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Controlled release antibiotics for dry powder lung delivery

Handoko Adi, Paul Michael Young, Hak-Kim Chan, Rania Salama and Daniela Traini

Advanced Drug Delivery Group, Faculty of Pharmacy (A15), University of Sydney, Sydney, NSW, Australia

Abstract

Introduction: Two controlled release (CR) antibiotics intended for inhalation therapy were evaluated. *Material and Methods*: Ciprofloxacin and doxycycline (both hydrochlorides) were selected as model drugs. Microparticles containing 90:10 ratio of polyvinyl alcohol (PVA) and single antibiotics or combinations were obtained via spray drying. The microparticles were evaluated in terms of particle size, morphology, thermal properties, aerosol performance, and in vitro release. *Results and Discussion*: Analysis of the microparticle morphology indicated comparable size distributions (2.04 ± 0.06, 2.15 ± 0.01, and 2.21 ± 0.01 μm for ciprofloxacin, doxycycline, and co-spray-dried antibiotic formulations, respectively). Thermal analysis of the microparticles suggested similar responses, which were dominated by the endothermic peaks observed for PVA alone. Analysis of the aerosol performance suggested that the individual antibiotic formulations had different aerosol profiles that were dependent on the antibiotic used. In comparison, the combination CR antibiotics had identical aerosol profiles, suggesting that the microparticles were homogeneous. The release of antibiotics from the CR microparticles showed that ≤50% was released over a 6-hour period in comparison to ≥90% being released in the first hour for microparticles containing no PVA. *Conclusions*: The potential for antibiotic therapy, and specifically CR antibiotic therapy using dry powder inhalers, provides a promising route for the treatment of pulmonary infection.

Key words: *Antibiotics; ciprofloxacin hydrochloride; controlled release; doxycycline hydrochloride; dry powder inhaler; polyvinyl alcohol*

Introduction

Respiratory tract infections are very common and affect all ages. In certain disease, such as cystic fibrosis (CF) or chronic obstructive pulmonary disease, reduced mucociliary clearance rates result in increased risk of infection with life-threatening consequences. Subsequently, lower respiratory tract infections are a major cause of morbidity and mortality among patients with CF and non-CF bronchiectasis[1–3]. Many patients are given long-term treatment with inhaled and/or systemic antibiotics. This therapy will help in maintaining their physical well being, as well as in decreasing the number of disease exacerbations and hospital admissions[4–6], with negligible drug hypersensitivity and little apparent increase in bacterial drug resistance or fungal superinfection[7–10]. Aerosolized antibiotics may also reduce symptoms by reducing the organism density in the airways and prevention of infection or delay of chronic colonization. Interestingly, although aerosolized antibiotics were first introduced in the 1950s, current antibiotic inhalation therapy is limited to nebulizers[11] which are cumbersome, expensive, require routine maintenance, and, in general, ineffective. To date, TOBI® (Tobramycin) is the only antibiotic by nebulization approved by the Food and Drug Administration. If given orally or intravascular, the major drawback of antibiotic therapy is the need for their relatively high-dose administration, which carries the potential for systemic toxicity[12,13]. Clearly, as with TOBI®, these limitations can be circumvented by direct delivery of antibiotic aerosol to the airways.

Recently, a trend in delivering respiratory medicaments via dry powder inhalers (DPIs) has become popular due to higher dosing potential and drug stability. Simply, DPIs consist of a formulated API(s) powder with an aerodynamic diameter suitable for respiratory delivery

Address for correspondence: Daniela Traini, PhD, Advanced Drug Delivery Group, Faculty of Pharmacy (A15), University of Sydney, Sydney, NSW 2006, Australia. Tel: +61 (2) 9351 2356, Fax: +61 (2) 93514391. E-mail: danielat@pharm.usyd.edu.au

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(<6 μm)[14] contained within a device that produces a therapeutic dose upon inhalation. Aerosol particles of micron size range have high surface area to mass ratios making the formulation and thus the development of powders and devices a significant challenge[15–18]. At present, no marketed dry powder antibiotic inhalation products exist. Thus a new method that can decrease the burden of treatment and offer patients more independence is highly sought after.

The local treatment of respiratory infection faces another challenge. Although antibiotics have rapid adsorption and clearance, antibiotics delivered to the lungs should remain resident at the site of action for as long as possible. Subsequently, a logical approach would be to formulate a powder that has certain release characteristics to ensure effective residence and prolonged treatment with minimal dosing. Controlled release (CR) products are formulated to release the drug's active ingredient gradually and predictably over extended periods. These formulations potentially provide greater effectiveness in the treatment of chronic conditions through more consistent delivery of the medication, reduced side effects, greater convenience, and higher levels of patient compliance due to a simplified dosage schedule, compared with those of immediate release drugs. Interestingly, little research has focussed on this delivery approach in the lung[19].

Here, the authors investigate the potential of CR antibiotics as an inhalable dry powder formulation. Ciprofloxacin hydrochloride and doxycycline hydrochloride were selected as model drugs for their broad-spectrum antibiotic characteristics. The CR component of the formulation, polyvinyl alcohol (PVA), was chosen as a model polymer since it was recently reported that viscous solutions containing PVA altered the in vivo release of 5(6)-carboxyfluorescein in an in vivo rat model[20]. Formulations were prepared as single and combination, as combination therapy for the treatment of respiratory infection has been reported as having improved efficacy[21]. The formulations were evaluated in terms of their physico-chemical properties, aerosol performance, and release profile using a diffusion model.

Materials and methods

Materials

Doxycycline and ciprofloxacin (both hydrochlorides) were chosen as model antibiotics and used as supplied (MB; Biomedical Australasia Pty Limited, NSW, Sydney, Australia). PVA with molecular weight of 22,000 was supplied from BDH Limited (Poole, UK) and had a reported degree of hydrolysis of 98%. Water was purified by reverse osmosis (MilliQ; Millipore, MA, USA). All solvents were obtained from Biolab (Victoria, Australia) and were of analytical grade.

Chemical analysis of ciprofloxacin and doxycycline

Simultaneous quantification of doxycyline and ciprofloxacin, with or without the presence of PVA, was achieved using high-performance liquid chromatography (HPLC). The HPLC system consisted of a Waters 717 plus autosampler, 600 pump, 486 detector, 600 controller with Millennium V.32 software (all Waters Ltd., Sydney, Australia). The stationary phase was a Phenosphere-NEXT C-18 column, 5 μm, 150 × 4.60 mm (Phenomenex, Sydney, Australia). The mobile phase consisted of a mixture of acetonitrile and 0.1 M sodium dihydrogen phosphate (30:70, v/v), adjusted to pH 3.35 with phosphoric acid. Standards and samples were prepared in 30:70 (v/v) acetonitrile : water. Experimental settings were as follows: detection wavelength, 350 nm; flow rate, 1.5 mL/min; injection volume, 100 μL. Linearity was obtained between 0.03 and –20 μg/mL (R^2 = 0.999) with a retention time of 1.8 and 3.3 minutes for ciprofloxacin and doxycycline, respectively.

Preparation of the spray-dried microparticulate systems

Microparticles containing 90:10 ratio of PVA and single antibiotics (either ciprofloxacin or doxycycline), or a combination at a 1:6 ratio (for ciprofloxacin and doxycycline, respectively) were obtained via spray drying. A 1:6 ratio was chosen based on antimicrobial susceptibility test standards[22].

PVA concentration of 90% (w/w) in the aqueous solution prior to spray drying was used, as this concentration previously resulted in the slowest drug release[23]. Briefly, PVA was dissolved in water until constant agitation at 90°C until a clear solution was obtained. After cooling the PVA solution to 40°C, an aqueous solution of the antibiotic component(s) was added. The feed solution was spray dried at the following conditions: feed rate of 4 mL/min, aspiration rate of 47.6 m^3/h, inlet and outlet temperatures of 120°C and 64°C, respectively, and an atomizing pressure of 800 kPa. Formulations were prepared such that the total feed concentration was 50 mg/mL. In addition, single microparticle systems containing either ciprofloxacin or doxycycline without the presence of PVA modifier were prepared as described previously to allow comparison of the release profiles[24].

PVA is generally considered as safe with no adverse toxicological or reproductive effects found in rat[25]. Furthermore, recently the toxicity of PVA 22,000 MW on lung epithelial cells has been assessed in vitro by

Salama et al.[26], using a human alveolar basal epithelium A549 cell line and found to be nontoxic.

Scanning electron microscopy

The morphology of the CR microparticles was investigated using scanning electron microscopy (SEM) (JSM 6000F; JEOL, Sydney, Australia) at 10 kV. All samples were mounted on adhesive black carbon tabs (premounted on aluminum stubs), and sputter-coated with gold (Sputter coater S150B; Edwards High Vacuum, Sussex, UK) at 40 nm thickness prior to analysis.

Particle size analysis

Particle size distributions of the spray-dried microparticle samples were determined by laser scattering (Mastersizer 2000; Malvern Instruments, Malvern, UK), using a dry powder feeder (Scirocco 2000; Malvern Instruments). Approximately 3 mg of powder was dispersed in air using 4-bar pressure. All samples were measured with a refractive index of 1.52. Each sample was analyzed in triplicate.

Differential scanning calorimetry

The thermal response of each powder was analyzed using a differential scanning calorimeter (DSC) (DSC823e; Mettler-Tolledo GmbH, Schwerzenbach, Switzerland). Approximately 3–5 mg of powder was accurately weighed into DSC sample pans, crimp sealed, and lid pierced. The thermal properties were analyzed over a 40–400°C at a ramp rate of 10°C/min. Data were normalized for initial mass and exothermal and endothermic peak temperatures, onset temperature, and heat of enthalpy (ΔH) for each peak were determined using STARe V9.0x software (Mettler-Tolledo GmbH).

In vitro drug release studies

Previous studies have suggested that a diffusion model is capable of differentiating between the release profiles of microparticles, intended for inhalation therapy[27]. Although the lung is a complex organ, if the active clearance mechanisms are not considered (i.e., phagocytosis or ciliary movement), the deposition and release of drug from solid particulates may be classified as (A) impaction, (B) wetting, and (C) diffusion/dissolution. Furthermore, as the total lung volume in the lower respiratory tract is estimated to be approximately 1 μL/cm^2/mL[28], current pharmacopeia methods that are based on dissolution in sink conditions are not representative.

The diffusion apparatus used for this study was based on a modified Franz diffusion cell described previously[27,29] . In general, 250 mL of degassed dissolution medium (consisting of phosphate buffer at pH 7.4) was pumped from a temperature-equilibrated water bath (37 ± 0.5°C) at 5 mL/min via a multichannel peristaltic pump (Minipuls 3; Gilson, Middleton, WI, USA) to the inlet port of the Franz cell. The sampling port of the cell was connected to a second pump channel, which returned the sample flow to the 250 mL sink for analysis. A 0.45 mm filter (MF™ Membrane Filters; Millipore, Bedford, MA, USA) was used as the model diffusion membrane with an internal diameter (when clamped to the Franz assembly) of 2.5 cm.

The Franz cell contained a heated water jacket that was maintained at 37 ± 0.5°C via a circulating water bath. The stirring rate in each cell was controlled via a multistation Franz cell station (VB6; PermeGear Inc, Hellertown, PA, USA). The continual renewal of dissolution medium, along with the agitation of the liquid due to the presence of a magnetic stirrer in the bottom of the Franz cell, ensured sink conditions below the diffusion membrane. Previous studies have suggested the aqueous solubility of ciprofloxacin and doxycycline to be 30 mg/mL, [30] and 50 mg/mL, [31,32] respectively, thus a 20 mg sample of ciprofloxacin dissolved in 250 mL would be 450 × less than the solubility limit. Once the system was equilibrated, the pre weighed powder sample (20 ± 0.2 mg) was evenly spread, on the previously pre-wetted membrane filter in the Franz cell, using a sieve to get an even distribution of powder. Samples of 2 mL were taken from the 250 mL reservoir at predetermined time intervals and assayed using the HPLC method described previously.

In vitro aerosol performance analysis

The aerosolization efficiency of the CR microparticle formulations was evaluated using a multi-stage Marple Miller Impactor (USP Apparatus 2; Copley Instruments Ltd., Nottingham, UK), which at 60 L/min produces cut-off diameters of 10, 5, 2.5, 1.25, and 0.626 μm for stage 1 to filter. The flow through the Marple Miller apparatus was controlled using a GAST rotary vein pump and solenoid valve timer (Copley Scientific, Nottingham, UK). Flow rate was tested prior to operation using a calibrated flow meter (TSI, Shoreview, MN, USA). A 20 ± 0.2 mg sample of microparticle formulation was accurately weighed into size 3 hard gelatin capsule (Capsugel, Sydney, Australia) which was placed into the dosage chamber of an Aerolizer® DPI (Novartis, Surrey, UK). A United State pharmacopoeia (USP) throat was attached to the Marple Miller apparatus and a custom-made rubber adaptor was used to connect the Aerolizer® to the mouthpiece. The device was tested for 4 seconds at 60 L/min. Temperature and humidity throughout the testing was 25°C and 45% RH, respectively. After actuation, the device, capsule, throat, all sample stages, and filter were washed with HPLC sample solvent into volumetric

flasks and adjusted to volume. Appropriate sample dilutions were made prior to testing by HPLC. Each CR microparticle formulation was tested in triplicate.

Results obtained from the in vitro impactor tests were analyzed to produce delivered dose (DD) (total drug concentration collected from all samples), fine particle dose (FPD; drug mass in stage 3 to filter), and fine particle fraction (FPF = FPD/DD × 100). The FPF and FPD refer to the deposited drug with an aerodynamic mass median diameter <5 μm.

Statistical analysis

Data were subjected to analysis of variance (ANOVA; Minitab 12.1; Minitab Ltd., Coventry, UK). Significant difference between formulations was analyzed using post hoc multiple comparisons (Fisher Pairwise) where a significant difference was classified as $p < 0.05$.

Results and discussion

Scanning electron microscopy

SEM images of the CR microparticles containing ciprofloxacin, doxycycline, and co-spray-dried ciprofloxacin/doxycycline are shown in Figure 1A–C, respectively. In general, all the particle systems had a 'doughnut' appearance, representative of particle collapse during the drying process. This morphology is similar to that reported for the same antibiotics when spray dried alone[24] and is most likely due to the spray-drying conditions (in comparison previous studies using spray-dried PVA particles showed spherical geometries but had different production conditions[23]).

Particle size analysis

Particle size distributions of the CR ciprofloxacin, doxycycline, and combination are shown in Figure 2. Analysis of the data suggested that all three CR microparticles formulations had similar particle size diameter; this is expected, because the three systems were spray dried under the same conditions and concentrations. Furthermore, the single and co-spray-dried CR antibiotics fell within the size range for respiratory delivery, with median volume diameters of 2.04 ± 0.06, 2.15 ± 0.01, and 2.21 ± 0.01 μm for the CR ciprofloxacin, doxycycline, and co-spray-dried antibiotics formulations, respectively ($n = 3$).

Differential scanning calorimetry

The response of each microparticle system to a temperature ramp is shown in Figure 3. In addition, a sample

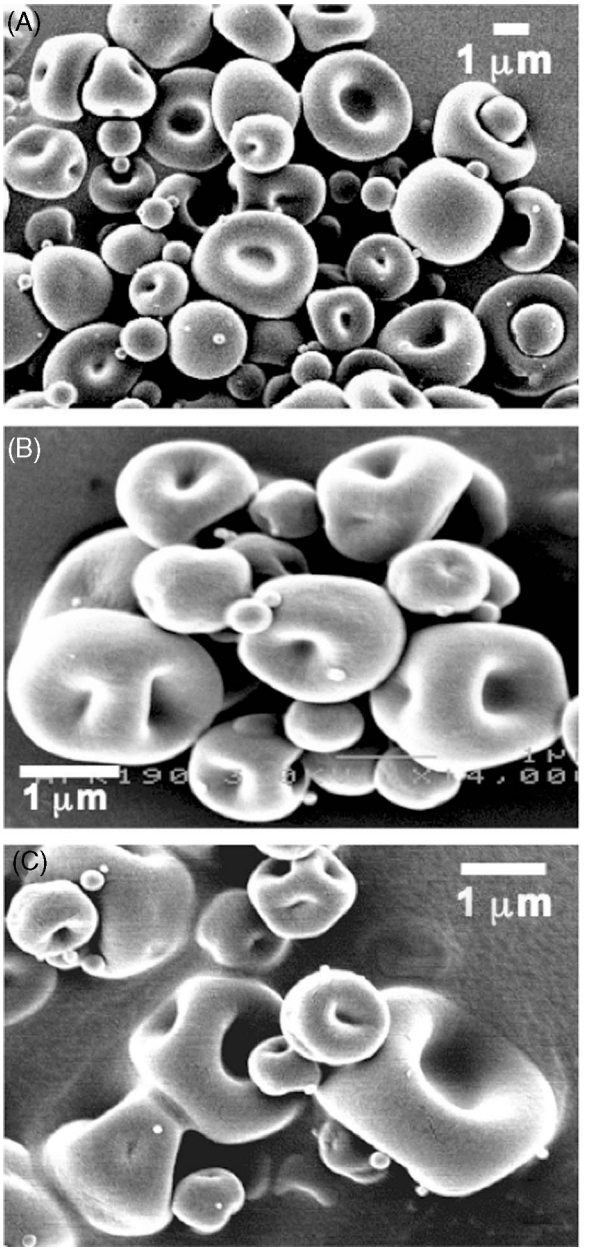

Figure 1. Scanning electron microscopy of controlled release antibiotics. (A) Spray-dried CR ciprofloxacin; (B) spray-dried CR doxycycline; (C) co-spray-dried CR ciprofloxacin/doxycycline.

containing only PVA was prepared, using the same methodology as for the CR particulates, and used as a control for comparison. The thermal response of PVA alone (Figure 3A) had a broad diffuse endothermic peak from around 30°C to 130°C indicative of water loss and a sharp endothermic peak at 228°C indicative of a melt[33]. The thermal response of either ciprofloxacin or doxycycline spray dried with PVA are shown in Figure 3B. Both formulations had profiles similar to that of PVA alone where a broad diffuse endothermic response was observed at lower temperatures followed by a sharp melting

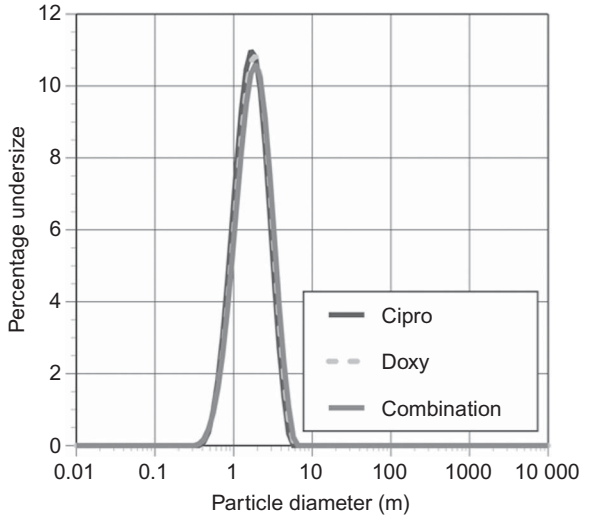

Figure 2. Particle size distribution of single and co-spray-dried controlled release antibiotics measured using laser diffraction.

endotherm at higher temperatures. Interestingly, the melting point of both co-spray-dried mixtures was slightly less (<225°C) than that for the pure PVA presumably due to an increase in entropy. In addition, a large endothermic peak was observed in the ciprofloxacin/PVA sample at around 270°C. This may be a melting peak for ciprofloxacin (spray dried alone, ciprofloxacin exhibited a melt around 333°C)[24]. As with the PVA alone, analysis of the doxycycline/PVA system showed only two primary endotherms; however, a complex series of small endothermic responses were observed between 125°C and 200°C. This was in good agreement for the thermal response of ciprofloxacin spray dried alone and is discussed in detail elsewhere[24]. In comparison, the combination of ciprofloxacin and doxycycline with PVA (Figure 3C) showed no such drug-specific features and was very similar to the PVA alone. Interestingly the melting temperature and the thermal energy were similar to that of the single systems, which is to be expected as the total 'impurity' concentrations remained equivalent (10%, w/w).

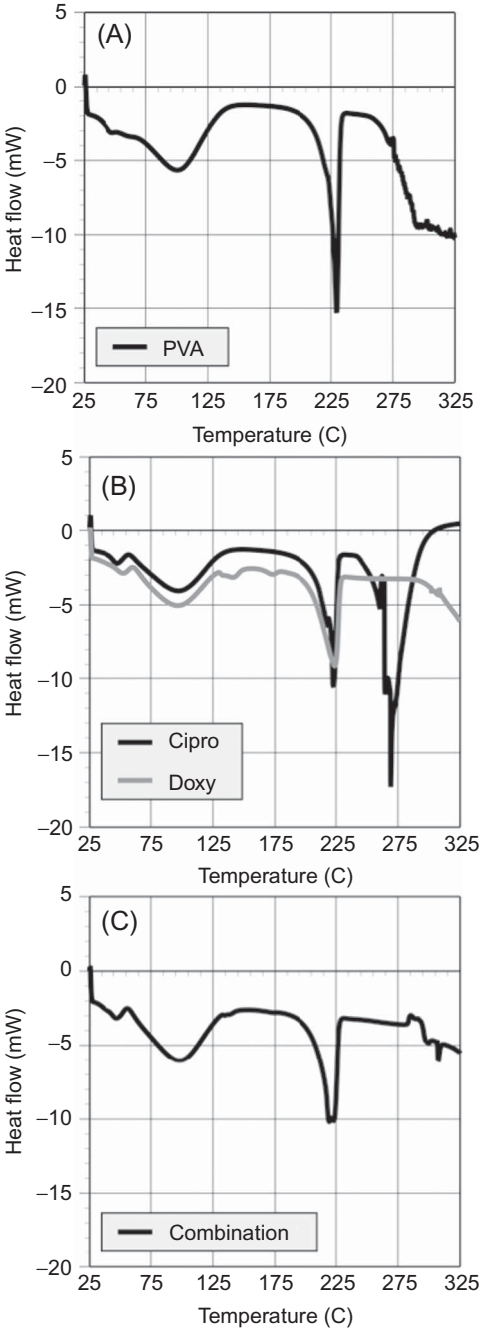

Figure 3. DSC thermograms of (A) PVA, (B) antibiotic PVA CR microparticles, and (C) co-spray-dried CR microparticles.

In vitro drug release

The amount of each antibiotic released through the diffusion cell as a function of time was calculated based on the theoretical drug mass and represented as percentage released. The percentage antibiotic released from the single and combined antibiotic CR microparticles and microparticles containing no PVA is shown in Figure 4. Analysis of the microparticle systems containing either ciprofloxacin or doxycycline without any CR agent showed similar and rapid release profiles with ≥90% being released in the first hour. In comparison, the systems containing 90% (w/w) PVA had significantly reduced diffusion rates with ≤50% being released over a 6-hour period. Furthermore, analysis of the profiles suggested that, in general, the release of doxycycline was faster than ciprofloxacin, most likely due to the relative difference in aqueous solubility (50 and 30 mg/mL), respectively[30–32]. Comparison of the release profiles of the CR doxycycline alone or with ciprofloxacin suggested no significant difference in release at all time points.

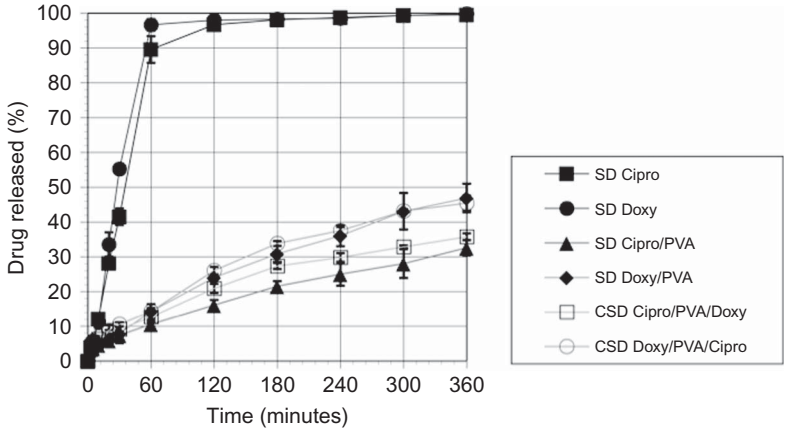

Figure 4. Release profile of spray-dried controlled release ciprofloxacin and doxycycline microparticles prepared as discrete entities or co-spray dried. Data are plotted against the spray-dried antibiotics without any control release agent. For the combination formulations the antibiotic under analysis is defined by the first drug in series.

Similar observations were found for the ciprofloxacin release rates, although a statistically significant difference between the single and combination formulations was observed at the 120 and 180 minutes time intervals, presumably due to the relative difference in ciprofloxacin concentrations between the two microparticle systems.

Previous study by Sweeney et al.[34] assessed the feasibility of spray freeze-dried liposomal ciprofloxacin powder for inhaled aerosol delivery. The formulation was dimyristoyl phosphatidylglycerol, lactose, and ciprofloxacin, with a weight ratio of 5:17:1. Upon administration of a 20-mg dose, the average mass of ciprofloxacin in the FPF per mass was determined to be 20.6 μg mg. This resulted in the minimum inhibition concentration (MIC) of ciprofloxacin of 5 mg/L, and this occurred in the most distal tracheobronchial generation. This concentration is well above the MIC of many bacteria causing respiratory infection including *Pseudomonas aeruginosa* (MIC90, 4 mg/L), *Streptococcus pyogenes* (MIC90, 1 mg/L), *Staphylococcus aureus* (MIC90, 1 mg/L), *Escherichia coli* (MIC90, 0.025 mg/L), and many other aerobes[35].

In this study, the formulation was ciprofloxacin and PVA, with the ratio of 1:9. Upon administration the average FPD of ciprofloxacin was 382.4 μg (n = 3), which, theoretically after 10 minutes of drug deposition in the lung, should be well above the therapeutic level.

It is important to note that the in vitro dissolution measurements are not representative of the powder quantity deposited on a specific stage of the Marple Miller Impactor. However, all the particles studied in the dissolution apparatus were of suitable size for delivery and thus may be considered representative of particles that would reach the lung. Previous studies by Salama et al. have confirmed, by endotracheal infusion, that drug particles containing 95% PVA of size similar to those studied here had in excess of 6 days a release in a ovine model, when compared to a control[26].

In vitro aerosol performance

The in vitro aerosol stage depositions of the single and co-spray-dried CR formulations are shown in Figure 5A and B, respectively. In general, the single spray-dried ciprofloxacin formulation had a lower aerosol performance (FPF = 21.2 ± 0.8%) when compared to the single doxycycline formulation (FPF = 24.5 ± 0.7%). Although these microparticle formulations contain a significant amount of PVA (90%, w/w), the significant difference in aerosol performance between particles containing ciprofloxacin and those containing doxycycline followed a trend similar to that reported previously[24], where doxycycline had a higher aerosol performance than ciprofloxacin. Interestingly, in this study, the relative difference in aerosol performance was not so high as that reported for microparticles containing only antibiotics (where ciprofloxacin had an FPF of 21% and doxycycline of 32%[24]). This is presumably due to the aerosol performance being dominated by the large percentage of PVA in the formulation.

Co-spray drying of ciprofloxacin and doxycycline in the presence of PVA resulted in a microparticle formulation that had similar aerosol performance (Figure 5B). Analysis of the percentage on the Marple Miller stage deposits showed no statistical difference in the relative amounts of ciprofloxacin and doxycycline (at a 1:6 ratio) deposited on each stage, suggesting that the microparticle formulations were homogeneous in nature. FPFs of 25.9% (±1.3) and 25.8% (±1.2) were observed for ciprofloxacin and doxycycline, respectively.

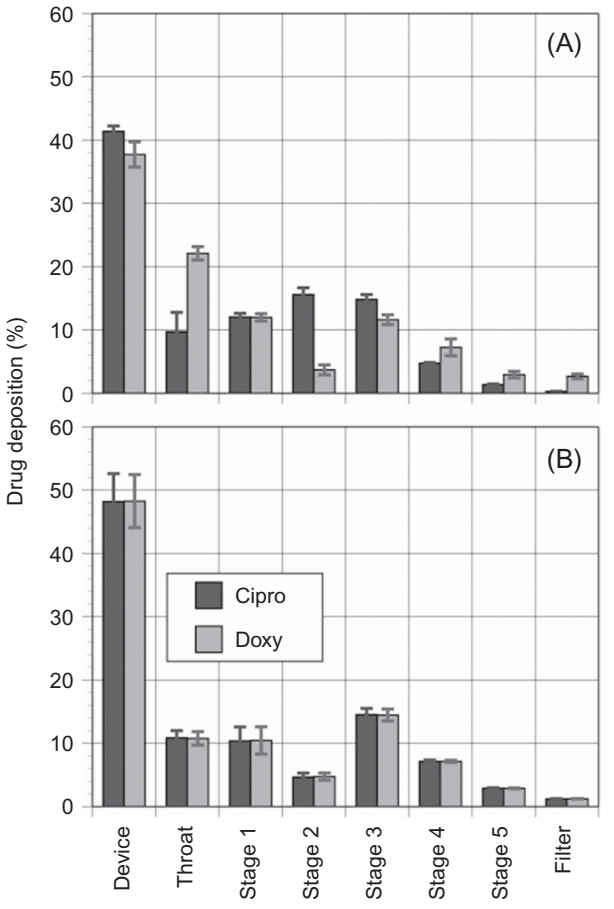

Figure 5. Percentage stage distribution in a Marple Miller Impactor of (A) simple mixes of ciprofloxacin and doxycycline CR microparticles and (B) combination of CR microparticles containing ciprofloxacin and doxycycline.

Conclusions

A series of antibiotic formulations containing ciprofloxacin, doxycycline, or a combination of both have been formulated with PVA with a view to control the release profile. In general, all powders had similar particle size distributions, thermal stability, acceptable aerosol performance, and modified release profiles. The novel approach presented in this investigation is to combine the antibacterial effect of an antibiotic with the advantages of CR formulations. Although this system would release lower antibiotic doses compared to current parenteral delivery, the advantage lies in the fact that the antibiotic would be delivered locally. Furthermore, the extended release profiles will result in increased local residence time and therefore improved therapeutic efficacy. With an established particle system, a future focus will be to investigate the local residency of such particles (as the lung has a series of active mechanisms for the removal of particulate systems), the in vivo release rate, and the antibacterial effectiveness.

Declaration of interest

The authors report no conflicts of interest.

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