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Sustained-release microparticle dry powders of chloramphenicol palmitate or thiamphenicol palmitate prodrugs for lung delivery as aerosols.

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Abstract

The purpose of this study was to design inhalable sustained-release nanoparticle-in-microparticles, *i.e.* nano-embedded microparticles, for the lung delivery of chloramphenicol or thiamphenicol as aerosols. The palmitate ester prodrugs of the two antibiotics were used to prepare PLGA-based nanoparticles or to form pure prodrug nanoparticles. Prodrug-loaded PLGA nanoparticles or pure prodrug nanoparticles were prepared using the emulsion-solvent evaporation method. Dry microparticle powders for inhalation were then produced by spray-drying the nanoparticle suspensions supplemented with lactose as a bulking agent and L-leucine as a dispersing enhancer. Examined under the scanning electron microscopy, the obtained microparticles appeared to be spherical and shriveled, with no crystal-like structures. Drug loading was satisfactory (14 to 34 % (m/m)) and the aerodynamic properties determined with a Next Generation Impactor were appropriate for lung delivery, with mass median aerodynamic diameters close to 3 µm. The in vitro release profiles showed that sustained released was achieved with these formulations, with an almost complete release over 14 days.

Keywords: chloramphenicol, thiamphenicol, aerosol, lung delivery, antimicrobial, palmitate prodrug, PLGA.

1. Introduction

Chloramphenicol (CHL) and thiamphenicol (THA) are antibiotics of the amphenicol class that possess similar broad spectra of activity against Gram positive and Gram negative bacteria (Eliakim-Raz et al., 2015; Serra et al., 2007). Commonly used in the past due to its broad spectrum and its good diffusion into tissues, chloramphenicol was reported to cause rare, irreversible and fatal idiosyncratic aplastic anaemia, which was attributed to the nitroso-chloramphenicol metabolite responsible for DNA damage (Dinos et al., 2016; Ferrari, 1984). Possessing a methyl sulfonyl group in place of the nitro group thiamphenicol was not associated with such a lateral effect (Ferrari, 1984; Yunis, 1984). Both chloramphenicol and thiamphenicol are however responsible for reversible dose-dependent bone marrow suppression in the case of prolonged systemic treatments (more than 7 days) (Eliakim-Raz et al., 2015; Ferrari, 1984). In the advanced countries, they have therefore been replaced in clinical practice with less toxic antibiotics, and their use is recommended as the second line treatments of life-threatening infections that do not respond to other antibiotics (Eliakim-Raz et al., 2015). Chloramphenicol is inexpensive, and is still widely used as topical preparations, e.g. in eye drops for the prevention and treatment of superficial eye infections, or as oral tablets or capsules or pediatric suspension in low-income countries (Lam et al., 2002). Though potentially useful for the treatment of life-threatening infections resistant to other antibiotics, chloramphenicol and thiamphenicol are not available world-wide and are considered as “forgotten” antibiotics in advanced countries (Pulcini et al., 2017). In particular, CHL and THA have been proposed to treat multidrug-resistant pulmonary bacterial infections. Their direct administration into the lungs as therapeutic aerosols should be considered to increase treatment efficiency and minimize whole body exposure responsible for adverse effects, particularly in the case of prolonged treatments. Thiamphenicol, in the form of its glycinate ester prodrug, was administered as an aerosol in oncological patients with respiratory infections and was effective in more than 95% of the patients (Macchi et al., 2011).

Due to their lipophilicity both CHL and THA (ALOGPS predicted logP = 1.15 and 0.33, respectively (Tetko et al., 2005)) were shown to have a high permeability across a broncho-alveolar epithelium model made of Calu-3 cell line (Nurbaeti et al., 2018), in consistency with the rapid intestinal absorption reported in human PK studies after oral administration (Ambrose, 1984; Ferrari, 1984). Due to their high apparent epithelial permeability, the pulmonary administration of CHL or THA will not improve lung exposure to these antibiotics compared to systemic administration. Therefore, in order to improve lung exposure to CHL or THA after their aerosolization, it is relevant to formulate them as sustained release drug delivery systems. Indeed, in previous works, we showed that antibiotics-loaded PLGA microspheres with sustained-release properties permitted to dramatically increase the concentration of high-permeability antibiotics in the pulmonary epithelial lining fluid over a prolonged time period (more than 72 h), compared to an intratracheally nebulized solution or an IV infusion (Doan et al., 2013; Gaspar et al., 2016). In preliminary experiments, the formulation and the preparation of CHL- or THA-loaded PLGA microspheres using the solvent evaporation method resulted in an insufficient drug loading (from 1 to 5% (w/w)) (data not shown). Previous studies showed that using lipophilic prodrugs dramatically increased the nanoparticle drug content compared to the parent drug and prolong the release time (Gomez-Gaete et al., 2008; Han et al., 2015). Lipophilic palmitate esters of chloramphenicol or of thiamphenicol are available. Chloramphenicol palmitate (CHLP) was synthetized in the past as a tasteless prodrug for oral administration in children. Different from chloramphenicol it is poorly absorbed, but it is quickly and almost completely hydrolyzed into the parent drug by esterases in the small intestine, which results in a CHL bioavailability similar to oral dosage forms of CHL (Ambrose, 1984). CHLP is marketed as a pediatric oral suspension in some countries. In the present work, lipophilic palmitate esters of CHL or of THA were formulated as dry nano-embedded microparticle powders for inhalation based on the nanoparticle aggregation process through the spray-drying of nanoparticle aqueous suspensions, based on methods described previously (Gomez-Gaete et al., 2008; Ruge et al., 2016; Torge et al., 2017). The nanoparticles were made with the prodrugs and PLGA polymer or with the prodrugs only using the solvent evaporation method and were then spray-dried in a solution of lactose and L-leucine in order to obtain nano-embedded microparticle dry powders. Once delivered by inhalation, the nano-embedded lactose microparticles are expected to dissolve and release the nanoparticles in the lungs. Nanoparticles will then

gradually release the prodrugs that will eventually be hydrolyzed by lung lipases into the active parent drugs (Camps et al., 1991).

2. Materials and methods

2.1. Materials

Thiamphenicol (THA) (98% pure), Chloramphenicol (CHL) (99% pure), Resomer® RG 502 H (PLGA 50:50, MW: 7,000–17,000, acid terminated), alpha-lactose monohydrate (purity ≥ 99%), L-leucine and lipase from porcine pancreas were purchased from Sigma-Aldrich (St. Quentin Fallavier, France). Chloramphenicol palmitate (CHLP) (Fig. 1) was purchased from Chem-Impex International, Inc. (Wood Dale, IL, USA). Thiamphenicol palmitate (THAP) (Fig. 1) was obtained from Abcam (Paris, France). Rhodoviol 4/125 (polyvinylalcohol, degree of hydrolysis of 88%) was purchased from Prolabo (Paris, France). Purified water was produced with a milli DI™ Millipore system and HPLC quality grade water used for HPLC analysis was purchased from Carlo Erba (Val de Reuil, France). Hank's Balanced Salt Solution (HBSS) was obtained from PAN Biotech GmbH (Aidenbach, Germany).

2.2. Methods

2.2.1. Nanoparticle preparation and characterization

Nanoparticles were prepared by an emulsion-solvent evaporation method. For the preparation of the prodrug-loaded PLGA nanoparticles, a solution of PLGA (100 mg) and prodrug (100 mg) in 20 ml of ethyl acetate (EA) was vortex mixed with 80 ml of an aqueous solution of polyvinylalcohol (0.5% w/v) supplemented with4.68 ml of EA. Then, in an ice bath, the mixture was subjected to magnetic stirring and ultrasonication for 6 min using a probe sonicator (Branson Sonifier 450) set at 20% maximum power. For the preparation of the prodrug nanoparticles, 200 mg of prodrug were dissolved in EA and processed as above. EA was evaporated off under vacuum at 30°C during 1 h using a rotary evaporator. Volume was adjusted to 80 ml with water and the suspension was filtered on a Fischerbrand glass microfibres filter (2.7 µm porosity). The mean diameter of the nanoparticle volume-weighted distribution (D_v) was determined in purified water by laser light diffraction (Microtrac® X100 particle size analyzer). The width of particle size distribution, or span, was calculated according to: span = $(d_{90} - d_{10})/ d_{50}$, where d_{10}, d_{50} and d_{90} are the particle

diameters corresponding to, respectively, 10, 50 and 90 vol.% on a relative cumulative particle size distribution curve (Vladisavljević and Schubert, 2003).

For the nanoparticle prodrug content determination, an aliquot of 4 ml of the nanoparticle suspensions was collected and nanoparticles were washed twice with water through two cycles of centrifugation at 14,610 *g* for 30 min (Eppendorf centrifuge 5804R). After the last centrifugation, pellets were dissolved in 3 ml of EA and dried at 45°C under a nitrogen flow. The dry residue was weighed and 3 ml EA was added. Then, 1.5 ml was collected and centrifuged at 16,900 g for 30 min (Eppendorf centrifuge 5418R) and the supernatant was collected for the prodrug spectrophotometric determinations as described in 2.2.4. For the nanoparticle yield (weight%) determination, the weight of dry residue recovered from the 4 ml aliquot of nanoparticle suspension was used to calculate the total amount of nanoparticle present in the initial 80 ml volume. The yield (weight%) was then calculated by dividing this amount with the weight inputs of the prodrug or prodrug plus PLGA (*i.e.*, 200 mg in both cases).

2.2.2. Microparticle preparation

The nanoparticle suspension was supplemented with L-leucine (2 mg/ml final concentration) and centrifuged for 30 min at 14,610 *g* (Eppendorf centrifuge 5804R). The supernatant was discarded and the pellet was re-dispersed in 20 ml of a solution of L-leucine (2 mg/ml) and lactose (8 mg/ml), which corresponded to a nanoparticle-to-additive weight ratio ranging from 0.60 to 0.85. Then the suspension was spray-dried using a Büchi® Mini Spray Dryer B-290 (Switzerland) set up in blowing mode and equipped with a 0.7 mm nozzle. Settings were: 5% pump rate for the feed, air flow rate set at maximum level (13.65 l/min) and aspiration rate of 100 %, with the pneumatic automatic nozzle cleaning operated 3 to 5 times per min. The inlet and outlet temperatures were 81-83°C and 52-54°C, respectively. The obtained powders were then characterized as described in 2.2.3.

2.2.3. Microparticle characterization

Scanning electron microscopy (SEM)

Microparticle powders were dispersed on double-sided adhesive carbon tapes that were fixed on copper stubs and sputter coated with a platinum film. SEM images were taken using a Teneo VolumeScope Electron microscope (FEI Thermo Scientific). The images were

obtained from the collection of secondary electrons under a voltage of 20 kV and a working distance of 11 mm.

Aerodynamic diameter determination

The aerodynamic diameter was determined using a Next Generation Impactor (NGI, Copley Ltd., Nottingham, UK), equipped with a TPK 2000 critical flow controller and a HCP5 vacuum pump (Copley HCP5, Nottingham, UK) according to the European Pharmacopeia test procedure 2.9.18. “Preparations for inhalation: aerodynamic assessment of fine particles” in the conditions specified in the monograph “Preparations for inhalation (Inhalation powders)”,as previously described (Gaspar et al., 2015). For each measurement, a size-three hard gelatin capsule was filled with 25 ± 1 mg of powder, inserted in a dry-powder inhaler (DPI) Handihaler® (Boehringer-Ingelheim, Germany) and pierced according to the DPI user’s manual. The DPI was tightly connected to the NGI induction port via a silicone adapter. The airflow rate was set at 41 ± 5% l/min in order to produce a pressure drop of 4 kPa through the inhaler. The test duration time was set in order to draw 4 l of air through the inhaler. The powder discharged from the capsule and deposited on the induction port, the stages and the MOC filter was dissolved with EA for the prodrug spectrophotometric assay. Powder deposited on the DPI and on the adapter was collected with ethanol, evaporated off at 45°C under a nitrogen flow and the dry residue was dissolved in EA.

The emitted dose (ED), *i.e.* the mass of prodrug deposited in the induction port, the stages and the MOC, was expressed as the percentage of the prodrug mass contained in the three hard gelatin capsules. The percent cumulative mass fractions of prodrug deposited in MOC and stages 1 to 7 were plotted versus the log aerodynamic diameters. The mass median aerodynamic diameter (MMAD) was calculated by linear interpolation using the equation of the linear interpolant that links the curve points immediately below and above 50% deposition. The fine particle fraction (FPF) (*i.e.* the mass fraction of the prodrug within the particles of 1-to-5 µm aerodynamic diameter) was expressed as the percentage of the ED.

Differential Thermal Analysis (DTA) and Thermal Gravimetric Analysis (TGA)

DTA and TGA were carried out on a SDT Q600 Instrument (TA Instruments, USA). Samples placed on platinum pans were heated from 30°C to 350°C at a heating rate of 10°C/min under a 100 ml/min air flow rate.

Prodrug content determination

For the prodrug extraction and quantification, 10 mg of microsphere powder (accurately weighed) was dispersed in 1 mL EA. After dissolution of the prodrug, the mixture was centrifuged at 14,610 g for 1 min (Eppendorf centrifuge 5804R). The supernatant was collected for the prodrug spectrophotometric determinations as described in 2.2.4. The prodrug content (%) was expressed as the amount of prodrug (mg) per 100 mg of microsphere power.

In vitro release studies

For in vitro release studies under sink conditions, the microparticle powders (50 mg) were dispersed in 10 ml of HBSS, pH 7.4, supplemented with 400 µl of a 1 mg/ml porcine lipase solution in HBSS, and incubated at 37 °C under magnetic stirring (300 rpm) (Gaspar et al., 2016). The lipase enzyme converted the released poorly water-soluble prodrugs into the soluble parent drugs, which permitted to maintain sink conditions. At pre-determined time points, aliquots were collected over 2 weeks and centrifuged at 16,900 *g* for 10 min (Eppendorf centrifuge 5418R). Then, supernatants were collected and CHL and THA were assayed by HPLC. In order to compensate for the loss of enzyme activity over time, the porcine lipase solution (400µl) was added every day to the release medium.

2.2.4. Prodrug spectrophotometric assay

The nanoparticle or spray-dried powder prodrug contents (weight%), *i.e.* the amount of prodrug (mg) per 100 mg of dry mass (including entrapped prodrug), were determined by spectrophotometry at 273 nm using a Varian Cary 50 UV-Visible spectrophotometer after dissolution in EA. The prodrug calibration curves in EA ranged from 3 to 60 µg/ml concentration for CHLP and from 100 to 1000 µg/ml for THAP.

2.2.5. Chloramphenicol and thiamphenicol HPLC assay

The chromatographic system consisted of Waters 717 plus autosampler, a Hitachi L-7110 pump and a Eurosep 785 A spectrophotometric detector (Applied Biosystem). For CHL assay, a C18 X Terra HPLC column (5 µm, 2.1x150 mm) with a Gemini C18 (4x2 mm) Phenomenex precolumn was used. The mobile phase was composed of a 75:25 (v/v) acetonitrile: water

mixture supplemented with 0.1% (v/v) formic acid and 0.2% (v/v) PIC B7 and was run at a 0.2 ml/min flow rate. The injection volume, the run time and the detection wavelength were 50 µl, 9 min and 275 nm, respectively. The calibration curve was constructed by linear regression of the peak areas versus concentrations (0.125 - 10 µg/ml in HBSS (pH 7.4)) with a weighting factor of $1/x^2$ (R^2 = 0.999).

For THA determination, a C18 X Terra HPLC column (5 µm, 3.9x150 mm) with a Gemini C18 (4x2 mm) Phenomenex precolumn was used. The mobile phase was composed of an 85:15 (v/v) acetonitrile: water mixture supplemented with 0.1% (v/v) formic acid and 0.2% (v/v) PIC B7 and was run at a 0.4 ml/min flow rate. The injection volume, the run time and the detection wavelength were 50 µl, 18 min and 225 nm, respectively. The calibration curve was constructed by linear regression of the peak areas versus concentrations (0.5 - 40 µg/ml in HBSS (pH 7.4)) with a weighting factor of $1/x^2$ (R^2 = 0.999).

3. Results and discussion

3.1. Nanoparticle preparation

A low molecular weight PLGA polymer with a rapid degradation rate (Diez and Tros de Ilarduya, 2006; Gaspar et al., 2016) was used for the preparation of PLGA nanoparticles in order to minimize the risk of polymer accumulation within the lung upon repeated dosing. The prodrug content of the PLGA nanoparticles ranged from 50 to 60% (m/m), with a loading efficiency of 100%, which indicated that the palmitate ester prodrugs had a high affinity for the polymer (Table 1). This high entrapment efficiency is in agreement with previous works where palmitate ester prodrugs were formulated within PLGA nanoparticles (Debotton et al., 2008; Tangsumranjit et al., 2006). The entrapment efficiency within PLGA nanoparticles was found to be dependent of the chain length of the carboxylic acid. Studying the effect of the chain length of the carboxylate ligands (acetate to decanoate) of a platinum derivative, Johnstone and Lippard showed a good correlation between the lipophilicity and the entrapment efficiency within PLGA nanoparticles, with the highest efficiency for the decanoate derivative (Johnstone and Lippard, 2013). In the case of dexamethasone acetate (ester) prodrug, Gómez-Gaete et al observed that the prodrug was partially incorporated within PLGA nanoparticles (Gomez-Gaete et al., 2008). It is interesting to note that using the same procedure as for preparing prodrug-loaded PLGA nanoparticles, nanoparticles were successfully prepared from pure CHLP or THAP prodrugs. Prodrug-loaded PLGA nanoparticles

and pure prodrug nanoparticles had virtually similar size, with a narrow size distribution (span value below 1) and the mean yield ranged from 63 to 86 wt.%, which was considered as satisfactory (Table 1). THAP-based nanoparticles were slightly larger than CHLP-based ones.

3.2. Dry microparticle powders

Dry microparticle powders were obtained by spray-drying the nanoparticle aqueous suspensions supplemented with lactose and L-leucine. The spray-drying production process was reported to produce monodisperse microparticles with high encapsulation efficiency and drug loading (Gomez-Gaete et al., 2008). Lactose was used as a bulking agent and L-leucine as a dispersibility enhancer in order to obtain powders with appropriate aerosolisation properties for inhaled delivery (Seville et al., 2007). Under 2,500x magnification the dry powders appeared to be made of microparticles with diameter below 5 µm and were similar whatever the nanoparticles used (Fig. 2 and 3). In agreement with reports showing that spray-dried powders area amorphous (Corrigan, 1995), no crystalline structures like the CHLP or THAP crystals of the commercial prodrug powder (Fig. 1) were observed on micrographs. Different from previous reported works (Gomez-Gaete et al., 2008; Ruge et al., 2016; Torge et al., 2017), it was not possible to distinguish nanoparticles even under the higher 10,000x and 40,000x magnifications. In these previous works the use of a different bulking agent and of polymers with higher glass transition temperatures may explain that the nanoparticle structures were preserved during the spray-drying process. The microparticles appeared to be shriveled like raisin (Fig. 2 and 3). This typical shape was attributed to the collapse of the microparticle structure during the spray-drying process and was reported to be promoted by L-leucine (Seville et al., 2007; Yang et al., 2015). This is an advantage for lung delivery, since particles with a high degree of surface corrugation were shown to possess better aerosolisation properties due to reduced contact area (Adi et al., 2008; Chew et al., 2005). Prodrug contents are presented in Table 2. As expected, the prodrug content was higher when the spray-dried powders were obtained from nanoparticles made of pure prodrugs, than with the prodrug-loaded PLGA nanoparticles.

3.3. DTA-TGA analysis

DTA-TGA analysis was conducted to analyze the interaction between CHLP and THAP with PLGA, L-leucine and lactose. Thermograms of CHL and THA (Fig. 4A and 4B) showed endothermic peaks at 153.6°C and 167.1°C corresponding to their respective melting points (Aiassa et al., 2015). Thermal degradation occurred at an onset temperature of around 200°C for CHL and THA as shown by the exothermic peaks (Fig. 4A and 4B) and the gradual weight loss (Fig. 4C and 4D). The prodrug CHLP exists as three described polymorphs: the stable form A and two metastable forms B and C (Kaneniwa and Otsuka, 1985), with different solubility values and oral bioavailability. Forms B and C are suitable for therapeutic use, but form A is considered as biologically inactive, which was attributed to a different susceptibility to pancreatic lipase in the upper intestinal tract. Forms A and B have respective melting points at 90.3 or 92°C and 86.7 °C respectively (Gamberini et al., 2006; Kaneniwa and Otsuka, 1985). The endothermic peak at 88.3°C on the CHLP thermogram (Fig. 4A) may correspond to form B or a mix of forms A and B, since the metastable form B tends to transform into the stable form A (Gamberini et al., 2006). The thermogram of THAP (Fig. 4B) showed an endothermic peak at 111.3°C which was attributed to its melting point. For both prodrugs, degradation occurred with an onset at around 230°C (Fig. 4A and 4B), as deduced from the exothermic peaks and from the weight loss (Fig. 4C and 4D).

For the four microparticle powders, the weight loss of 2 to 5 % observed over 40 to 70°C (Fig. 4C and 4D) was due to the evaporation of residual water (Raula et al., 2012). A further weight loss with an onset at around 140°C was simultaneous with an endothermic event (over 145 to 190°C) and was attributed to the sublimation of the L-leucine (Lähde et al., 2009). The exothermic event with an onset at 190-200°C (Fig. 4A and 4B) which is simultaneous with an important weight loss (Fig. 4C and 4D) was attributed to the thermal degradation of the microparticle components. The thermograms of microparticle powders made with CHLP nanoparticles or with CHLP-loaded PLGA nanoparticles were roughly similar (Fig. 4A) with a small endothermic peak at 85.8°C attributed to the melting of the CHLP crystal form B. The small size of this endothermic peak suggests that CHLP is mainly in an amorphous or solubilized form. Microparticle powders made with THAP nanoparticles or THAP-loaded PLGA nanoparticles showed also similar thermograms (Fig. 4 C&D). The small endothermic peak at 108-109°C was attributed to the melting of THAP (see the pure THAP thermogram as a reference) and, taking into account the microsphere THAP content, its size indicates that THAP is mainly in an amorphous or solubilized form.

3.4. Aerodynamic properties

For all the formulations, the high ED% values (above 70%) indicated that the microparticle powders were efficiently emitted from the DPI (Table 2). MMAD values were around 3 µm, suggesting that the microparticles powders successfully de-aggregated during the aerosolisation process within the DPI. FPF (%) were very satisfactory in the range of values reported for marketed formulations (15-30%) for pulmonary administration (Smith and Parry-Billings, 2003), indicating that the powders should be efficient to deliver CHLP and THAP to the lungs through inhalation. With MMAD around 3 µm microparticles mainly deposit in the central airway, where bacteria are difficult to eradicate using systemic administration of antibiotics due to a low vascularization, thicker epithelium, mucus and biofilm formation, compared to alveoli (Bjarnsholt et al., 2009).

3.5. Prodrug release studies

Prodrug release studies were carried out at 37°C in HBSS buffer, pH 7.4. In order to maintain sink conditions, porcine lipase was added to the release medium, which converted the poorly soluble prodrugs released in HBSS buffer into the more soluble parent drugs, which were eventually determined by HPLC. In the case of the microparticle powder formulations prepared with CHLP- nanoparticles (with or without PLGA), the release profiles were rather linear and showed a sustained release of the prodrugs (Fig 5) with 70 (without PLGA) or 90% (with PLGA) release at 14 days.

With the THAP powders, the profiles were characterized with a two-rate release. A higher rate over 3 or 4 days with 60% or 50% cumulated release without or with PLGA respectively, followed by lower rate resulting in 80% cumulated release at 14 days. This was attributed to the higher water solubility of the THA moiety than the solubility of the CHL moiety, leading to a higher dissolution rate in the release medium. Release rates were also higher with the microparticle powders made with prodrug-loaded PLGA nanoparticles than with those made with pure prodrug nanoparticles. In the case of prodrug-loaded PLGA nanoparticles, prodrugs are dispersed within the PLGA matrix that gradually degrades. Such degradation is likely to produce pores within the nanoparticles that permit the entry of water and increase the exchange surface area with the release medium.

For both prodrugs, whatever the formulation, the releases were much slower than in previously reported results obtained with dexamethasone acetate-loaded PLGA nanoparticles (Gomez-Gaete et al., 2008). This can be explained by a higher affinity of the CHLP or THAP prodrugs towards the PLGA polymer, resulting in a slower drug release.
It is difficult to anticipate whether such relatively slow sustained release profiles of the prodrugs and their hydrolysis rate into the active drugs within the lungs will be appropriate to reach efficient CHL or THA concentrations in the lung areas. Previous PK studies with sustained release rifampicin or levofloxacin-loaded PLGA microparticles showed that such delivery systems permitted to reach high epithelial lining fluid (ELF) to blood plasma drug concentration ratios due to the local release in the small ELF volume. In addition, little is known about the esterase activity within the lungs (Camps et al., 1991). Therefore, PK studies will be necessary to further investigate the therapeutic potential of the present formulations.

4. Conclusion

Dry powder formulations of CHLP or THAP with sustained release profiles and appropriate aerodynamic properties for lung delivery as aerosols were successfully prepared with the nanoparticle-within- microparticle spray-drying production technology. Further PK investigations are needed to evaluate the potential efficiency of such innovative CHLP or THAP drug delivery systems.

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Conflict of interest

The authors report no conflicts of interest.

References

Adi, S., Adi, H., Tang, P., Traini, D., Chan, H.K., Young, P.M., 2008. Micro-particle corrugation, adhesion and inhalation aerosol efficiency. European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences 35, 12-18.

Aiassa, V., Zoppi, A., Albesa, I., Longhi, M.R., 2015. Inclusion complexes of chloramphenicol with beta-cyclodextrin and aminoacids as a way to increase drug solubility and modulate ROS production. Carbohydrate polymers 121, 320-327.

Ambrose, P.J., 1984. Clinical pharmacokinetics of chloramphenicol and chloramphenicol succinate. Clinical pharmacokinetics 9, 222-238.

Bjarnsholt, T., Jensen, P.Ø., Fiandaca, M.J., Pedersen, J., Hansen, C.R., Andersen, C.B., Pressler, T., Givskov, M., Høiby, N., 2009. Pseudomonas aeruginosa biofilms in the respiratory tract of cystic fibrosis patients. Pediatric pulmonology 44, 547-558.

Camps, L., Reina, M., Llobera, M., Bengtsson-Olivecrona, G., Olivecrona, T., Vilaro, S., 1991. Lipoprotein lipase in lungs, spleen, and liver: synthesis and distribution. Journal of lipid research 32, 1877-1888.

Chew, N.Y., Tang, P., Chan, H.K., Raper, J.A., 2005. How much particle surface corrugation is sufficient to improve aerosol performance of powders? Pharmaceutical research 22, 148-152.

Corrigan, O.I., 1995. Thermal analysis of spray dried products. Thermochimica Acta 248, 245-258.

Debotton, N., Parnes, M., Kadouche, J., Benita, S., 2008. Overcoming the formulation obstacles towards targeted chemotherapy: in vitro and in vivo evaluation of cytotoxic drug loaded immunonanoparticles. Journal of controlled release : official journal of the Controlled Release Society 127, 219-230.

Diez, S., Tros de Ilarduya, C., 2006. Versatility of biodegradable poly(D,L-lactic-co-glycolic acid) microspheres for plasmid DNA delivery. European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V 63, 188-197.

Dinos, G.P., Athanassopoulos, C.M., Missiri, D.A., Giannopoulou, P.C., Vlachogiannis, I.A., Papadopoulos, G.E., Papaioannou, D., Kalpaxis, D.L., 2016. Chloramphenicol Derivatives as Antibacterial and Anticancer Agents: Historic Problems and Current Solutions. Antibiotics (Basel, Switzerland) 5.

Doan, T.V., Gregoire, N., Lamarche, I., Gobin, P., Marchand, S., Couet, W., Olivier, J.C., 2013. A preclinical pharmacokinetic modeling approach to the biopharmaceutical characterization of immediate and microsphere-based sustained release pulmonary formulations of rifampicin. European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences 48, 223-230.

Eliakim-Raz, N., Lador, A., Leibovici-Weissman, Y., Elbaz, M., Paul, M., Leibovici, L., 2015. Efficacy and safety of chloramphenicol: joining the revival of old antibiotics? Systematic review and meta-analysis of randomized controlled trials. The Journal of antimicrobial chemotherapy 70, 979-996.

Ferrari, V., 1984. Salient features of thiamphenicol: review of clinical pharmacokinetics and toxicity. Sexually transmitted diseases 11, 336-339.

Gamberini, M.C., Baraldi, C., Tinti, A., Rustichelli, C., Ferioli, V., Gamberini, G., 2006. Solid state characterization of chloramphenicol palmitate. Raman spectroscopy applied to pharmaceutical polymorphs. Journal of Molecular Structure 785, 216-224.

Gaspar, M.C., Gregoire, N., Sousa, J.J., Pais, A.A., Lamarche, I., Gobin, P., Olivier, J.C., Marchand, S., Couet, W., 2016. Pulmonary pharmacokinetics of levofloxacin in rats after aerosolization of immediate-release chitosan or sustained-release PLGA microspheres. European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences 93, 184-191.

Gaspar, M.C., Sousa, J.J., Pais, A.A., Cardoso, O., Murtinho, D., Serra, M.E., Tewes, F., Olivier, J.C., 2015. Optimization of levofloxacin-loaded crosslinked chitosan microspheres for inhaled aerosol

therapy. European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V 96, 65-75.
Gomez-Gaete, C., Fattal, E., Silva, L., Besnard, M., Tsapis, N., 2008. Dexamethasone acetate encapsulation into Trojan particles. Journal of controlled release : official journal of the Controlled Release Society 128, 41-49.
Han, J., Michel, A.R., Lee, H.S., Kalscheuer, S., Wohl, A., Hoye, T.R., McCormick, A.V., Panyam, J., Macosko, C.W., 2015. Nanoparticles Containing High Loads of Paclitaxel-Silicate Prodrugs: Formulation, Drug Release, and Anticancer Efficacy. Molecular pharmaceutics 12, 4329-4335.
Johnstone, T.C., Lippard, S.J., 2013. The effect of ligand lipophilicity on the nanoparticle encapsulation of Pt(IV) prodrugs. Inorganic chemistry 52, 9915-9920.
Kaneniwa, N., Otsuka, M., 1985. Effect of grinding on the transformations of polymorphs of chloramphenicol palmitate. Chemical and pharmaceutical bulletin 33, 1660-1668.
Lähde, A., Raula, J., Malm, J., Kauppinen, E.I., Karppinen, M., 2009. Sublimation and vapour pressure estimation of l-leucine using thermogravimetric analysis. Thermochimica Acta 482, 17-20.
Lam, R., Lai, J., Ng, J., Rao, S., Law, R., Lam, D., 2002. Topical chloramphenicol for eye infections. Hong Kong medical journal= Xianggang yi xue za zhi 8, 44-47.
Macchi, A., Terranova, P., Macchi, S., Roselli, R., Castelnuovo, P., 2011. Aerosol therapy with thiamphenicol glycinate: a retrospective study on efficacy and safety in a group of sixty-six oncological patients. International journal of immunopathology and pharmacology 24, 189-193.
Nurbaeti, S.N., Olivier, J.-C., Adier, C., Marchand, S., Couet, W., Brillault, J., 2018. Active Mediated Transport of Chloramphenicol and Thiamphenicol in a Calu-3 Lung Epithelial Cell Model. Journal of pharmaceutical sciences 107, 1178-1184.
Pulcini, C., Mohrs, S., Beovic, B., Gyssens, I., Theuretzbacher, U., Cars, O., Bosevska, G., Bruch, M., Bush, K., Cizmovic, L., 2017. Forgotten antibiotics: a follow-up inventory study in Europe, the USA, Canada and Australia. International journal of antimicrobial agents 49, 98-101.
Raula, J., Seppälä, J., Malm, J., Karppinen, M., Kauppinen, E.I., 2012. Structure and dissolution of l-leucine-coated salbutamol sulphate aerosol particles. AAPS PharmSciTech 13, 707-712.
Ruge, C.A., Bohr, A., Beck-Broichsitter, M., Nicolas, V., Tsapis, N., Fattal, E., 2016. Disintegration of nano-embedded microparticles after deposition on mucus: A mechanistic study. Colloids and Surfaces B: Biointerfaces 139, 219-227.
Serra, A., Schito, G., Nicoletti, G., Fadda, G., 2007. A therapeutic approach in the treatment of infections of the upper airways: thiamphenicol glycinate acetylcysteinate in sequential treatment (systemic-inhalatory route). International journal of immunopathology and pharmacology 20, 607-617.
Seville, P., Learoyd, T., Li, H.-Y., Williamson, I., Birchall, J., 2007. Amino acid-modified spray-dried powders with enhanced aerosolisation properties for pulmonary drug delivery. Powder technology 178, 40-50.
Smith, I.J., Parry-Billings, M., 2003. The inhalers of the future? A review of dry powder devices on the market today. Pulmonary pharmacology & therapeutics 16, 79-95.
Tangsumranjit, A., Pellequer, Y., Lboutounne, H., Guillaume, Y., Lamprecht, A., Millet, J., 2006. Enhanced ascorbyl palmitate stability by polymeric nanoparticles. Journal of drug delivery science and technology 16, 161-163.
Tetko, I.V., Gasteiger, J., Todeschini, R., Mauri, A., Livingstone, D., Ertl, P., Palyulin, V.A., Radchenko, E.V., Zefirov, N.S., Makarenko, A.S., Tanchuk, V.Y., Prokopenko, V.V., 2005. Virtual computational chemistry laboratory--design and description. Journal of computer-aided molecular design 19, 453-463.
Torge, A., Grützmacher, P., Mücklich, F., Schneider, M., 2017. The influence of mannitol on morphology and disintegration of spray-dried nano-embedded microparticles. European Journal of Pharmaceutical Sciences 104, 171-179.
Vladisavljević, G.T., Schubert, H., 2003. Influence of process parameters on droplet size distribution in SPG membrane emulsification and stability of prepared emulsion droplets. Journal of Membrane Science 225, 15-23.

Yang, F., Liu, X., Wang, W., Liu, C., Quan, L., Liao, Y., 2015. The effects of surface morphology on the aerosol performance of spray-dried particles within HFA 134a based metered dose formulations. asian journal of pharmaceutical sciences 10, 513-519.
Yunis, A.A., 1984. Differential in-vitro toxicity of chloramphenicol, nitroso-chloramphenicol, and thiamphenicol. Sexually transmitted diseases 11, 340-342.

Table 1: Characterization of nanoparticles (mean±SD, n = 8-9)

Nanoparticle type	Dv (nm) of the PSD	Span of the PSD[1]	Prodrug content (%, m/m)[2]	Yield (%)
CHLP nanoparticles	159±6	0.54±0.07	82±11	86±5
CHLP-loaded PLGA nanoparticles	160±4	0.64±0.09	50±13	63±18
THAP nanoparticles	177±6	0.79±0.07	91±22	84±9
THAP-loaded PLGA nanoparticles	178±12	0.77±0.04	60±14	70±7

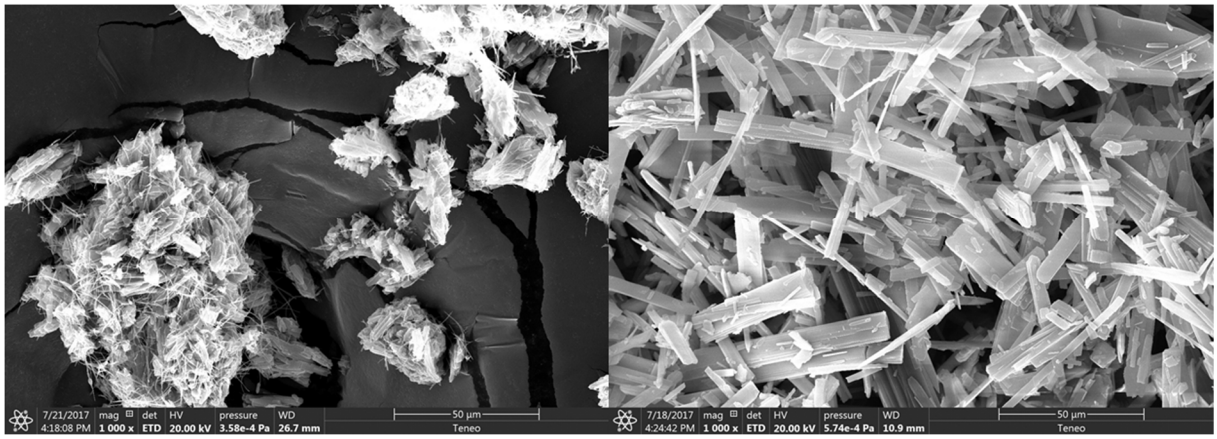
[1]PSD: particle size distribution

[2]mg prodrug per 100 mg nanoparticle

Table 2: Prodrug contents and aerodynamic characterization of the dry microparticle powders (mean±SD, n = 3-4)

Dry microparticle powder made with :	**Prodrug content (%, m/m)[1]**	**ED (%)**	**FPF (%) (1-5µm)**	**MMAD (µm)**
CHLP nanoparticles	30.2±1.0	75±9	33±10	3.1±0.2
CHLP-loaded PLGA nanoparticles	13.9±3.0	79±8	27±13	3.3±0.5
THAP nanoparticles	34.5±4.8	91±8	47±9	2.9±0.2
THAP-loaded PLGA nanoparticles	21.0±0.7	82±19	36±10	2.8±0.3

[1]mg prodrug per 100 mg dry microparticle powder

506 

507 **Figure 1:** SEM of the commercial CHLP (left) and THAP (right) powders

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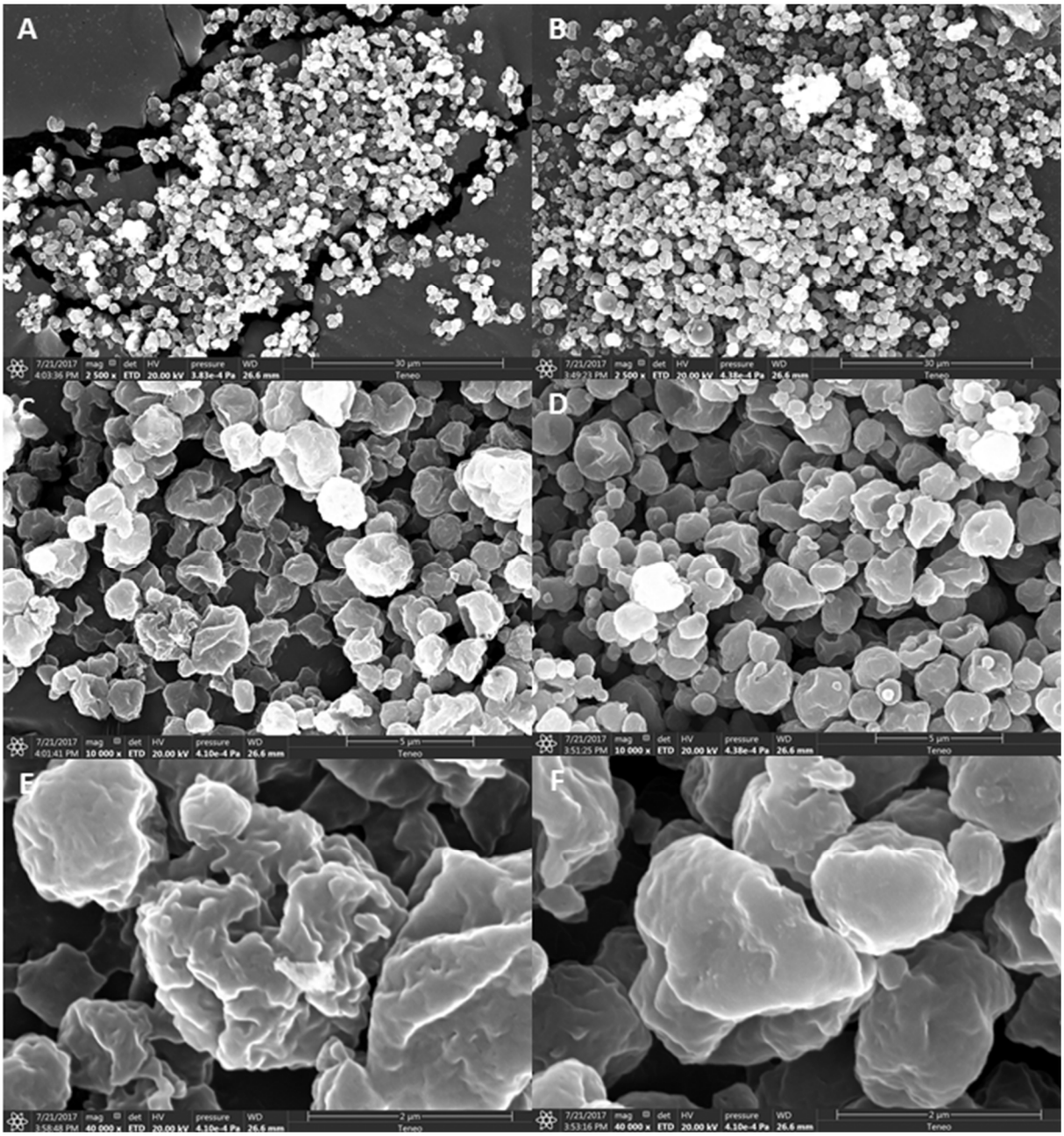


Figure 2: SEM of microparticle powders prepared with CHLP nanoparticles (A, C and E) or CHLP-loaded PLGA nanoparticles (B, D and E) with magnifications of 2,500x for A and B, 10,000x for C and D and 40,000x for E and F.

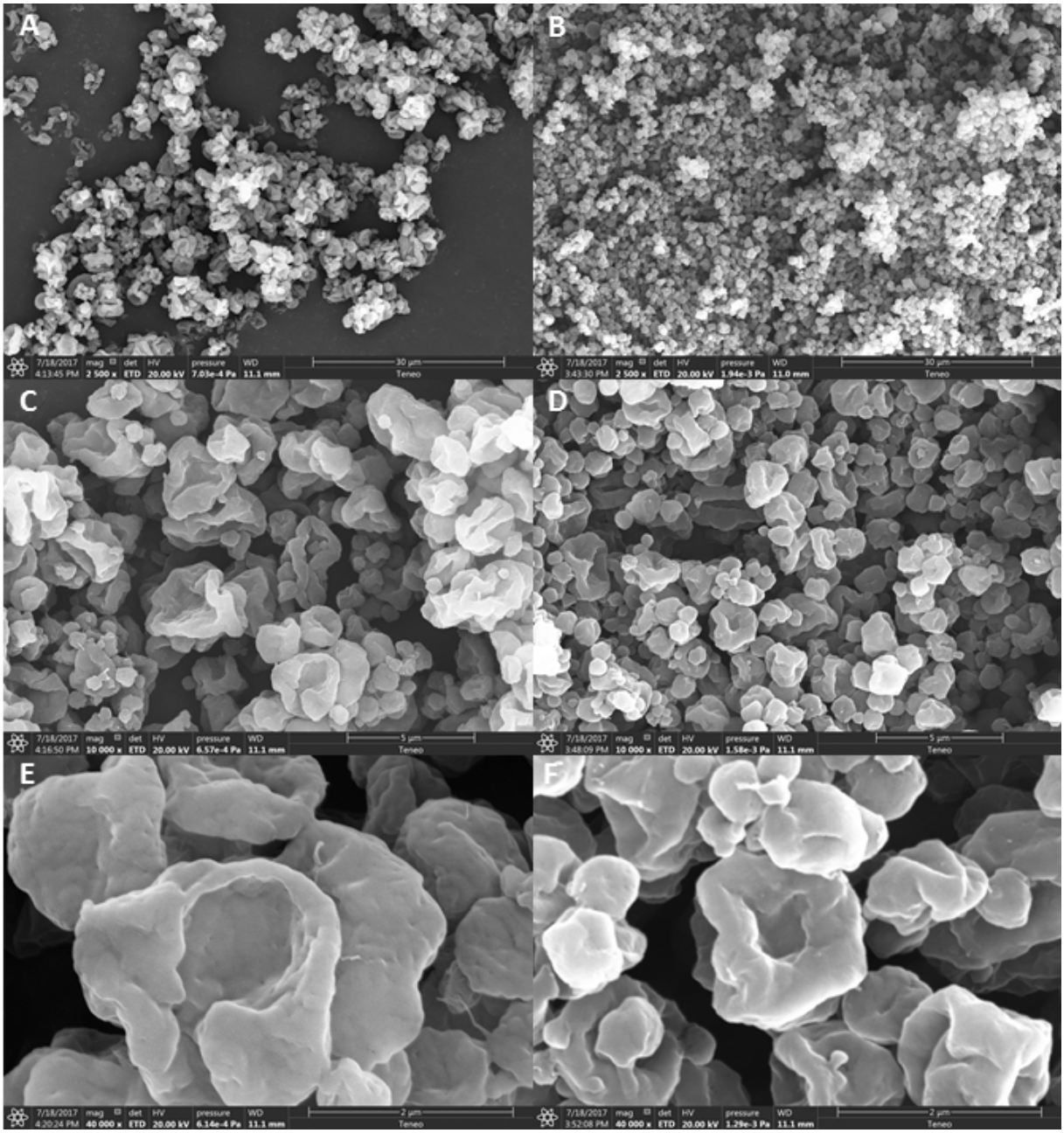
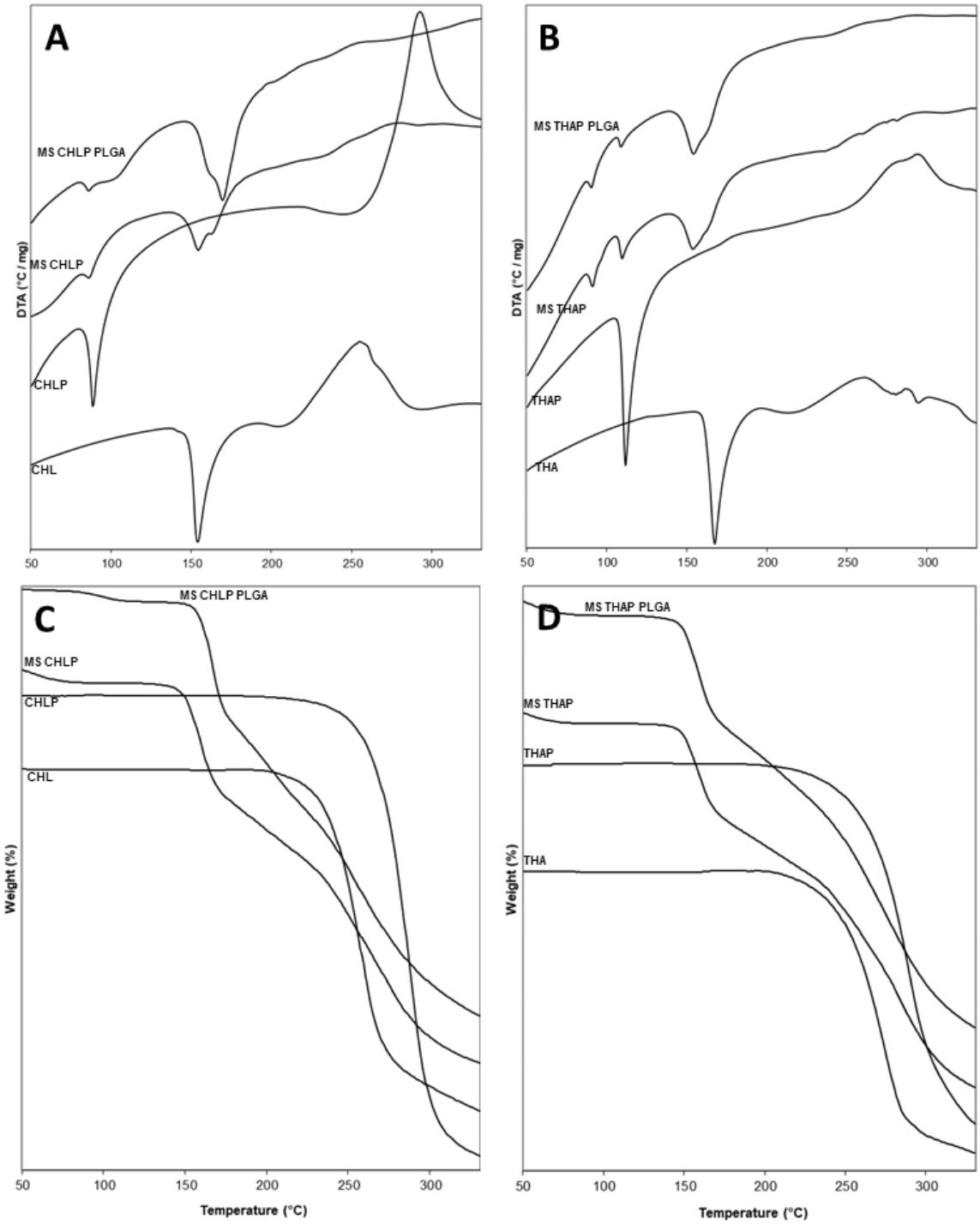


Figure 3: SEM of microparticle powders prepared with THAP nanoparticles (A, C and E) or THAP-loaded PLGA nanoparticles (B, D and F) with magnifications of 2,500x for A and B, 10,000x for C and D and 40,000x for E and F.



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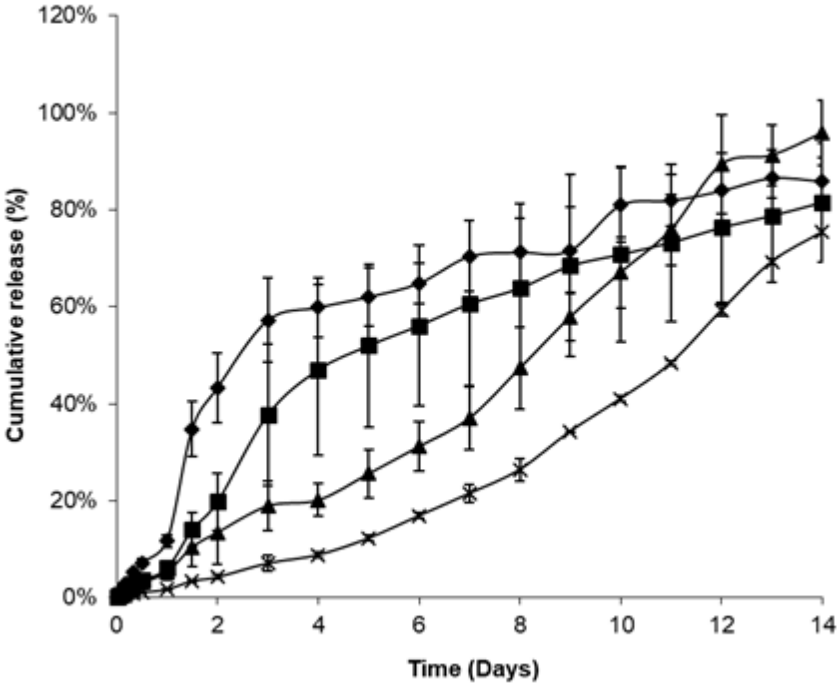
521 **Figure 4:** DTA (A & B) and TGA (C & D) thermograms of raw materials (CHL, CHLP, THA and
522 THAP) and of dry microparticle powders made with CHLP or THAP nanoparticles (MS CHLP or
523 MS THAP) or with CHLP- or THAP-loaded PLGA nanoparticles (MS PLGA CHLP or MS PLGA
524 THAP). Total weight loss (%) is indicated on TGA curves.

525



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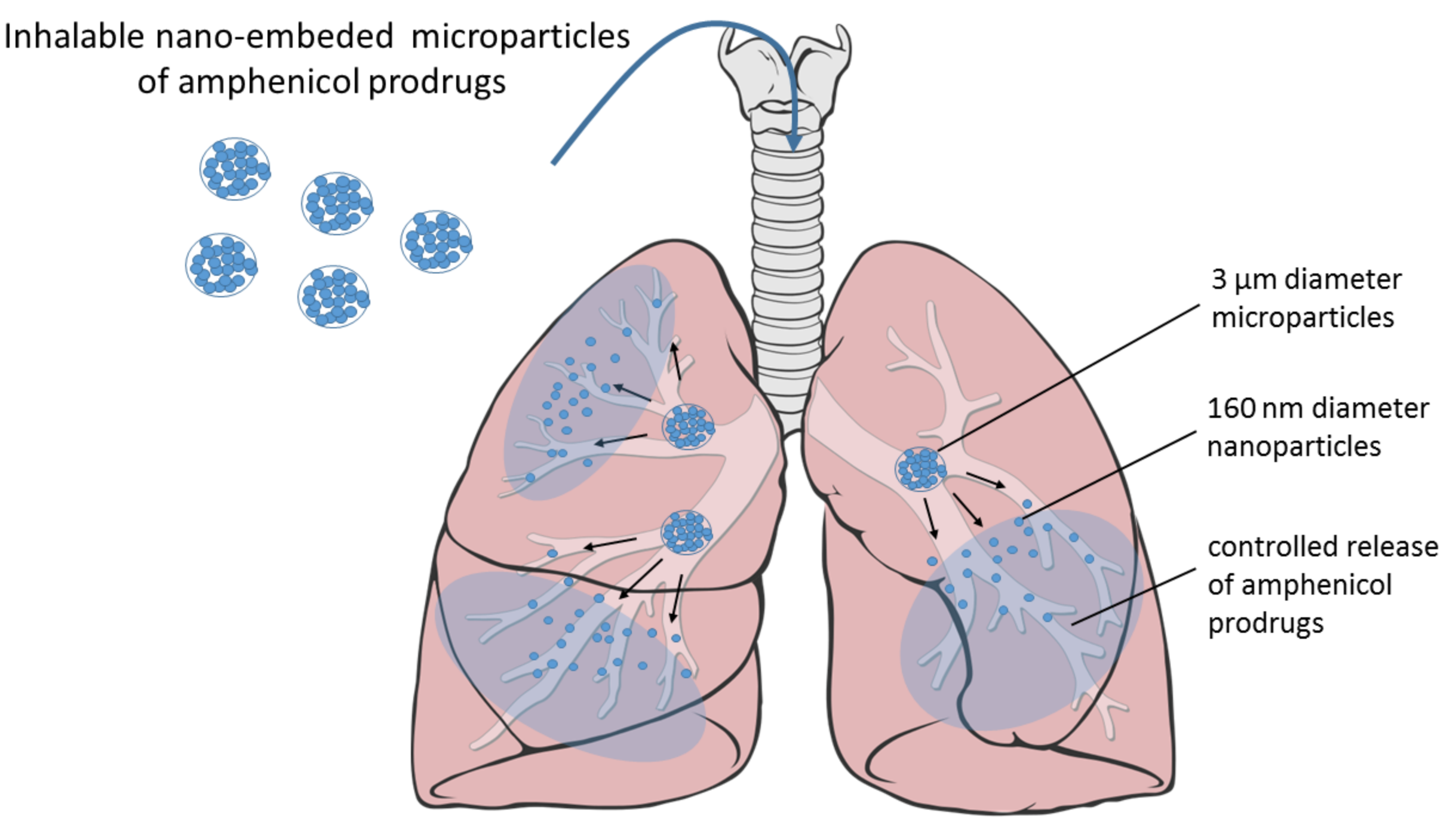
527 **Figure 5:** In vitro prodrug release profiles in HBSS medium, 37°C, for dry microparticle
528 powders made with CHLP nanoparticles (×), with THAP nanoparticles (■), with CHLP-loaded
529 PLGA nanoparticles (▲) or with THAP-loaded PLGA nanoparticles (◆).


Inhalable nano-embeded microparticles of amphenicol prodrugs
3 µm diameter microparticles
160 nm diameter nanoparticles
controlled release of amphenicol prodrugs