A novel dry powder inhalable formulation incorporating three first-line anti-tubercular antibiotics

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Abstract
Treatment for tuberculosis (TB) using the standard oral antibiotic regimen is effective but inefficient, requiring high drug dosing and lengthy treatment times. Three concurrent first-line antibiotics recommended by the World Health Organization (WHO) guidelines are pyrazinamide, rifampicin and isoniazid. Combining these antibiotics in a novel formulation for dry powder inhalation (DPI) may facilitate rapid and efficient resolution of local and systemic infection. However, spray-dried individually, these antibiotics were found to be physically unstable. A solution of the three antibiotics, at the WHO-recommended ratio, was spray-dried. The collected powder was assessed by a series of in vitro methods to investigate aerosol performance, particle physico-chemical characteristics and dissolution profile. Particles obtained were spherical with a surface composed primarily of rifampicin, as identified by TOF-SIMS. A mass median aerodynamic diameter of 3.5 ± 0.1 μm and fine particle fraction (<5 μm) of 45 ± 3% indicated excellent aerosol performance. The combination powder was differentiated by the presence of rifampicin dihydrate and the delta polymorph of pyrazinamide. Quantitative analysis indicated individual particles contained the three antibiotics at the expected proportions (400:150:75 w/w). This excipient-free triple antibiotic DPI formulation could be used as a significant enhanced treatment for TB.

1. Introduction
Tuberculosis (TB) remains a significant medical issue worldwide, despite implementation of a highly effective standard treatment regimen. The World Health Organization recommended regimen [1] consisting of oral co-administered isoniazid, rifampicin, pyrazinamide and often ethambutol is primarily threatened by poor patient adherence [2,3]. This is partly related to side effects of oral administration, which require high drug dosing and lengthy treatment times. These high doses are necessary for the drugs to reach poorly or non-vascularised sections of the body such as granulomas, tubercles and infected alveolar macrophages [4]. The long treatment times (up to 6 months) treat the slow-growing populations of bacteria but also decrease patient adherence [5]. Premature self-termination of treatment by patients in turn leads to drug resistant strains of TB which further complicates treatment efficacy.

Inhalable anti-tubercular formulations have been proposed as a potential solution to these issues [6–15]. Furthermore, since tuberculosis is primarily communicated via the pulmonary route, with 75–80% of cases remain localised in the lungs [5,15,16], aerosolised therapy appears the most logical route. Pulmonary therapeutics increase the chance of arresting TB infection before dissemination to other organs by maximising drug concentration at infected sites in the lungs, thereby reducing overall drug dosing and related side effects [11,17,18]. These local drug concentrations may even be high enough to overcome some drug resistance [15]. More specifically, inhaled antibiotic particles can target alveolar macrophages which, when infected with TB, are maintained in a state of ‘alternative activation’ whereby macrophage cytosol conditions are suitable for TB bacilli replication and survival [8,15]. However, phagocytosis of microparticles has been shown to revert these macrophages to a ‘classical activation’ state that resurrects their innate bactericidal mechanisms [6,14,15,19]. Thus, inhaled antibiotic microparticles are expected to perform a dual action: to have antibiotic drug activity and to be able to activate innate bactericidal mechanisms.

Despite extensive research into particle-engineering techniques for both dry powder and liquid formulations employing various [9–11,13,18,19], no commercial aerosolised anti-tubercular
therapy is currently available. Misra et al. [8] suggests this may be related to hurdles such as difficulties in formulation development, unknown safety of inhaled excipients and production scalability. The current investigation focuses on pulmonary dry powder delivery as it offers shorter treatment times, enhanced patient compliance and device portability.

The aim of this research was to present a novel method whereby three anti-tubercular antibiotics could be effectively combined into an inhaled dry powder formulation for efficient treatment of TB-infected patients. Bypassing the oral route of administration would allow for reduced oral dosing and related side effects. In addition, this triple antibiotic dry powder may address aforementioned issues to effectively treat pulmonary TB in a timely manner. The micron-sized dry powder is produced in a single step spray-drying process without excipients and combines three first-line antibiotics for TB, as suggested by the WHO, (pyrazinamide (PYR):rifampicin (RIF):isoniazid (IZD) in a ratio of approximately 5:2:1) [1]. The physico-chemical characteristics and aerosol performance of this triple-DPI powder were characterised using various in vitro and analytical techniques.

2. Methods

2.1. Dry powder aerosol production

The combined antibiotic dry powder was produced by spray-drying. A Buchi-290 Mini spray-dryer was operated in a closed loop, connected in series with a Buchi-296 dehumidifier and Buchi B-295 inert-loop (all from Buchi Laboratories, Flawil, Switzerland), using nitrogen as the drying gas. For the triple antibiotic dry powder, the feed solution consisted of the three antibiotics – pyrazinamide (8 mg/mL), rifampicin (3 mg/mL) and isoniazid (1.5 mg/mL) (all antibiotics from Hangzhou ICH Imp & Exp Co. Ltd., Hangzhou, China) – in an ethanol:water (50:50 v/v) solution. The same concentrations and solvent mixture were used to individually spray-dry the three antibiotics for analytical comparison. Spray-drying was undertaken with the following settings: inlet temperature 60 °C, atomiser 40 mm (approximately 500 L/h), aspirator 100% (40 m3/h) and feed rate 5% (2 mL/min). Powders were protected from light and moisture in a non-transparent desiccated container at 25 °C and used within 3 days.

2.2. Particle morphology

Particle surface morphology was characterised using a Zeiss Ultra Plus scanning electron microscope (Carl Zeiss, Oberkochen, Germany) at an acceleration voltage of 5 kV. The sample powders was placed onto carbon tape and sputter-coated with approximately 15 nm of gold (Emitech K550X) prior to imaging.

2.3. Particle sizing

Each single and triple antibiotic spray-dried formulation was analysed using laser diffraction with the associated software calculating particle size distributions by the Mie theory (Malvern Mastersizer 2000, Malvern Instruments Ltd., Worcestershire, UK) to give their respective volumetric median diameter and span – defined as the difference between the 10th and 90th percentile particle diameters, divided by the volumetric median diameter. The refractive index (RI) for pyrazinamide (1.577) was used for all measurements, as it composes more than 50% of the triple antibiotic powder. Furthermore, the RIS of rifampicin (1.613) and isoniazid (1.588) are similar.

Powder was loaded into a Scirocco 2000 dry powder feeder and dispersed in a 3.5 bar airstream. The dispersive air pressure (3.5 bar) was chosen by comparing median particle size over the minimum to maximum pressures (0.5–4.0 bar). Measurements were performed in triplicate.

2.4. Aerosol dispersion and drug quantification

Aerosol performance of the antibiotic microparticles was assessed using a multi-stage liquid impinger (MSLI) (Copley Scientific, Nottingham, UK) coupled with a USP throat. The first four MSLI stages were each filled with 20 mL of rinsing solvent (methanol:50 mM phosphate buffer pH3.0, 50:50 v/v), and the fifth stage fitted with a 0.2 µm glass filter (Pall Corporation, Surry Hills, Australia). To minimise evaporation of the solvent, the MSLI airflow was equilibrated to approximately 100 L/min when empty, then after filling with solvent readjusted to exactly 100 L/min within 5 s, using a GAST Rotary vein pump (Erweka GmbH, Heusenstamm, Germany) and calibrated flow meter (TSI 3063, TSI instruments Ltd., Buckinghamshire, UK). An airflow of 100 L/min represents the flow rate achievable by patients using a comfortable inspiratory effort with an Aeroliser® DPI device (Novartis, Mulgrave, Australia) [20]. Size 3 hydroxypropyl methylcellulose capsules (Capsugel, West Ryde, Australia) were filled with approximately 20 mg of the powder and actuated for 2.4 s using an Aeroliser® connected to the USP via a mouthpiece adapter.

After actuation, the device, capsule, throat and stages 1–4 of the MSLI were washed using varying amounts of rinsing solvent, specifically: 10 mL each for the throat, device and stage 5, and 5 mL each for the adaptor and capsule. Each sample was tested in triplicate.

Quantification of the dispersed drug was performed using high performance liquid chromatography (HPLC). The method used was adapted and modified from Calleri et al. [21]. The Shimadzu HPLC system comprised of a CBM-20A controller, LC-20AT pump, SPD-20A UV/VIS detector, SIL-20A HT autosampler and LCsolution software (all from Shimadzu Corporation, Kyoto, Japan). It was coupled with a µBondapak® C18 (3.9 × 300 mm) column (Waters, Milford, MA, USA) with a sample injection volume of 100 µL.

The mobile phases were 50 mM phosphate buffer (pH 3.0) (orthophosphoric acid from Ajax Finechem Pty. Ltd., Taren Point, Australia; potassium dihydrogen orthophosphate from Biolab Ltd., Clayton, Australia) (A), acetonitrile (B) and methanol (solvents both from V.S.Chem House, Bangkok, Thailand) (C). The gradient profile was A:C (9:1 v/v) for 5.25 min, followed by a simultaneous linear decrease to A:C (1:0 v/v) by 10.5 min and increase to A:B (50:50 v/v) by 14.5 min. The latter was maintained for 30 min and then a gradient initiated down to A:C (9:1 v/v) by 35 min, which was maintained until 40 min. The flow rate was kept at 1.0 mL min⁻¹ throughout.

The UV wavelengths for detection were adjusted to detect the antibiotics microparticles at the following retention times: 261 nm initially for isoniazid at 3.7 min, then 265 nm from 5.25 min followed by 254 nm at 9.5 min, to detect pyrazinamide and rifampicin at 6.2 min and 22 min, respectively. Standards solutions were prepared for each of the three antibiotics at 0.01, 0.05, 0.1 and 0.2 mg/mL and gave an R² value of 1.00 for all antibiotics.

Total emitted dose describes the post-aerosolisation weight difference between the initial weight of drug loaded into the capsule and the amount retained within the Aeroliser®. The mass median aerodynamic diameter (MMAD) was obtained from the log-probability plot of the MSLI results. Fine particle fraction (FFP) was defined as the percent mass of aerosol particles with an aerodynamic diameter less than 5 µm.

2.5. Qualitative surface analysis

Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS) was used to image spectral data (surface chemical mapping based on mass fragment analysis) of the sample powder to provide
 qualitative information on its surface chemistry. Chemical maps were produced to investigate the spatial distribution of the various identified mass fragments of interest.

A PHI TRIFT V nanoTOF instrument (Physical Electronics Inc., Chanhassen, MN, USA) equipped with a pulsed liquid metal \(^{79}\)Au primary ion gun working at 30 kV energy was operated under a vacuum of \(5 \times 10^{-6}\) Pa or better. Dual charge neutralisation was provided by an electron flood gun and 10 eV Ar\(^+\) ions. ‘Unbunched’ Au; instrumental settings were used to optimise spatial resolution (beam size \(\sim 175\) nm) for the collection of images from areas roughly \(50 \times 50\) µm, with an acquisition time of four minutes.

Samples were prepared by spraying the fine spray-dried antibiotics powder onto adhesive paper, with loose material shaken free. A finely dispersed powder surface was obtained with a low background signal from the substrate. The powder was not compressed at any time to prevent altering the surface properties of the sample.

The powder was analysed using a series of characteristic peaks correlating to the protonated molecular ions of each antibiotic. The protonated molecular ion for rifampicin (m/z \(\sim 824\) amu) was of low intensity, so a characteristic higher intensity fragment ion (m/z \(\sim 97\) amu) was used instead. The identifying ion peaks utilised were at m/z = 97, 124 and 138 amu, for rifampicin, isoniazid and pyrazinamide, respectively. Sample spectra and images were processed and interpreted using Wincadence\(^\text{TM}\) software (Physical Electronics Inc., Chanhassen, MN, USA).

### 2.6. Dissolution profile

Microparticle dissolution profiles of the various spray-dried antibiotics were determined using Franz dissolution cells in a heated station stirrer (V6B, PermeGear Inc., Bethlehem, USA). Deposition and dissolution of the drug on the stationary wetted filter as used in this method have been proven to better simulate drug release in the lung surface environment [22].

Cell reservoirs were filled with 22.7 mL of simulated lung fluid (SLF, pH 7.4) [23] to create a slight positive meniscus. At this volume, complete sample dissolution would result in less than 2% saturation of the medium for all samples – ensuring sink conditions. The medium in each cell was stirred using a magnetic stirrer bar at a constant speed and maintained at 37 °C throughout the experiment. Powder was deposited on a 0.45 µm nitrocellulose membrane (MF\(^\text{TM}\) Membrane Filters, Millipore, Bedford, Bedford, USA) by placing the filter under the dispersion jets in Stage 3 of a Next Generation Impactor (NGI).

For each antibiotic powder dissolution experiment, two 20 mg pre-filled capsules were actuated using the Aeroliser device into a NGI, at an airflow of 100 L/min. The drug particles deposited on the filter had a total mass ranging from 0.5 to 1.0 mg and an aerodynamic diameter between 3.42 and 2.18 µm – an aerosol size range suitable for delivery to the deep lung [18]. To start the dissolution process, the filter was then secured between the SLF meniscus and the top compartment of the Franz cell. At pre-determined time intervals, 350 µL of dissolution medium was sampled from the cell and replaced with equivalent volume of fresh SLF. Following the final sampling time-point at 3 h, the filter was further removed and rinsed with 3 mL of SLF for sampling. Dissolution testing was performed in triplicate for each sample antibiotic powder.

Quantitative analysis was conducted using the previously described HPLC method. Standards solutions were prepared for each antibiotic at 0.0015, 0.015, 0.725 and 0.15 mg/mL to give an \(R^2\) value of 1.00 for all antibiotics.

### 2.7. Powder crystallinity

X-ray powder diffractometry (XRD) (D5000, Siemens, Karlsruhe, Germany) was used to ascertain powder crystallinity. The sample powder was spread into the cavity of the XRD glass slide then analysed by CuK\(\alpha\) radiation (30 mA, 40 kV) from 5° to 40° 2\(\theta\), at a step rate of 0.04° 2\(\theta\) per second.

### 2.8. Thermal analysis

Thermal response profiles for the sample powder were assessed by differential scanning calorimetry (DSC, 821e, Mettler Toledo, Melbourne, Australia). Aluminium crucibles were loaded with 5 mg of the sample powder and crimped with a perforated lid to ensure equal pressure. Heating was initiated in 10 °C/min increments from 40 to 400 °C. Exothermal and endothermal peak temperatures were determined using STARE software V.9.0x (Mettler Toledo, Greifensee, Switzerland).

### 2.9. Moisture sorption characteristics

Moisture sorption isotherms of the sample powders were determined using a Dynamic Vapour Sorption (DVS) instrument (Surface Management Systems, London, United Kingdom). Ten milligrams of triple antibiotic powder were exposed to two 0–90% RH cycles at 10% RH increments. Equilibrium moisture content at each humidity step was determined by a dm/dt of 0.002% per minute. Chamber temperature was maintained at 25 °C and mass changes over time recorded.

### 2.10. Statistical analysis

Data were statistically analysed using the \(t\)-test (Minitab 16, Minitab Pty. Ltd., Sydney, Australia), with \(p\)-values <0.05 considered significant.

### 3. Results and discussion

Research into inhaled therapeutics for tuberculosis has focused on two approaches – (1) inhalable particles co-formulated with drug-release controlling polymers such as poly(DL-lactide-co-glycolide) (PLGA), which primarily target infected alveolar macrophages, and to some extent the local lung tissue [7,10,24–27] and (2) microparticles with maximal drug loading (>75% drug) that target lung epithelial tissue and systemic circulation [13,28–31]. This work focuses on the latter approach to maximise antibiotic delivery to the patient, although some drug particle uptake by infected alveolar macrophages is still expected.

The efficacy of inhaled microparticles with high drug loading has been established using animal models of tuberculosis. Garcia-Contreras et al. [29] and Fiegel et al. [28] manufactured and demonstrated the usefulness of spray-dried capreomycin in a TB-infected guinea pig model. High drug concentrations were present in lung tissue, and systemic plasma half-life of the drug was double that achieved compared to drug administered intravenously or intramuscularly [28,29]. Using a similar in vivo model, these results were replicated for spray-dried PA-824, a nitrimidazoppyran antibiotic. Systemic and pulmonary drug concentrations in the lung were sustained for 24 and 32 h, respectively, after initial administration – more than twice as long compared to delivery by the oral route [30]. Notably, inhaled treatment not only achieved high pulmonary drug concentrations, but at high enough dosing could achieve therapeutic levels of the drugs systemically. However, in all aforementioned formulations, addition of an excipient in the form of 20% w/w l-leucine was necessary to improve aerosol dispersion characteristics.

Clearly, further maximising total drug loading per particle is paramount for inhaled therapy that targets both local and systemic delivery. Particles suitable for inhalation consisting only of the
primary TB antibiotic, rifampicin, has previously been reported by Son et al. [13]. In this investigation, an excipient-free formulation has been developed through the incorporation of three first-line antibiotics into a single inhalable dry powder. The formulation reflects the standard principle of treating tubercular infection with a combination of antibiotics to suppress drug resistance.

Scanning electron micrographs of the various spray-dried antibiotic powders are presented in Fig. 1a–d. Fig. 1b shows spray-dried rifampicin alone as light, filamentous particles, whilst isoniazid (Fig. 1c) had irregular, globular shapes with rough granular surfaces. Pyrazinamide (Fig. 1d) formed geometric, microcrystalline structures. When the antibiotics were spray-dried in combination, the resulting particles (Fig. 1a) were surprisingly distinct, forming non-aggregating spherical particles that did not show similar characteristics to any of the individually spray-dried antibiotics. Particle surfaces of the triple therapy powder appear dimpled (Fig. 2a), corrugated (Fig. 2b) or contained a mixture of both features, which have been reported as useful morphologies for reducing particle–particle cohesion [32].

These combination microparticles were within a physical size range of 0.2–5.0 μm. Laser diffraction measurements were performed at a representative dispersive pressure of 3.5 bar based on dispersion results seen in Fig. 3. There was a considerable decrease in span and $d_{50}$ from 0.5 to 1 bar, suggesting the break-up of agglomerates during the aerosolisation. The $d_{50}$ continued to decrease followed by a plateau between 3.5 and 4.0 bar, indicative of maximum powder deagglomeration. At 3.5 bar pressure, the triple antibiotic powder had a median volume diameter of 2.4 ± 0.1 μm with a span of 2.0 ± 0.1. The combined physical and volumetric sizing data suggest a median particle size suitable for phagocytosis by the infected alveolar macrophages [8,18,33].

Fig. 4 presents the distribution of the triple antibiotic powder following in vitro MSLI deposition. The mass median aerodynamic diameter of 3.5 ± 0.1 μm and span of 1.88 ± 0.01 obtained from aerosol dispersion demonstrates the powder is suitable for deep lung delivery. The calculated emitted dose was 89 ± 3%, with the majority of particles deposited in stages 1–5 of the MSLI, and less than 12% of the powder was retained in the device, capsule and adaptor. The resulting high fine particle fraction (≤5 μm, 46 ± 3%) of an aerosol composed exclusively of the three first-line antibiotics maximises therapeutic delivery to the pulmonary site of tubercular infection. Subsequent drug particle uptake by alveolar macrophages is expected to clear infection by the dual action of antibiotic activity and reversion of infected macrophages to a more ‘classical activation’ state with associated innate defence mechanisms [6,15].

Furthermore, quantification results indicate that individual particles consist of the three drugs at the intended ratio. Table 1 shows the theoretical (5:2:1 – PYR–RIF–IZN) versus total recovered ratios of the antibiotics, following MSLI aerosol dispersions of the triple antibiotic powder. The ratio of drugs closely matched (within 1%) those expected from a uniform powder, suggesting individual drug particles readily formed with the intended amount of the three antibiotics. Quantitative HPLC analysis did not show any additional peaks that would indicate degradation products. Interestingly, when comparing the distribution of individual antibiotics as a percentage of their individual recovered amounts, a statistically significantly ($p < 0.05$) lower amount of rifampicin – 16.7 ± 0.9%, compared to 20.7 ± 0.8% and 21.6 ± 0.4% for isoniazid and pyrazinamide, respectively – was found in the throat. This was balanced by a significantly ($p < 0.05$) higher rifampicin quantity – 13.9 ± 0.6% and 6.8 ± 0.4% – recovered in the final two MSLI stages which capture...
particles smaller than 2.4 and 1.32 μm, respectively. A possible explanation may be that aerosolisation of the dry powder in the inhaler during dispersion or subsequent impaction of microparticles in the throat release particle surface fragments that are then captured in the lower stages of the MSLI. These sub-micron surface fragments are expected to compose mostly of rifampicin, as TOF-SIMS showed microparticles with a high rifampicin concentration at the particle surface. There may also be differences in antibiotic distribution with particle size. For example, the sub-micron satellite particles observed in Fig. 1a may also contain a higher proportion of rifampicin relative to the other two antibiotics. However, although statistically significant, the differences in rifampicin content may not be clinically important.

The ToF-SIMS surface chemical micrographs of the triple antibiotic DPI formulation are shown in Fig. 5. From the total ion signals, the outline of the triple antibiotic powder can be identified in the scan area of 50 μm × 50 μm. The black areas indicate regions where no signal was acquired. An overwhelmingly high quantity and more extensive distribution of rifampicin (red colour) was identified at the surfaces of the triple antibiotic particles.
Pyrazinamide (blue colour) exhibited a low intensity but ubiquitous presence on particle surfaces, which may be a result of its dominant proportion in the composition of the formulation. Isoniazid (green colour) was much less prominent, suggesting it is isolated at the core of the particles. However, in some imaged particles, it was present at higher concentration ‘hotspots’.

In the spray-drying process, evaporation of solvent from individual droplets results in a rapidly shrinking liquid body. For a hydrophilic solvent, hydrophobic and heavier molecules tend to remain on the surface of this solvent body to form the outer layer of the final dried particles after total solvent evaporation. Results from TOF-SIMS confirm that rifampicin (log$P_{2.7}$, MW 823) remained at the particle surface whilst isoniazid (log$P_{-1.12}$, MW 137) and pyrazinamide (log$P_{-1.31}$, MW 123) migrated inwards with the relatively polar solvent mixture during particle formation [34–36]. The presence of isoniazid ‘hotspots’ at the particle surface may result from damage to the outer rifampicin layer or incomplete surface formation, resulting in the corrugated surface morphology of the triple antibiotic particles, as seen in Fig. 2b.

Incomplete rifampicin surface coverage is further supported by the similarity in the dissolution profile of the combination and individually spray-dried antibiotic powders (Fig. 6a and b). For both sets of powders, the maximal rate of dissolution for isoniazid and pyrazinamide is reached within 20 min, with 59–62% of the total drug being dissolved. The fast wetting and dissolution profiles are indicative of the relatively high water solubilities of these drugs. Rifampicin, being the most hydrophobic of the three antibiotics under investigation, had a slower rate of dissolution when spray-dried alone. This may be associated with the higher proportion of more soluble rifampicin dihydrate in the triple combination powder as ascertained from the XRPD and DSC results discussed below. The presence of the slower dissolving rifampicin on the antibiotic particle surface was expected to reduce the overall rate of particle dissolution, thereby allowing time for some uptake by TB-infected alveolar macrophages. However, rifampicin appears to have little impact on dissolution of isoniazid and pyrazinamide in the triple antibiotic particles. Its relatively low 25% w/w amount in the triple formulation may have resulted in a thin rifampicin surface layer (rifampicin being the most hydrophobic of the three antibiotic present) that was easily compromised leading to rapid dissolution of the other two antibiotics. Whilst there may be some macrophage phagocytosis of the antibiotic particles, these results suggest it would be unlikely to be on a significant scale.

XRPD analysis diffraction patterns of the single spray-dried and co-spray-dried antibiotic powders are shown in Fig. 7. Rifampicin spray-dried alone gave a mostly diffuse pattern with some small peaks, suggesting mostly amorphous rifampicin with a minor proportion of dihydrate form [13]. Contrastingly, isoniazid and pyrazinamide spray-dried individually showed multiple high intensity
peaks indicating crystalline structures. The XRPD profile for pyrazinamide matches its gamma polymorph [37]. Relative to these latter antibiotics, the triple antibiotic powder presented lower intensity peaks, as the powder now contains amorphous rifampicin. Whilst some peaks can be matched with spray-dried isoniazid and rifampicin, some new peaks are present at \(2\theta = 9.12^\circ, 16.36^\circ, 16.76^\circ, 18.24^\circ, 20.4^\circ\) and \(27.72^\circ\), which match the delta polymorph of pyrazinamide. Furthermore, there is a loss of peaks beyond \(27.72^\circ\) which were associated with the gamma polymorph [37]. Spray-drying pyrazinamide in the presence of the other antibiotics therefore favoured transformation into its delta rather than gamma polymorphic form.

The thermograms obtained from DSC analysis (Fig. 8) show the individually spray-dried antibiotics – rifampicin, isoniazid and pyrazinamide – had peaks at 124, 172 and 191 °C, respectively, that correspond with their melting points [13,38]. For rifampicin and isoniazid, these peaks are duplicated in the thermal profile of the combination powder. For rifampicin, the broad peak shape indicates rifampicin dihydrate is partly in the amorphous form [13]. All three antibiotics experience exothermic peaks due to decomposition above 300 °C. Interestingly, although the delta form of pyrazinamide transitions into the gamma form upon heating to approximately 135 °C, the thermogram for the triple antibiotic powder does not show the expected endothermic peak at approximately 190 °C [37]. This missing peak may be associated with altered thermal activity in the presence of the other antibiotics. This potential drug interaction may also result in lower crystallinity of one or more of the antibiotics, which together with the shared sample weight between three antibiotics contribute to the relatively reduced peak intensities in the combination powder thermogram.

Moisture sorption profiles for the sample powders are presented in Fig. 9a–d. The dual cycle analyses show all spray-dried powders undergo reversible moisture sorption, indicating the materials were mostly crystalline. When individually spray-dried, pyrazinamide and isoniazid had a maximum mass change at 90% RH of 0.13% (Fig. 9c) and 0.12% (Fig. 9d), respectively. Their initial sorption profiles were not reproduced during the second cycle. Rifampicin experienced a significantly greater 14.3% mass change with a second moisture sorption cycle that was almost identical to the first, suggesting greater physical stability. The minor difference in the shapes of the first and second sorption profiles of the combination powder may indicate expulsion of residual solvent from spray-drying during the first cycle desorption. Similarly, the combined antibiotic powder sorption profile was closely reproduced during the second cycle. This is notable considering the less stable profiles for the aforementioned pyrazinamide and isoniazid, which together compose 75% of the formulation. It was inferred that the maximal mass change of 4.4% at 90% RH was attributed to the rifampicin component, considering its proportion in the powder by weight. Thus, the triple combination powder appears to enhance formulation stability with regard to moisture uptake relative to the three individually spray-dried antibiotics, as demonstrated by low powder moisture sorption (<2% at 40% RH) and sorption profile reproducibility.

4. Conclusions

This study presents a dry powder inhalable formulation consisting of three first-line antibiotics used in standard oral or intravenous tuberculosis treatment. The excipient-free powder is produced in a single step by an industrially scalable process (spray-drying) and has been tailored for pulmonary delivery, with the ultimate goal of targeting infected alveolar macrophages. The
combination of three antibiotics displayed better physical stability characteristics than powders of the antibiotics sprayed-dried alone. This engineered inhaled triple antibiotic dry powder could be used as an enhanced therapeutic alternative of the standard oral anti-tubercular regimen, reducing oral dosing, shortening drug regimen and limiting toxicity. In this, this will improve patient compliance and minimise the development of anti-tubercular drug resistance. The efficacy and long-term storage stability of this novel formulation will be assessed in upcoming further investigations using in vitro and in vivo TB infection models.

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