen, Nagelschmitz, & Staab, 2014; Velkov *et al.*, 2015; Wilson *et al.*, 2013). However, the CIP apparent permeability measured through the well-known Calu-3 lung epithelium model is high compared to current commercially available inhaled ATBs (S. Marchand *et al.*, 2015). Consequently, CIP is rapidly absorbed from the lung after pulmonary inhalation as a solution (Gontijo *et al.*, 2014; Lamy *et al.*, 2018) compared to the marketed ATBs, such as tobramycin (S. Marchand *et al.*, 2015) or colistin methane sulfonate (Sandrine Marchand *et al.*, 2010).

It has been previously shown that CIP interacts with metal cations to form complexes that decrease its pulmonary epithelial permeability (Brillault, Tewes, Couet, & Olivier, 2017; F. Tewes *et al.*, 2016). In a previous study, we showed in a rat model that the intratracheal (IT) delivery of a dry powder loaded with CIP-copper (Cu^{2+}) complex allows a 100-times higher epithelial lung fluid (ELF) CIP exposure to be obtained compared to the IT delivery of a CIP solution (Lamy *et al.*, 2018). The dry powder formulation used previously, which comprised amorphous calcium carbonate-based microparticles, could be a very promising approach in the treatment of lung infection. However, the physical properties of this formulation could still be improved to obtain a powder with better aerosol performance, i.e. narrower geometric standard deviation (GSD) and a mass median aerodynamic diameter (MMAD) around 3 μm allowing a more precise targeting of the bronchioles. The amorphous calcium carbonate constituting these particles is the most soluble calcium carbonate form (Meiron *et al.*, 2011) and rapidly released the $(\text{CIP-Cu})^{2+}$ complexes and calcium ions in the lung after intratracheal administration to healthy rat (Lamy *et al.*, 2018). However, a recent study has shown that calcium ions can interact with the mucins present in the lung of CF patients, impairing their expansion to a fully functional structure. Large aggregates of mucin are formed at pH lower than 6.2 and calcium ion concentrations higher than 10 mM (400 mg/L) (Ambort *et al.*, 2012). Albeit the effect of amorphous calcium carbonate on the mucin expansion process is unknown, and that calcium concentrations found in rat lung ELF after the amorphous calcium carbonate microparticles IT delivery in rat was 10-times lower than the Ca^{2+} concentration need to aggregate the mucins (Ambort *et al.*, 2012; Lamy *et al.*, 2018), a decrease in Ca^{2+} proportion in the formulation was also envisaged. Therefore, a change in the amorphous calcium carbonate excipient by the maltose, with the aims to improve the particles' aerodynamic properties and avoid the potential calcium-mucin interaction was investigated. Maltose was selected as it has previously been studied as a potential excipient for dry powder for inhalation produced by spray drying and showed interesting characteristics, such as low aerodynamic diameter, and good flow properties and fine particle fraction (FPF) (Kawakami, Sumitani, Yoshihashi, Yonemochi, & Terada, 2010; Marriott, MacRitchie, Zeng, & Martin, 2006). Spray dried particles were

spherical with rough surfaces, which generally enhances dispersion from the dry powder inhaler (Kawakami *et al.*, 2010; Marriott *et al.*, 2006). Thus, the aim of this study was to develop, characterize, and evaluate the stability of (CIP-Cu) complex-loaded microparticles for inhalation made of maltose as the main excipient.

2. Materials and Methods

2.1. Chemicals

Ciprofloxacin powder (purity $\geq 98,0\%$), copper hydroxide $\text{Cu}(\text{OH})_2$, hyaluronic acid (HA) sodium salt from *Streptococcus equi*, ammonium carbonate $(\text{NH}_4)_2\text{CO}_3$, formic acid, L-leucine and maltose were purchased from Sigma (Wicklow, Ireland). All chemicals used were of analytical grade, and solvents were of high-performance liquid chromatography (HPLC) grade.

2.2. Preparation of CIP microparticles

Microparticles were prepared based on a modification (in order to include maltose) of a spray drying method which was previously used (F. Tewes *et al.*, 2016). Briefly, two aqueous solutions, one containing CIP, copper hydroxide, calcium hydroxide, leucine, formic acid and hyaluronic acid, and the other containing ammonium carbonate and maltose were prepared separately, to avoid the early CuCO_3 and CaCO_3 precipitation. These two solutions were mixed during the microparticle preparation process using a Y-tube connected to the feeding tube of the Büchi B-290 mini spray dryer. Ammonium carbonate was used as a blowing agent in order to obtain hollow particles with low density. The spray dryer was operated in the sucking mode and settings were: 30% peristaltic pump rate, 6 bar nitrogen flow rate, 630 L/h drying air flow rate, 120°C inlet temperature. A total of 6 formulations with different percentages of maltose were prepared (Table 1). The amount of CIP, copper hydroxide, formic acid and hyaluronic acid were kept constant. The calcium hydroxide to maltose ratio (w:w) was varied in the following proportions: 1:0, 3:1, 1:1, 1:3, 0:1 (Table 1)(note that the ratio 1:0 was previously characterized by Lamy *et al.*). Leucine was added only in the formulation containing a ratio of $\text{Ca}(\text{OH})_2$: maltose of 0:1 (w:w).

2.3. Scanning electron microscopy (SEM)

SEM was performed with a TescanMira Variable Pressure Field Emission Scanning Electron Microscope (Brno, Kohoutovice, Czech Republic) as previously described (Lamy *et al.*, 2018). Briefly, samples were fixed on aluminum stubs using double-sided adhesive tape and sputter-coated with gold. Visualization was performed at 5kV and micrographs were taken at different magnifications in more than one region of the sample.

2.4. Thermogravimetric analysis (TGA)

TGA measurements were performed as previously described

Table 1: Compositions of the solutions used for the preparation of microparticles by spray drying. Ca(OH)₂:maltose ratios (w:w) 1:0, 3:1, 1:1, 1:3, 0:1 were named CIP-Cu, CIP-Cu mix 1, CIP-Cu mix 2, CIP-Cu mix 3 and CIP-Cu-maltose respectively (the terminology "mix" was used when maltose and calcium carbonate were both present in formulation). The formulation containing a ratio of Ca(OH)₂:maltose of 0:1 (w:w) and leucine was named CIP-Cu-maltose-Leucine.

	CIP (g/L)	Ca(OH) ₂ (g/L)	Cu(OH) ₂ (g/L)	L-Leucine (g/L)	Formic acid (% (v/v))	Hyaluronic acid (g/L)	Maltose (g/L)
CIP-Cu	1.6	0.8	0.44	-	0.1	0.4	-
CIP-Cu mix 1	1.6	0.6	0.44	-	0.1	0.4	0.2
CIP-Cu mix 2	1.6	0.4	0.44	-	0.1	0.4	0.4
CIP-Cu mix 3	1.6	0.2	0.44	-	0.1	0.4	0.6
CIP-Cu-maltose	1.6	-	0.44	-	0.1	0.4	0.8
CIP-Cu-maltose-leucine	1.6	-	0.44	0.9	0.1	0.4	0.8

(Lamy *et al.*, 2018) under nitrogen purge using a Mettler TG50 module with attached Mettler MT5 balance (Mettler Toledo Ltd., U.K.). Samples were placed into open aluminum pans. A heating rate of 10°C/min and temperature range of 25-300°C was used for all experiments.

2.5. Volume-weighted geometric particle size distribution (PSD)

The PSD was determined using a Malvern Mastersizer 2000 laser diffraction instrument (Malvern Instruments Ltd. Worcestershire, UK) as previously described (Lamy *et al.*, 2018). The $d_{(0.5)}$ reported is the geometric median particle size of the volume distribution, while 10 percent and 90 percent of the distribution lie below $d_{(0.1)}$ and $d_{(0.9)}$ respectively. Results presented are the average of three determinations.

2.6. Powder X-ray diffraction (XRD)

X-Ray powder diffraction was measured using a Rigaku Miniflex II desktop X-Ray diffractometer (Rigaku, Japan) with Ni-filtered Cu K α radiation ($\lambda = 1.54 \text{ \AA}$) as previously described (Lamy *et al.*, 2018; F. Tewes *et al.*, 2016).

2.7. Specific surface area (SSA)

SSA was determined by gas adsorption at 77 K using a Micromeritics Gemini 2835c (SMS Ltd., London, UK) as previously described (Lamy *et al.*, 2018). Briefly, samples were degassed under a nitrogen purge for 24h at 25°C (Ogain, Li, Tajber, Corrigan, & Healy, 2011). The amount of nitrogen gas adsorbed at six relative pressure values ($0.05 < P/P_0 < 0.30$) was determined in order to calculate SSA according to the Brunauer, Emmett, and Teller (B.E.T.) method. Analyses were performed in triplicate for CIP-Cu-maltose-leucine and once for CIP-Cu-maltose.

2.8. Aerodynamic particle size distribution

The aerodynamic particle size distribution was measured using

a Next Generation cascade Impactor (NGI, Copley Scientific). The air flow rate was adjusted to 60 L/min and the time of aspiration was adjusted to 4 seconds by using a critical flow controller (TPK 2000, Copley Scientific) to obtain 4 L of aspiration. In this condition, critical sonic flow was assured ($P_3/P_2 < 0.5$) and the flow rate was assumed to be stable. The inhaler (Handihaler[®], Boehringer Ingelheim) was filled with a gelatin size 3 capsule loaded with 10 ± 2 mg of powder ($n = 3$ for CIP-Cu-maltose-leucine and $n = 1$ for CIP-Cu-maltose). After inhaler actuation, particle deposition on the NGI was determined by the CIP assay method described below. The mass of particles with aerodynamic diameter $\leq 5.0 \mu\text{m}$, expressed as a percentage of the emitted recovered mass, was considered to be the fine particle fraction (FPF). The mass median aerodynamic diameter (MMAD) and FPF were calculated as previously described (F. Tewes *et al.*, 2016).

2.9. Analytical assays

CIP concentrations were determined by reversed-phase HPLC coupled to a fluorometer for detection ($\lambda_{\text{exit}} = 280 \text{ nm}$, $\lambda_{\text{em}} = 460 \text{ nm}$) as previously described (Lamy *et al.*, 2018). Briefly, reversed-phase chromatography was performed by using a security guard cartridge (Gemini C18, Phenomenex) and a C18 X Terra MS column (5 μm pore size, 100 x 2.1 mm). The mobile phase, flowing at a rate of 0.25 mL/min, was made of 0.1% formic acid in water, acetonitrile and sodium heptane sulfonate (PIC[®] B7, Waters) mixed in a volume ratio of 80:20:1 (v:v:v). Seven calibration standards (from 1.56 to 10 ng/mL) and 3 levels of control (3.125, 2.5, 7.5 ng/mL) were prepared in the mobile phase. For the calculation of the slope and the intercept of the calibration curve, a $1/X^2$ -weighted linear regression was applied.

2.10. Solid state stability

Solid state stability studies of the CIP-Cu-maltose-leucine formulations were conducted at different conditions of temperature (4 and 25°C) and relative humidity (RH) (0 and 60%) in a closed cold room or incubator for a period of 21 months. Samples were

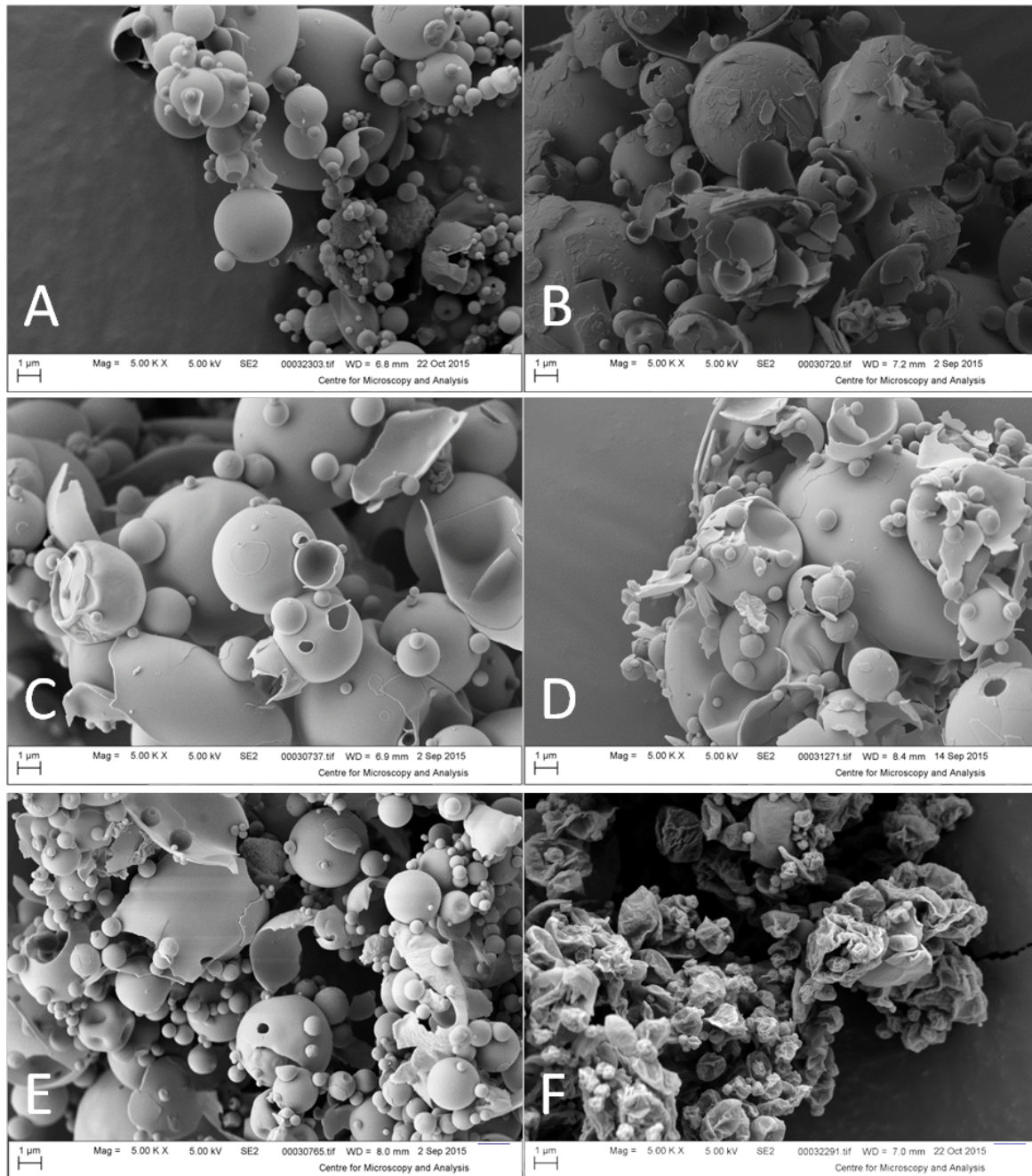


Figure 1: SEM micrographs of the microparticles. (A) CIP-Cu, (B) CIP-Cu mix 1, (C) CIP-Cu mix 2, (D) CIP-Cu mix 3, (E) CIP-Cu-maltose, (F) CIP-Cu-maltose-leucine. Samples were coated with a 10 nm-thick gold film. Primary electrons were accelerated under a voltage of 5 kV. Images were formed from the collection of secondary electrons.

placed in test chambers with Amebis humidity capsules (containing saturated salt solutions) (Amebis Ltd., Ireland) designed to achieve the required relative humidity (Serrano *et al.*, 2018). Test chambers

were kept in an incubator or fridge according to test conditions. Samples were removed at appropriate time intervals for XRD analysis.

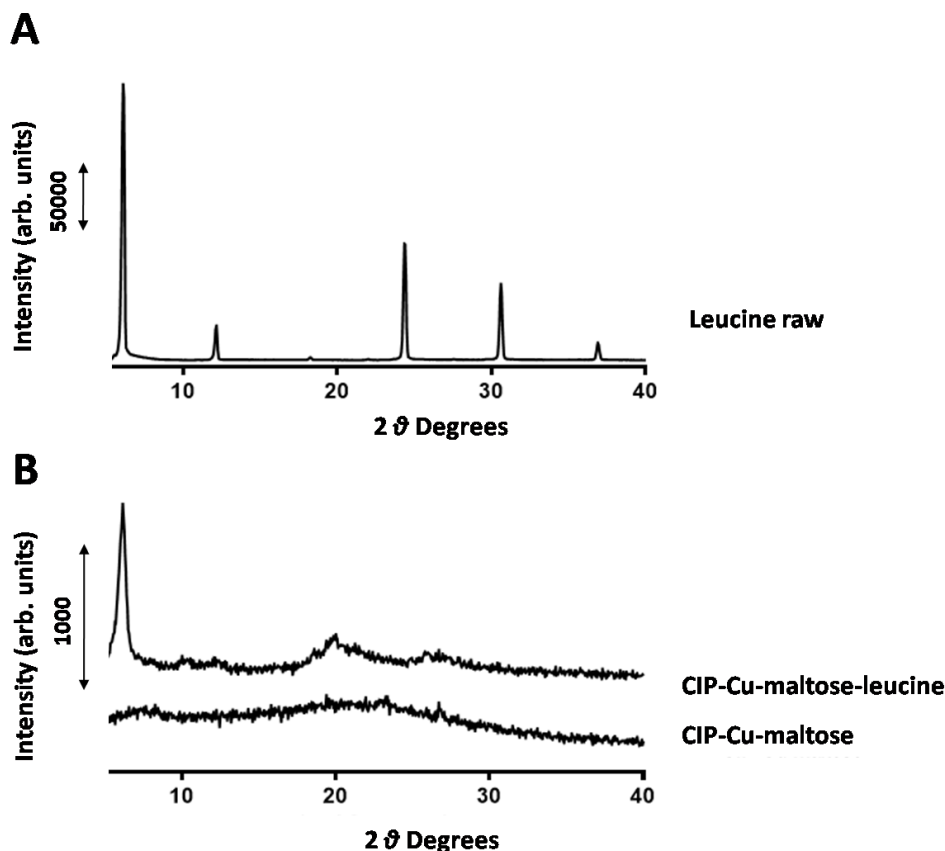


Figure 2: XRD patterns of L-leucine raw material (A), and CIP-Cu-maltose and CIP-Cu-maltose-leucine (B). (XRD pattern baselines of CIP-Cu-maltose and CIP-Cu-maltose-leucine were shifted upward to make the graph more readable).

3. Results and discussion

The aim of this study was to improve the aerodynamic properties of microparticles loaded with a CIP-Cu²⁺ complex developed to treat lung infection by inhalation. Recently, pharmacokinetic (PK) studies were performed to validate proof of concept studies which showed that CIP pulmonary residency time can be controlled by the affinity of its complex formed with metallic divalent cations (such as Cu²⁺) (Lamy *et al.*, 2018). This PK study was achieved in rats by intratracheal administration of a powder comprised of amorphous calcium carbonate-based microparticles. To improve the aerosol performance of the formulation, maltose was selected for the current study as a potentially more inert excipient material to replace calcium carbonate. Formulations with different percentages of maltose (Table 1) were developed and screened based on their micromeritic properties and compared with

calcium carbonate particles.

Powders prepared by spray drying solutions with varying maltose concentrations had similar CIP loadings, and this latter parameter was thus not considered as a selection criterion to screen the formulations. In a previous work (Lamy *et al.*, 2018), it was shown that CIP-Cu particles enabled high CIP concentrations to be maintained in the lung up to 18 hours after intratracheal administration to rats of a CIP dose of 2.5 mg/kg, i.e. 3 to 4-times lower than the currently used oral dose for an adult of 70 kg (Follath *et al.*, 1986; Mandell *et al.*, 2007). This suggests that a CIP dose lower than the usual oral dose could be used by inhalation of this formulation.

The increase in percentage maltose used in the excipient component of the formulation did not influence the particle morphology, as observed by SEM (Figure 1). The microparticles obtained

Table 2: Micromeritic properties of the spray dried powders. Thermogravimetric analysis (TGA) results represented the moisture content determined by the loss-of-mass measured between 25°C to 100°C. Geometric particle size distribution (PSD): d(0.5) is the median of the volume-weighted PSD, 10% in volume of the particles have a diameter below d(0.1) and 90% have a diameter below d(0.9), SSA is the specific surface area of the particles. Results are expressed as mean \pm SD (n=3), except for CIP-Cu for TGA and geometric PSD where n=1.

	Loading	TGA	Geometric PSD				
	CIP % (m/m)	% (m/m) of water	d(0.1) (μ m)	d(0.5) (μ m)	d(0.9) (μ m)	Span	SSA* (m ² /g)
CIP-Cu	41.9 \pm 10.4	7.2	1.5	5.7	14.5	2.3	1.9
CIP-Cu mix 3	45.4 \pm 0.1	4.8 \pm 1.3	1.4 \pm 0.4	6.9 \pm 1.9	18.1 \pm 6.1	2.4 \pm 0.2	1.9 \pm 0.5
CIP-Cu-maltose	53.5 \pm 14.2	6.1 \pm 0.2	1.7 \pm 0.4	8.9 \pm 0.3	29.1 \pm 1.8	3.1 \pm 0.4	1.6 \pm 0.2
CIP-Cu-maltose-leucine	35.9 \pm 3.2	6.5 \pm 0.8	0.9 \pm 0.1	3.2 \pm 0.6	10.8 \pm 0.2	3.2 \pm 0.6	3.2 \pm 0.4

* Calculated from geometric particle size distribution, assuming solid spherical particles

throughout all the batches formulated without leucine were spherical, apparently hollow particles (Figure 1 A to E).

Similar morphologies were obtained by Kawakami *et al.* (Kawakami *et al.*, 2010) by spray drying 9.5% w/v maltose solutions supplemented with various surfactants. However, SEM micrographs showed that mainly particles containing maltose appeared broken. Less broken microparticles were visible in the micrographs of the formulations made only with calcium carbonate (CIP-Cu, Figure 1A). The apparent brittleness of these hollow particles suggests that the dispersion process in the inhaler may break them down to smaller, more easily inhalable particles (Watts, Wang, Johnston, & Williams III, 2013).

Pure maltose particles can have a tendency to aggregate (Kawakami *et al.*, 2010), which can lead to poor flowability and limits their performance in a dry powder inhaler (Maggi, Bruni, & Conte, 1999; Nichols *et al.*, 2001). In order to improve the flowability and aerosolization performance of the maltose-based formulation, L-leucine was added as dispersant.

L-leucine has been previously used in dry powder inhaler (DPI) formulations, and shows anti-adherent properties, and the ability to disrupt particle-particle interactions (Begat *et al.*, 2009; Chow *et al.*, 2017; Hoppentocht, Hagedoorn, Frijlink, & de Boer, 2014; Lähde, Raula, Malm, Kauppinen, & Karppinen, 2009; Li *et al.*, 2017). The macroscopic aspect of the powder containing L-leucine was less aggregated and seemed less dense than the other formulations. SEM micrographs showed corrugated particles (Figure 1F), which should decrease the surface contact between the particles, and thus decrease particle-particle interactions (Nolan, Li, Tajber, Corrigan, & Healy, 2011).

The solid state nature of the formulations was characterized by powder XRD analyses (Figure 2). All formulations without L-leucine were XRD amorphous and presented only a diffuse halo in their diffractograms, similar to the one displayed in Figure 2B for the CIP-Cu-maltose (diffractograms were superimposable to that of CIP-Cu-maltose, in order to improve the readability of the graph,

data are not shown for CIP-Cu, CIP-Cu mix 1, 2 and 3). In the presence of L-leucine in the formulation, diffraction peaks at 6, 20, and 25 2θ degrees corresponding to L-leucine (Li *et al.*, 2017) were observed (Figure 2B)(note that XRD patterns were artificially separated in order to improve the readability of the graph). These peaks were broader and of lower intensity compared to the initial L-Leucine raw material powder (Figure 2A), showing the low degree of crystallinity of L-leucine in the formulation. A part of this decrease of intensity may be attributing to a dilution effect of the leucine inside the formulation, but no experiment was performed to investigate it. Also, the crystal orientation of L-leucine in the raw material powder and spray dried powder were different. In the spray dried powder (Figure 2B), the L-leucine crystal preferred the orientation favouring the peak at 20 2θ degrees in addition to the peak at 6 2θ degrees. This change in crystal orientation after spray-drying was previously observed (Li *et al.*, 2017; Raula *et al.*, 2007) and suggested to be as a result of crystallization of L-leucine at the surface of the particles (Raula *et al.*, 2007). Furthermore, the presence of L-leucine at the surface of the particles may prevent moisture-induced deterioration of the particles (Li *et al.*, 2017; Li *et al.*, 2016).

As previously stated, the aim of this study was to replace part or all of the calcium carbonate in the formulation with maltose, while improving the properties of the CIP-Cu formulation. During preliminary analysis, it was clear that the properties (loading, geometric diameter, residual moisture) of CIP-Cu mix 1 and 2 were not improved relative to the CIP-Cu formulation, and thus these formulations were not selected for further investigated and their data are not shown.

Residual moisture content is a key quality parameter for amorphous spray dried particles as it can correlate with poor stability. The presence of moisture in amorphous materials generally reduces its glass transition temperature (T_g), which can affect its properties. For example, T_g reduction accelerates the crystallization rate of the material and makes it softer and more cohesive, decreasing aerosol performance (Kaialy, 2016; Maggi *et*

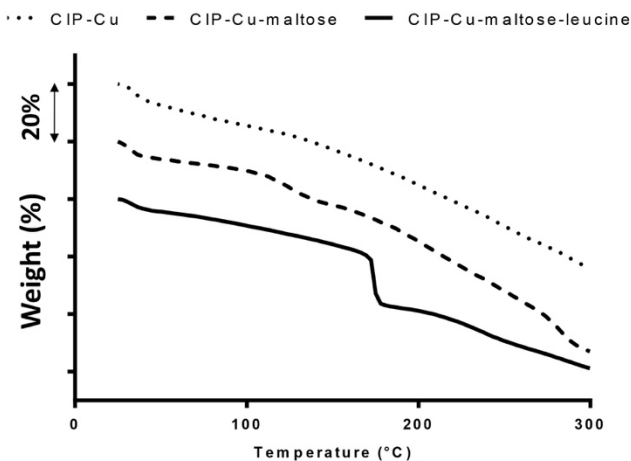


Figure 3: TGA thermograms. Powder weight (%) versus temperature profiles for: CIP-Cu (small dashes), CIP-Cu-maltose (large dashes), and CIP-Cu-maltose-leucine (solid line). Thermograms were shifted to improve the readability of the graph. The interval between ticks on the Y axis represents 20 % of mass loss.

al., 1999; Zafar, Vivacqua, Calvert, Ghadiri, & Cleaver, 2017). TGA thermograms (Figure 3) gave two types of information about the spray dried powders: the moisture content and the thermal degradation characteristics (note that thermographs were artificially separated in order to improve the readability of the graph). The powder moisture content, determined by the loss-of-mass measured between 25°C to 100°C, were comparable for all formulations, with or without L-leucine (Table 2). Given the relatively low spray-drying temperature used (120°C), these residual moisture values, ranging from 4.8 to 7.2 %, can be considered to be low. TGA thermograms (Figure 3) were similar for all formulations made without L-leucine (data not shown for CIP-Cu mix 3). They all showed a gradual loss of mass when the temperature was increased from 100°C to 300°C. For the CIP-Cu-maltose-leucine formulation, the thermogram showed a sharp decrease in mass around 175°C. This mass loss can be attributed to the sublimation of the crystalline L-leucine (Lähde *et al.*, 2009).

The volume-weighted median geometric diameters ($d_{(0.5)}$) of formulations without leucine (Table 2) might be considered too high (at $> 5 \mu\text{m}$) for good lung deposition, but in the presence of L-leucine the $d_{(0.5)}$ was between 1 and $5 \mu\text{m}$ ($3.2 \pm 0.6 \mu\text{m}$), which is the target range for good bronchial deposition. With respect to the results summarized in Table 2, CIP-Cu mix 3 did not demonstrate better micromeritic properties than CIP-Cu-maltose so it was not selected for further studies.

Albeit they have a more crumpled surface morphology (Figure 1F), the CIP-Cu-maltose-leucine particles have a similar specific surface area ($\text{SSA } 5.3 \pm 0.5 \text{ m}^2/\text{g}$), as measured by N_2 sorption

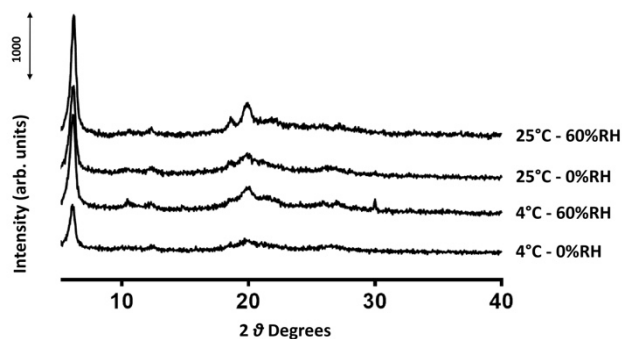


Figure 4: XRD patterns of CIP-Cu-maltose-leucine after storage for 21 months in different conditions: 4°C/0% RH, 4°C/60% RH, 25°C/0% RH and 25°C/60% RH. Different patterns are superimposable and are artificially separated in order to improve the readability of the graph. Relative humidity (RH) was controlled with Amebis humidity capsules in test chambers. Y axis is in arbitrary units, the scale is given by the double arrow.

(Table 3), to the smooth CIP-Cu-maltose particles ($3.7 \text{ m}^2/\text{g}$) (Table 3). These SSA values were 2-time higher than SSA calculated from geometric PSD (Table 2), this difference may be explained by the porosity of the hollow particles (Lamy *et al.*, 2018). In comparison, CIP-Cu had a 3-times higher SSA ($14.8 \pm 0.6 \text{ m}^2/\text{g}$) (Lamy *et al.*, 2018). This reduction in SSA through the inclusion of maltose/leucine might be seen as a drawback in the formulation, change since high SSA is thought to be one of the key parameters necessary to achieve both good dispersibility and high lung deposition of powders (Amaro *et al.*, 2015).

In order to achieve a good lung deposition, particles should have an aerodynamic diameter between 1 and $5 \mu\text{m}$ (d'Angelo *et al.*, 2014; Dudley *et al.*, 2008; A. M. Healy, Amaro, Paluch, & Tajber, 2014). The aerodynamic diameter is the diameter of a sphere of unit density, which reaches the same velocity in the air stream as the particle analyzed. It is related to the volume-equivalent geometric diameter by the particle shape and density (F Tewes, Ehrhardt, & Healy, 2013).

The volume-weighted median geometric diameters ($d_{(0.5)}$) of maltose-based formulations of 6.9 ± 1.9 and $8.9 \pm 0.3 \mu\text{m}$ for CIP-Cu mix 3 and CIP-Cu-maltose respectively (Table 2), might be considered too high for good lung deposition. However, the particles constituting these powders were hollow, suggesting an apparent particle density lower than $1 \text{ g}/\text{cm}^3$, and an aerodynamic diameter lower than the geometric diameter. In fact, the mass median aerodynamic diameter (MMAD) of the CIP-Cu-maltose particles ($4.9 \mu\text{m}$, Table 3) was nearly half its $d_{(0.5)}$ ($8.9 \mu\text{m}$, Table 2). This MMAD is similar to that of amorphous calcium carbonate particles previously obtained ($5.0 \pm 0.6 \mu\text{m}$) (F. Tewes *et al.*, 2016).

Table 3: Micromeritic and aerodynamic properties of the spray dried powders. Aerodynamic particle size distribution was measured using a Next Generation cascade Impactor (NGI) and expressed as mean \pm SD for the mass median aerodynamic diameter (MMAD), the geometric standard deviation (GSD) and the fine particle fraction (FPF). Brunauer, Emmet and Teller (BET) method was used to calculate the specific surface area (SSA) of the particles. n=3 for CIP-Cu-maltose-leucine and n=1 for CIP-Cu-maltose.

	NGI			N ₂ sorption
	MMAD (μ m)	GSD	FPF % (<5 μ m)	BET SSA (m ² /g)
CIP-Cu-maltose	4.9	2.4	46.1	3.7
CIP-Cu-maltose-leucine	3.4 \pm 0.2	1.9 \pm 0.1	65.4 \pm 7.7	5.3 \pm 0.5

In the presence of L-leucine, the $d_{(0.5)}$ and the MMAD decreased from 8.9 \pm 0.3 μ m to 3.2 \pm 0.6 μ m and from 4.9 μ m to 3.4 \pm 0.2 μ m, respectively (Tables 2 and 3, respectively). Also, the fine particle fraction (FPF) was higher for the formulation containing L-leucine (65.4 \pm 7.7 %) than without (46.1%). Thus, a larger percentage of CIP-Cu-maltose-leucine particles should reach the infectious site compared to the CIP-Cu-maltose particles. If larger quantities of drug can reach the target site, less powder would need to be administered. This could limit potential excipients adverse effects, even though they are biocompatible and bio-assimilable.

The amorphous form is a metastable state, which is thermodynamically driven towards crystallization with modification of its pharmaceutical properties (Anne Marie Healy, Worku, Kumar, & Madi, 2017). To evaluate whether the semi-amorphous CIP-Cu-maltose-leucine formulation was stable with respect to crystallization in the long term, powder XRD patterns were recorded after 21 months of storage in different conditions of temperature and relative humidity (RH) (Figure 4). Overall, minor changes were observed on the diffractograms of the powders stored in the various conditions compared to the diffractogram of the freshly prepared samples. Based on the intensity values of diffraction peaks at 6 and 20 2θ degrees, the formulation was stable when it was stored in dry conditions (0% RH), regardless of the temperature (4 and 25°C). However, when stored at 60% RH, the intensity of the peaks increased two-fold, showing an increase in the degree of crystallinity. The relatively slow crystallization rate of L-leucine seems to be mainly driven by the increase in the RH. These results suggest that the CIP-Cu-maltose-leucine formulation should be stable throughout the shelf life of the product in desiccated conditions.

The pulmonary delivery to rats of CIP-Cu-loaded microparticles allowed 100-times higher pulmonary ELF CIP exposure to be achieved compared to the pulmonary delivery of a CIP solution (Lamy *et al.*, 2018). We can presume that the pulmonary delivery of the CIP-Cu-maltose-leucine formulation will display similar pharmacokinetic characteristics. Interestingly, maltose is a compound that can induce chemotaxis in *P. aeruginosa* (Rico-Jiménez *et al.*, 2016; Sampedro, Parales, Krell, & Hill, 2014). This

characteristic could favor the attraction of bacteria close to the dissolving particles and could increase bacterial exposure to the antibiotic.

4. Conclusion

Microparticles made of maltose and L-leucine and loaded with a ciprofloxacin-Cu complex were developed to treat chronic bacterial lung infection. These particles were physically stable for 21 months and showed aerodynamic properties suitable for use with a dry powder inhaler. Considering the enhanced pulmonary epithelial lining fluid ciprofloxacin exposure obtained with the pulmonary delivery of CIP-Cu-loaded microparticles, this new formulation seems promising for future clinical development.

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