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Development of excipients free inhalable co-spray-dried tobramycin and diclofenac formulations for cystic fibrosis using two and three fluid nozzles

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ABSTRACT

This study aims to investigate the effect of physicochemical properties and aerosol performance of two (2FN) and three-fluid nozzles (3FN) on the inhalable co-formulation of tobramycin and diclofenac dry powders. Combination formulations of tobramycin and diclofenac at 2:1 and 4:1 w/w ratios were prepared at a laboratory scale using a spray dryer in conjunction with a 2FN or 3FN. Powder size, morphology, solid-state characteristics, and aerodynamic and dissolution properties were characterised. The nozzle types and the formulation composition influenced the yield, particle size, solid-state properties, aerosolization behaviour and dissolution of the co-spray dried formulations. In particular, using the 2FN the co-spray dried formulation of tobramycin and diclofenac at 2:1 w/w showed smaller particle size (D50, 3.01 ± 0.06 μm), high fine particle fractions (FPF) (61.1 ± 3.6% for tobramycin and 65.92 ± 3 for diclofenac) and faster dissolution with approx. 70% diclofenac released within 3 h and approx. 90% tobramycin was released within 45 min. However, the 3FN for the co-spray dried formulation of tobramycin and diclofenac at a 2:1 w/w ratio showed a larger particle size (D50, 3.42 ± 0.02 μm), lower FPF (40.6 ± 3.4% for tobramycin and 36.9 ± 0.84 for diclofenac) and comparative slower dissolution with approx. 60% diclofenac was released within 3 h and 80% tobramycin was released within 45 min. A similar trend was observed when the tobramycin to diclofenac ratio was increased to 4:1 w/w. Overall results suggest that spray drying with 2FN showed a superior and viable approach to producing excipients-free inhalable co-spray dried formulations of tobramycin and diclofenac. However, the formulation produced using the 3FN showed higher enrichment of hydrophobic diclofenac and an ability to control the tobramycin drug release *in vitro*.

1. Introduction

Cystic fibrosis (CF) is a genetic disease caused by the mutation in the cystic fibrosis transmembrane conductance regulator (CFTR). The loss of CFTR functions in CF makes patients more susceptible to opportunistic bacteria such as *Pseudomonas aeruginosa* that lead to recurrent infections. Patients with CF suffer from progressive decline of lung functions caused by small airway obstruction and bronchiectasis that subsequently leads to low quality of life, morbidity and mortality (De Boeck, 2020; Elborn, 2016). One of the standard treatment regimens for the management of CF is a combination therapy of inhaled aminoglycosides (tobramycin) or macrolides (azithromycin) and oral non-steroidal anti-inflammatory drugs (NSAIDs) (ibuprofen) or corticosteroids (Sheikh et al., 2021a). However, this treatment regimen uses two different routes (oral and inhalation) and therefore it is often cumbersome in terms of administration and potentially leads to low patient compliance. A higher level of compliance is expected if antibiotics and anti-inflammatory drugs are co-delivered as a combination therapy *via* a single administration. Drug delivery *via* inhalation is an attractive strategy against respiratory infections, allowing direct drug deposition at the diseased site while minimising systemic absorption and potential toxicity associated with high systemic doses.

Tobramycin is a water-soluble cationic molecule that exerts antibiotic and toxic effects *via* its positive charge. When delivered by the inhalation route, tobramycin shows low epithelial permeability (due to poor lipid solubility), high bronchial secretion retention and low

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systemic bioavailability thus minimising systemic side effects (ototoxicity and nephrotoxicity) as compared to intravenous administration (Akkerman-Nijland et al., 2021; Govaerts et al., 1990; Wargo and Edwards, 2014). In the case of CF, inflammation is highly prevalent that could further worsen lung functions. Therefore, it is important to overcome infection-induced hyper-inflammatory conditions which could be achieved by using oral anti-inflammatory drugs such as NSAIDs. A meta-analysis of existing clinical information in younger patients with CF has revealed that a high dose of ibuprofen slows the deterioration and destruction of lungs (Lands and Dauletbaev, 2010; Lands and Stanojevic, 2019). However, there are growing concerns about the use of oral ibuprofen in chronic CF due to the high dosage regimen and potential side effects such as gastrointestinal bleeding and abdominal pain. High-dose ibuprofen when combined with aminoglycosides is also often associated with acute renal toxicity. Therefore, it is important to identify safer alternative NSAIDs to reduce the inflammation in the lungs.

On the other hand, diclofenac is a strong oral anti-inflammatory drug with the potential to be repurposed as an alternative NSAID in CF. To bypass the potential gastrointestinal side effects from oral diclofenac and enhance local (lungs) availability, inhalation is a viable delivery option. Our previous work demonstrated that low-dose inhalable diclofenac (150 μg/dose) delivered *via* a pressurised metered-dose inhaler (pMDI) could efficiently reduce inflammation in both healthy (NuLi-1) and CF (CuFi-1) cell models (Sheikh et al., 2021b). While aerosolization of diclofenac *via* pMDI could be a viable option, pMDI often has some limitations. For instance, pMDI may contain additional components such as propellants [Hydrofluoroalkane (HFA)] and excipients (i.e., ethanol). While HFA does not deplete the ozone layer, they are still considered a potent greenhouse gas with the potential for global warming effect (Wilkinson et al., 2019). Therefore, it is imperative to move towards substitution of the current propellent with a lesser carbon footprint or use an alternative approach of inhalable delivery that could reduce the environmental impact. A dry powder inhaler (DPI) is a potential alternative and viable approach for drug delivery to the lungs as it incorporates the majority of powder within the respirable range (<5 μm). In contrast to pMDI, DPI is breath actuated and mainly driven by the patient's inspiratory flow. Being highly stable powders, DPI can be delivered as a propellent and excipients-free formulation.

Despite recent advances in particle engineering techniques, there are still challenges to produce dry powders that satisfy the aerodynamic properties for effective aerosolization and enhanced deposition. This is especially problematic with combination drugs that have large differences in aqueous solubility. Spray-drying is a popular particle-engineering technique for dry powder production as it enables efficient control of the particle sizes and surface morphology making them suitable for inhalation. The spray-drying process involves atomisation of the liquid feed into fine droplets, followed by immediate drying of these droplets in the atmosphere of heated air and subsequently collection of the dried powders facilitated by the streams of the exhaust gas. Various processing parameters including inlet temperature, feed flow rate, drug concentration and atomisation typically guide the physiochemical properties of the dry powder. However, the traditional spray-drying equipped with a 2-fluid nozzle (2FN) is mainly suitable for drugs or excipients with similar solubility profiles, and it is less suitable for spray-drying compounds with varying solubilities. The 2FN uses only one liquid channel where a single feed suspension or solution can be delivered to the atomiser at a time. While it may be possible to dissolve the compounds with varying solubilities in a mixture of miscible organic and aqueous solvents, this approach is less eco-friendly as it may require a large concentration of organic solvents. In addition, the possibilities of drug precipitation, low yield and uncontrolled particle formation make it unsuitable for bulk manufacturing. Alternatively, a 3-fluid nozzle (3FN) that consists of two different feed channels for simultaneous delivery of two different solution feeds could be used to circumvent the above issue (Shetty et al., 2020). In a 3FN, the pumped liquid feeds are met at the tip of the nozzle and undergo transient mixing between the solvents for miscible solvents or surface coverage of the inner feed by the outer feed in non-miscible or slightly miscible solvents. Since the exact volume of organic solvents can be titrated and controlled to meet at the tip during particle formulations, 3FN may decrease the use of a large volume of organic solvents and energy. The mixing of drugs and excipients at the tip of the nozzle makes them suitable to control drug encapsulation with efficient control of coating materials to control drug distribution on the surface of the particles followed by drug release.

Building upon the above hypothesis, the main aim of this study is to develop a co-spray dried inhalable powder formulation of tobramycin and diclofenac with a high aerosolization efficiency using traditional 2FN and compared with the newer 3FN. We hypothesised that using 3FN, diclofenac (hydrophobic) could be fed from the outer channel while tobramycin could be fed from the inner channel that could make a fine particle with a majority of the diclofenac molecule on the surface. This unique approach is expected to facilitate the faster release of the hydrophobic diclofenac to control the inflammation and sustained release of tobramycin (hydrophilic) for prolonged antibiotic effect. A series of formulations were co-spray dried using traditional 2FN and 3FN to generate microparticles and their aerosolization behaviour, morphology, and release characteristics were compared. Formulating DPI-based inhalable diclofenac and tobramycin as a combination therapy, delivered by a single unit device could provide a new and convenient therapeutic option for CF.

2. Materials and methods

Tobramycin and diclofenac sodium were purchased from Hangzhou ICH Biofarm Co. Ltd (Hangzhou, China). Sodium phosphate dibasic, Tris base (Tris(hydroxymethyl)aminomethane), 1-Fluoro-2,4-dinitrobenzene, acetonitrile, ethanol, methanol and phosphoric acid were purchased from Sigma Aldrich (Australia). Phosphate buffer saline (PBS) was purchased from Gibco, Invitrogen (Australia). All solvents used were of analytical grade.

2.1. Preparation of spray-dried powder

The combination spray-dried formulations of tobramycin and diclofenac dry powder were prepared at weight ratios of tobramycin to diclofenac (2:1 w/w and 4:1 w/w) using a Buchii mini spray dryer B-290 (Buchi Laboratories, Flawil, Switzerland). The spray-dried formulations were prepared by atomizing the liquid feed using two-fluid (2FN) and three-fluid (3FN) nozzles in the stream of hot air. The spray-dried individual drugs (tobramycin and diclofenac) were prepared using a 2FN nozzle for comparison. The spray-drying yield is expressed in w/w % and was calculated by dividing the total mass of the drug introduced in the feed solution by the total mass of the spray-dried powder collected in the sample collector.

2.1.1. Two-fluid nozzle

Tobramycin (1.6 g and 1.33 g) were separately dissolved in 80 mL of 100% v/v Milli-Q water and diclofenac (0.4 g and 0.67 g) was separately dissolved in 20 mL of 100% v/v ethanol. Both drugs were mixed at predetermined ratios to make up to 100 mL and sonicated for 10 min at room temperature. The final solid concentration (drugs) in the solution was maintained at 2.0% w/v to ensure that the hydrophobic diclofenac remained solubilised in the mixture without precipitation. Two batches of the co-spray dried powder of tobramycin and diclofenac at 2:1 and 4:1 referred as 2FN (2:1) or 2FN (4:1) were prepared. The mixed ethanolic solution was spray-dried using a 2FN. The spray drying conditions for 2FN were as follows: open mode, inlet temperature 130 °C, outlet temperature 78–80 °C, feed rate 3 mL/min, aspiration 100% (40 m^3/h) and atomiser setting at 50 mm (~600 L/h). The spray-drying settings along with feed rate were selected based on our previous results (Parlati et al., 2009).

2.1.2. Three-fluid nozzle

Tobramycin (4.0 g) was dissolved in 200 mL of 100% v/v Milli-Q water and diclofenac (either 1 or 2 g) was separately dissolved in 200 mL of 20% v/v ethanol solution. Both drugs tobramycin (100% v/v aqueous solution) and diclofenac (20% v/v ethanol) were separately fed using different channels at pre-determined drug ratios in three fluid nozzles (3FN). Tobramycin was pumped from the inner channel and diclofenac from the outer channel with a total feed of 3 mL/min. This ensured that during the formation of droplets, tobramycin may effectively remain in the inner core with the majority of the diclofenac remaining on the surface of the tobramycin. All other conditions in the spray dryer were kept similar to 2FN. Two batches of co-spray dried powder of tobramycin and diclofenac at a ratio of either 2:1 or 4:1 were prepared, which were referred to as 3FN (2:1) or 3FN (4:1) throughout the manuscript.

2.2. Drug content analysis

High-pressure liquid chromatography (Shimadzu, Japan) was used to chemically quantify the content of tobramycin and diclofenac in the formulations. The HPLC system was equipped with SPD-20A UV–Vis detector, an LC 20AT liquid chromatography system, a SIL-10 A HT autosampler (Shimadzu) and HyperClone C-18 column (250 × 4.6 mm, 5 μm, Phenomenex, Torrance, USA).

Tobramycin is a highly polar compound and lacks UV absorbing chromophore, therefore, a pre-column derivatization procedure with slight modification from USP 31 was used for the preparation of the HPLC samples (Zhu et al., 2016). In brief, 125 μL of reagent A (30 mg of Tris base, 2 mL of water and 8 mL DMSO) and 125 μL of reagent B (5 mg/mL of 1-Fluro-2,4-dinitrobenzene in ethanol) was added to 50 μL of tobramycin to initiate the reaction. The reaction was carried out at a constant 60 °C for 1 h in a block heater. The reaction was seized by placing reaction vials in the ice, and 350 μL of acetonitrile was added to the reaction mixture before being subjected to analysis. The mobile phase was prepared freshly using 1 g Tris base, 10 mL of 1 N sulfuric acid, 450 mL water and 550 mL acetonitrile. The injection volume was 50 μL and the drug compound was eluted at 0.8 mL/min from HyperClone ODS C18 120A. The tobramycin was detected at a wavelength of 365 nm. A linear calibration curve between 10 and 200 μg/mL (R^2 = 0.99) was obtained and used for quantification of the drug content.

Diclofenac was quantified using a mobile phase mixture of methanol: phosphate buffer (0.8 g/L sodium dihydrogen phosphate) at a ratio of 70:30 v/v (pH 4.3, adjusted using phosphoric acid). The injection volume was 20 μL and the flow rate of the mobile phase was adjusted to 1.5 mL/min. Diclofenac was detected at a wavelength of 284 nm and a well correlated linear calibration curve at concentrations between 0.1 and 100 μg/mL (R^2 = 0.99) was used to quantify the amount of diclofenac. The limit of detection (LoD) and limit of quantification (LoQ) for diclofenac was calculated as 0.0025 and 0.00825 μg/mL, respectively. Similarly, the LoQ and LoD for tobramycin were calculated as 0.5 and 1.65 μg/mL, respectively.

2.3. Powder density

Bulk and tapped densities of the spray-dried powder were determined by slight modification from the previously reported method (Eedara et al., 2016). Powder (~0.75–1 g) was filled in a 5 mL measuring cylinder through a linear scale increment of 0.2 mL. The volume occupied by the powder in a measuring cylinder was recorded as a bulk volume. To determine the tapped volume, the cylinder was tapped on a flat bench at 100 taps/min until the change in powder volume was constant. The bulk and tapped density were further determined by dividing the mass of the powder by its volume before and after tapping.

2.4. Scanning electron microscopy

The surface morphology of the co-spray dried powders was imaged using a bench-top scanning electron microscope (JCM-6000PLUS Neoscope, JEOL, Japan). Before imaging, 2 to 5 mg of samples were deposited onto the carbon tape mounted on an aluminium stub and coated with a 15 nm gold layer using a sputter coater (Smart Coater, JEOL, Japan). Powder samples were mounted in carbon tape and coated with 15 nm thickness for 2 min. Images were taken at different magnifications at an accelerating voltage of 15 kV.

2.5. X-ray diffraction

The X-ray diffraction (XRD) pattern of raw diclofenac, raw tobramycin and co-spray dried powders was assessed using a Panalytical Aeris (Malvern Panalytical Ltd, Malvern, UK) equipment at a room temperature. The diffraction patterns were recorded with an angular increment of 0.022° C/s over diffraction angle (2θ) in the range of 5–40 °C using a cobalt X-ray source at a voltage of 15 kV and 40 mA current.

2.6. Particle size distribution by laser diffraction

The size distribution of the powder samples was analysed by laser diffraction technique using a Mastersizer 3000 (Malvern Instruments, Malvern, UK) combined with a dry powder dispersion system (Malvern Aero S). A sample amount of 10–20 mg was passed through the Scirocco dry powder feeding cells at a dispersive compressed air pressure of 2.6 bar, obscuration between 0.1% and 15% and a vibration feed rate at 30%. Volume weighted particle distribution that represented as D10, D50 and D90 as 10%, 50% and 90%, respectively of the cumulative total percentage of populations were calculated from built-in software. The dispersity of the particle population was represented by the span value using the following equation.

$$Span\ \ value = (D90 - D10)/D50$$

2.7. Energy dispersive X-ray analysis

Elemental analysis of the powder was carried out by energy dispersive X-ray (EdX) spectroscopy using field-emission scanning electron microscope (FE-SEM) (Nova NanoSEM450, FEI, USA) equipped with Bruker X-Flash 6|30 detector. Briefly, the powder samples were mounted on copper tape and coated with platinum at a thickness of 10 nm before analysis. An acceleration voltage of 15 kV and a spot size of 4 was used during the imaging of samples. The elemental percentage was calculated using Bruker Esprit 1.9 software and average readings from 10 points are reported.

2.8. Differential scanning calorimetry

The thermal property of the powders was scanned by using differential scanning calorimetry (Mettler-Toledo DSC 823e, Schwerzenbach, Switzerland). Samples (~5–10 mg) were placed in aluminium pans, sealed and lids were pierced. The aluminium pan was heated at a rate of 10 °C/min over the range of 25–440 °C under a nitrogen environment. An empty aluminium pan was heated as a reference sample. Nitrogen was directly connected to the flow controller to maintain the nitrogen level in the system (dry gas) and oven (purge gas) to approx. 30 mbar and 10–20 mbar, respectively. DSC data were analysed using STARe software version 11.00a (Build 4393) (Mettler-Toledo, Schwerzenbach, Switzerland).

2.9. Dynamic vapour sorption

The moisture sorption capacity of the powders was assessed by dynamic vapor sorption (DVS-1 Intrinsic, Surface Measurement Systems

Ltd.). Samples (10–30 mg) were placed in a pan and the experiment was set at 0% relative humidity (RH) as a baseline at isothermal conditions (25 °C). The samples were then exposed to 2 sorption and desorption cycles from 0 to 90% RH with 10% increments, and the change in mass over time was recorded. The equilibrium moisture content at each step was determined by a change in mass to time ratio (dm/dt) of 0.0005% per minute.

2.10. In vitro aerodynamic performance

Pulmonary deposition profiles of the co-spray dried formulations were assessed using a next-generation impactor (NGI®, Apparatus X, Copley, Nottingham, UK) as per United States Pharmacopoeia (USP). To ensure minimal particle bounce, the throat and stages of the NGI were coated with a mixture of Brij 35 (1 g), ethanol (4 mL) and glycerol (5 mL). The airflow rate was adjusted to 60 L/min using a pump and calibrated flow meter (TSI 4040; TSI Instruments Ltd., USA). Gelatin capsule (size 3, Capsugel®, Sydney, Australia) was filled with powder (~20 ± 2 mg) and loaded into the dosage chamber of a low-resistance dry powder inhaler device (DPI, RS01, Plastiape, Osnago, Italy). The inhaler was further connected to a 3D printed mouthpiece adaptor and USP induction port. The capsule was pierced, and powders were actuated for a period of 4 s corresponding to one breath. Following actuation, the powder deposited in the device, capsule, USP throat, stages (1–7) and micro-orifice contactor (MOC) were collected by rinsing with a mixture of methanol and water at 70:30 v/v%. The samples were further diluted and filtered with a 0.45 μm nylon filter before the contents of the drug were quantified by HPLC. The aerosolization performance of the powders was tested in triplicates. The emitted dose was calculated as the total amount of drug recovered minus the drug retained in the device and capsule. Fine particle fractions (FPF) (correspond to the ratio of the total mass of the emitted drug below 5 μm to the delivered dose), mass median aerodynamic diameter (MMAD), geometric standard deviation (GSD) were calculated using NGI results from Copley Inhaler Testing Data Analysis Software (CITDAS) software version 3.1 (Copley, Nottingham, UK). The cut-off size of stages 1, 2, 3, 4, 5, 6 and 7 of the NGI at 60 L/min are 8.06 μm, 4.46 μm, 2.82 μm, 1.66 μm, 0.94 μm, 0.550 μm and 0.340 μm, respectively.

2.11. Dissolution studies

The dissolution profiles of the various spray-dried microparticles were investigated with a slight modification of established procedures (Chan et al., 2013; Parlati et al., 2009) using Franz dissolution cells equipped with heated water jackets that are mounted under a heated station stirrer (V6B, permeGear, Inc, Bethlehem, USA). In brief, the cells were filled with 22.7 mL of phosphate buffer saline (PBS) as a dissolution media and a paper filter (Nitrocellulose 0.45 μm, MF^{TM} membrane filters, Millipore Bedford, USA) pre-soaked in PBS was secured between the donor and receptor compartments. The temperature of the media was maintained at 37 ± 0.5 °C throughout the experiment using a circulating warm water peristaltic pump (Carter-Manostat) at 5 mL/min and stirred at a constant speed using a magnetic stir bar. The system was equilibrated for approximately 30 min prior to the experiment. The powder samples (~40 mg) were evenly distributed on the donor side of the membrane. A complete dissolution of the co-spray dried formulation of tobramycin and diclofenac at both ratios (2:1 and 4:1) in dissolution media (22.7 mL PBS) ensured <10% of total saturation in the media for both drugs confirming total sink conditions (Durairaj et al., 2009; Parlati et al., 2009). The samples (1 mL) were taken from the receiver compartment at pre-determined time intervals and replaced with an equal volume of pre-heated PBS (37 ± 0.5 °C). After the last sampling point, the filter membrane was removed and washed with 5 mL PBS at the apical side which allowed exact quantification of the remaining content on the apical side of the membrane. The dissolution experiments were carried out in triplicates for each formulation, and drug release was quantified by established HPLC assays as described above. The similarity (f_2) factor in dissolution profiles was calculated as previously reported by using an excel add-in program "DDsolver" (Zhang et al., 2010). The f_2 values between 50 and 100 are considered as sameness or equivalence between two profiles, respectively (Diaz et al., 2016).

2.12. Statistical analysis

Data was analysed either by one-way analysis of variance (ANOVA) followed by Tukey posthoc multiple comparisons or independent student *t*-test using Graph pad prism (Graph pad Software version 9.3.1, CA, USA). A p-value < 0.05 was considered statistically significant. Unless stated otherwise, experiments were performed in triplicates and values expressed as mean ± standard deviation.

3. Results

3.1. Spray dried powder preparation-evaluation of process yield, density and total drug content

Four batches of co-spray dried tobramycin and diclofenac at 2:1 and 4:1 w/w ratios were prepared using either 2FN or 3FN (Table 1). The resultant spray drying yield obtained were between 63 and 80% (Table 2). Diclofenac content was shown to influence the total yield. In both nozzles, the yield obtained after co-spray drying tobramycin and diclofenac at 2:1 ratio was relatively higher than 4:1. Similarly, the 2FN formulation showed a higher yield than 3FN when co-spray dried at 4:1 ratio. The batch size used in the study for the 2FN nozzle was moderately lower (100 mL) than 3FN (200 mL). The higher batch size (200 mL) for 3FN was used to match the diclofenac concentration (20% w/v ethanolic solution) from the outer nozzle in 3FN. Despite using twice the batch size for 3FN compared to 2FN, the final yield at a 2:1 ratio was similar for both the nozzles, showing no impact of batch sizes. The choice of nozzles was found to influence the density of the powder. Powders prepared *via* 3FN showed a significantly high bulk and tapped density than powders prepared via 2FN (Table 2). The lower bulk density of powders from 2FN is attributed to the higher solvent evaporation rate and formation of porous particles (Fig. 1A and B). Interestingly, powder density remained unchanged when the ratio of tobramycin was increased for both 2FN and 3FN. The actual drug content in all co-spray dried powder was closed to its theoretical amount, further indicating a minimum drug loss during spray drying (Table 3). To further understand the influence of co-spray drying of hydrophilic (tobramycin) and hydrophobic (diclofenac) drugs *via* 2FN and 3FN, each formulation was assessed for their physicochemical properties.

3.2. Scanning electron microscopy

The representative scanning electron micrographs of the spray-dried powders prepared using 2FN and 3FN are shown in Fig. 1 (A-D). In general, particles were heterogeneous, and spherical with corrugated morphologies regardless of the production process and drug ratios. Interestingly, particles prepared *via* 2FN at a lower tobramycin concentration 2FN (2:1) showed a mixture of both smooth and corrugate

Table 1
Composition of the stock and final feed concentration of each formulation.

Nozzle-type	Formulation	Tobramycin (% w/v)	Diclofenac (% w/v)	Final feed concentration (% w/v)
Two-fluid	2FN (2:1)	1.33	0.67	2
Two-fluid	2FN (4:1)	1.6	0.40	2
Three-fluid	3FN (2:1)	2.0	1.0	3
Three-fluid	2FN (4:1)	2.0	0.5	2.5

Table 2
Spray-drying powders yield and their corresponding densities (Mean ± SD, n = 3).

	Powder	Density (g/cm^3)	
	Yield (%)	Bulk	Tapped
2FN (2:1)	80	0.215 ± 0.008	0.331 ± 0.002
2FN (4:1)	75	0.216 ± 0.002	0.337 ± 0.008
3FN (2:1)	80	0.299 ± 0.003	0.423 ± 0.008
3FN (4:1)	63	0.303 ± 0.003	0.438 ± 0.016

surface morphologies (Fig. 1A).

3.3. X-ray diffraction

The powder XRD of raw diclofenac and tobramycin and their co-spray dried powders are presented in Fig. 2. Raw tobramycin and diclofenac exhibited a highly crystalline nature as indicated by numerous high-intensity intrinsic peaks at different angles from 7.5 to 40°. The 3FN formulations showed intrinsic characteristics peaks of mostly diclofenac, but at a reduced intensity indicating marginal crystallinity. In contrast, the 2FN formulations showed an absence of the characteristic crystalline peaks from both drugs, indicating the formation of amorphous material.

3.4. Particle size analysis

The particle size distribution as measured by laser diffraction of co-spray dried formulations is depicted in Table 4. Overall, all formulations showed monomodal distribution with the majority of particles falling within the inhalable range (<5 μm). A smaller peak (Fig. 1E) ranging from 40 to 100 μm of <0.9% and 0.2% by volume were observed in all formulations indicating some degree of particle aggregation. The particle size of powders prepared *via* 2FN was significantly smaller than

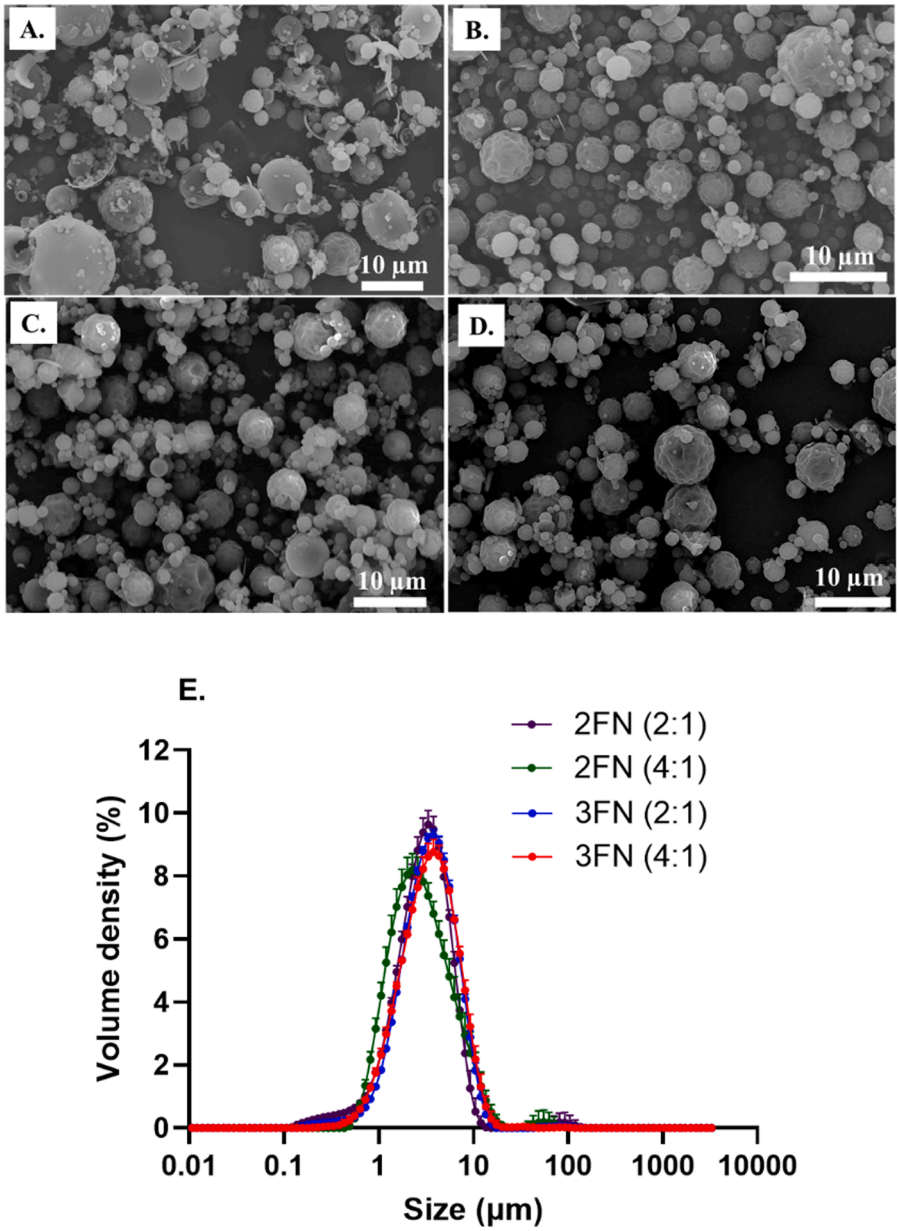

Fig. 1. Representative scanning electron microscopy images of different ratios of co-spray dried formulations of tobramycin and diclofenac prepared using **A.** 2FN (2:1) (Magnification ×1700) **B.** 2FN (4:1) (Magnification ×2700) **C.** 3FN (2:1) (Magnification ×2000) and **D.** 3FN (4:1) (Magnification ×2000). **E.** Particle size distribution of **A-D** as measured by laser diffraction (Mean ± SD; n = 5).

Table 3
The total amount of drug recovered from co-spray dried powders of tobramycin and diclofenac prepared at a different ratio by either 2FN or 3FN nozzles versus theoretical drug content in the formulations (Mean ± SD, $n = 5$).

	Tobramycin (mg)		Diclofenac (mg)	
Theoretical		Actual	Theoretical	Actual
2FN (2:1)	13.33	12.30 ± 0.45	6.67	6.46 ± 0.34
2FN (4:1)	16.00	16.40 ± 0.02	4.00	3.90 ± 0.30
3FN (2:1)	13.33	13.25 ± 0.24	6.67	6.73 ± 0.33
3FN (4:1)	16.00	15.10 ± 0.83	4.00	4.16 ± 0.38

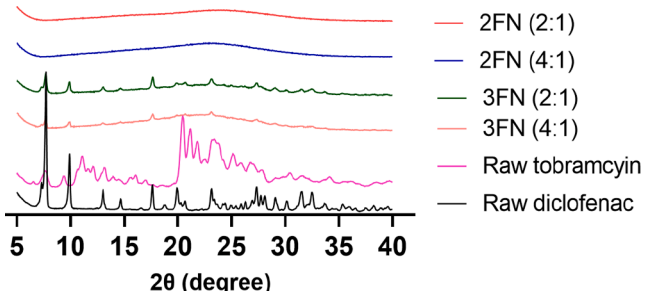

Fig. 2. X-ray powder diffraction pattern of raw diclofenac, raw tobramycin and co-spray dried formulations of tobramycin and diclofenac prepared at different ratios using either 2FN or 3FN.

Table 4
Size distribution of co-spray dried tobramycin and diclofenac powders (Mean ± SD, $n = 5$).

	D10 (μm)	D50 (μm)	D90 (μm)	Span
2FN (2:1)	1.14 ± 0.052	3.01 ± 0.06	6.26 ± 0.37	1.70
2FN (4:1)	1.13 ± 0.045	2.66 ± 0.20	7.42 ± 0.85	2.36
3FN (2:1)	1.38 ± 0.023	3.42 ± 0.02	7.37 ± 0.18	1.75
3FN (4:1)	1.32 ± 0.012	3.43 ± 0.06	7.67 ± 0.34	1.85

3FN. For instance, D50 (particle size distribution within 50th percentile) for 2FN (2:1) and 3FN (2:1) was approximately 3.01 ± 0.06 μm and 3.42 ± 0.02 μm, respectively. Similarly, a significant difference in D10 (particle size distribution within 10th percentile) was observed for all formulations.

For 2FN formulations, the mode of size distribution as indicated by its span value was dependent on the drug ratio (tobramycin to diclofenac). For instance, span values for 2FN (2:1) and 2FN (4:1) were approximately 1.7 and 2.36, respectively. Formulation with a higher amount of hydrophilic tobramycin displayed a more heterogeneous dispersion of powders. In contrast, for 3FN, tobramycin amount had little or no effect on particle size distribution.

3.5. Elemental analysis by energy dispersive X-ray analysis

The percentage distribution of the two elements (sodium and chlorine) by weight and atomic percentage as obtained from X-ray dispersive spectroscopy is shown in Table 5. EdX is a powerful tool that maps and quantifies the distribution of the surface elements up to the depth of 1–2 μm with a high spatial resolution. The atomic and weight percentages of five major surface elements (nitrogen, oxygen, carbon, sodium, and chlorine) on the surface of the spray-dried powder were evaluated. Among these elements, sodium and chlorine which are distinctly present in diclofenac powder but not present in tobramycin were used for our analysis (Table 5). Typically, a linear increase in sodium and chlorine percentages was observed when the diclofenac ratio increased in the formulations, regardless of its nozzle types (Table 5). As expected in both nozzles, a higher proportion of sodium or chlorine (atomic and weight %) was present when the co-spray dried powder of tobramycin to diclofenac was prepared at 2:1 than 4:1. Similarly, based on chloride percentage in 2FN (4:1) and 3FN (4:1), a decrease in surface enrichment of diclofenac was observed.

Table 5
Elemental composition of dry powder formulations expressed as average atomic (At), and weight (Wt) percentage calculated from EDX analysis of 10 random points in each sample.

	Sodium (%)		Chlorine (%)	
	Wt	At	Wt	At
Diclofenac (raw)	4.3	4.8	83.4	69.0
Tobramycin (raw)	0.0	0.0	0.0	0.0
2FN (2:1)	4.6	3.3	33.1	15.9
2FN (4:1)	2.6	1.6	18.5	7.5
3FN (2:1)	7.8	6.2	42.7	22.4
3FN (4:1)	3.2	2.0	13.8	5.8

3.6. Differential scanning calorimetry

The influence of heat flow on the thermal properties of the spray-dried powders is shown in Fig. 3. Spray-dried diclofenac sodium showed a single sharp endothermic peak at 292 °C, close to the melting point of raw diclofenac sodium (approx. 290 °C) (Balogh et al., 2015) and a sharp exothermic peak at 306 °C due to degradation. Spray-dried tobramycin showed a broad endothermic peak at 97.5 °C and a small endothermic peak at 140 °C which may be attributed to the phase transition of amorphous tobramycin. The broad exothermic peak of tobramycin at 301 °C was most likely attributed to oxidative degradation of tobramycin.

In general, all formulations showed characteristic broad endothermic peaks similar to spray-dried tobramycin ranging from 25 to 153 °C due to the glass transition (Tg), suggesting the co-spray dried formulations were in an amorphous state.

Particularly, for 2FN, the broad endothermic peak was observed at ~ 25–153 °C, which could be a residual peak from the solvent or related to the differential pattern of diclofenac distribution. This peak was followed by an exothermic peak at ~ 247 and 260 °C for 2FN (2:1) and 2FN (4:1), respectively. However, for 3FN (2:1), the broad endothermic peak was observed at ~ 60–90 °C. This peak was followed by low-intensity endothermal peaks at 133 and 145 °C before degradation at 254 °C. In contrast, the 3FN (4:1) showed a broader endothermic peak at ~ 25–125 °C and a similar degradation profile to 3FN (2:1).

3.7. Dynamic vapor sorption

To determine how the moisture affects the powder properties,

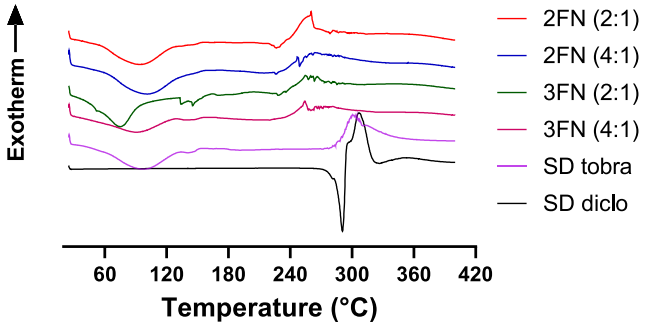

Fig. 3. Differential scanning calorimetry thermograms of co-spray dried formulations of tobramycin and diclofenac prepared at different ratios using either 2FN or 3FN. For comparison, tobramycin and diclofenac were individually spray-dried using 2FN (SD). SD tobra- Spray-dried tobramycin; SD diclo- Spray-dried diclofenac.

moisture sorption profiles of individually and co-spray dried drugs powders were measured (Fig. 4). When individually spray-dried, tobramycin and diclofenac showed maximum mass changes at 90% RH of 18.5% and 20% w/w, respectively. Spray-dried diclofenac started to absorb water at 50% RH during the first sorption process and the moisture uptake was rapidly inclined at 5% and 20% RH. Notably, spray-dried tobramycin absorbed water vapour at a steady rate until 50% RH with a change in mass of 14% w/w. However, at 60% RH, the change in mass dropped to 13% w/w and thereafter the mass steadily rose to 18.5% at 90% RH. A similar pattern in a change in mass, however, at a higher RH (60%) was observed for co-spray dried tobramycin and diclofenac at a 4:1.

Overall, spray-dried powders showed a significant increase in their mass (15–23% w/w) at 90% RH level regardless of their compositions in formulations and nozzle types. In all samples, the sorption profile from the first cycle was not reversible possibly due to the removal of residual solvents during the first desorption cycle. The co-spray dried powder from 2FN showed similar moisture sorption profiles at 90% RH regardless of their drug ratio. In contrast, there was a difference in the moisture sorption capacity of the 3FN formulations. At 90% RH, 3FN (4:1) adsorbed lower amount of water vapour (mass increased by 15.5% w/w) compared to 3FN (2:1) (mass increased by 22% w/w).

3.8. *In vitro aerosol performance by the next generation impactor (NGI®)*

The deposition profiles and crucial *in vitro* aerosolization parameters of the co-spray dried powders of tobramycin and diclofenac in device/capsules, throat and NGI stages are depicted in Fig. 5 and Table 6,

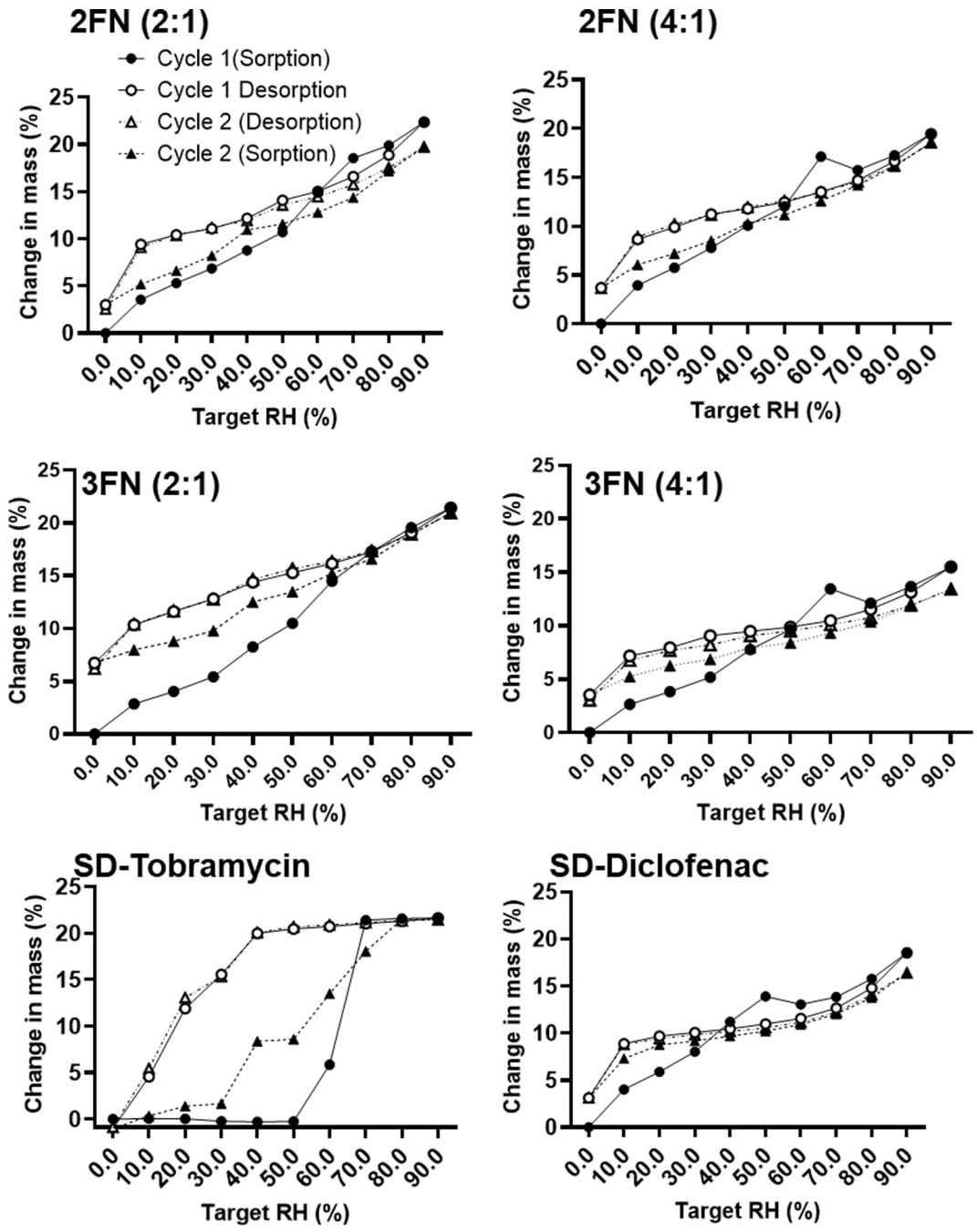

Fig. 4. Moisture sorption isotherms of spray-dried powders prepared by either using 2FN or 3FN. For comparison, drugs were spray-dried individually using a 2FN (SD-tobramycin and SD-diclofenac). All samples were exposed at 0 to 90% RH at 25 °C and the change in mass were recorded.

respectively. In general, nozzle types played a significant role in the aerosolization performance of the co-spray dried powders. The calculated emitted dose of all formulations was within the range of 78–85% and most powders were deposited from stages 3 to 7 as well as MOC. A large fraction of both drugs was recovered in 2FN formulations (~95%), while the recovery of diclofenac from 3FN formulations was comparatively lower.

The 2FN formulations showed statistically significant higher drug deposition at the lower stages (stage 3-MOC) than 3FN which could be correlated to higher FPF and lower MMAD of 2FN. The FPF of tobramycin and diclofenac prepared *via* 2FN were in the range of 61–64% and 54–66%, respectively. In contrast, 3FN formulations showed significantly reduced FPF of tobramycin and diclofenac and these values were in the range of 41–44% and 37–46%, respectively. The MMAD of dry power in 2FN formulations was significantly low as compared to 3FN (2.8–3.4 μm *vs* 3.8–4.4 μm) demonstrating its suitability for deep lung delivery. This suggests that the 3FN formulations likely form larger particles (Table 3) and following aerosolization, they are mostly deposited in the throat as evidenced from the deposition data (Fig. 5).

Table 6
Aerosolization performance of co-spray dried tobramycin and diclofenac powders prepared by either 2FN or 3FN using next-generation impactor (NGI®). Fine particle fraction (FPF), emitted dose (ED), median mass aerodynamic diameter (MMAD) and geometric standard deviation (GSD) were calculated based on NGI data (mean ± SD, $n = 3$). * Statistical analysis was performed using student's *t*-test and compared with similar formulations for both nozzles and $p < 0.05$ is considered significant.

Tobramycin	2FN (2:1)	2FN (4:1)	3FN (2:1)	3FN (4:1)
FPF [%]	61.1 ± 3.6	64.34 ± 2.7	40.6 ± 3.4*	44.2 ± 7.3*
MMAD [μm]	3.17 ± 0.2	3.15 ± 0.17	4.36 ± 0.25*	4.22 ± 0.1*
GSD	1.93 ± 0.1	1.85 ± 0.04	1.75 ± 0.00	1.79 ± 0.0
ED (%)	84.6 ± 1.2	80.6 ± 7.76	82.72 ± 3.65	80.0 ± 0.0
Recovery (%)	94.9 ± 2.7	96.3 ± 4.6	95.5 ± 2.3	84.9 ± 6.4
Diclofenac	**2FN (2:1)**	**2FN (4:1)**	**3FN (2:1)**	**3FN (4:1)**
FPF [%]	65.92 ± 3	54.4 ± 3.23	36.9 ± 0.84*	45.5 ± 4.1*
MMAD [μm]	2.85 ± 0.29	3.37 ± 0.41	4.09 ± 0.25*	3.75 ± 0.2
GSD	1.97 ± 0.1	1.93 ± 0.16	1.84 ± 0.05	1.92 ± 0.04
ED (%)	85.14 ± 0.4	80.2 ± 2.8	78.52 ± 5.75	84.02 ± 1.1
Recovery (%)	98.0 ± 6.5	96.33 ± 4.6	83.3 ± 4.2*	86.6 ± 3.2*

3.9. Dissolution profiles

Drug release/diffusion profiles for various co-spray dried powders of tobramycin and diclofenac are shown in Fig. 6. The similarity (f_2) in drug dissolution was compared and shown in Table 7. Overall, drug release from the co-spray dried powders was significantly dependent upon the diclofenac content in the formulation and the types of nozzles (Table 7). Diclofenac was released at a slow and sustained rate from 3FN formulations and at least 60–70% of the drug was released over 3 h (Fig. 6A and B). Increasing the diclofenac content in 2FN formulation was shown to increase its release.

For 3FN, tobramycin release was influenced by the types of nozzles

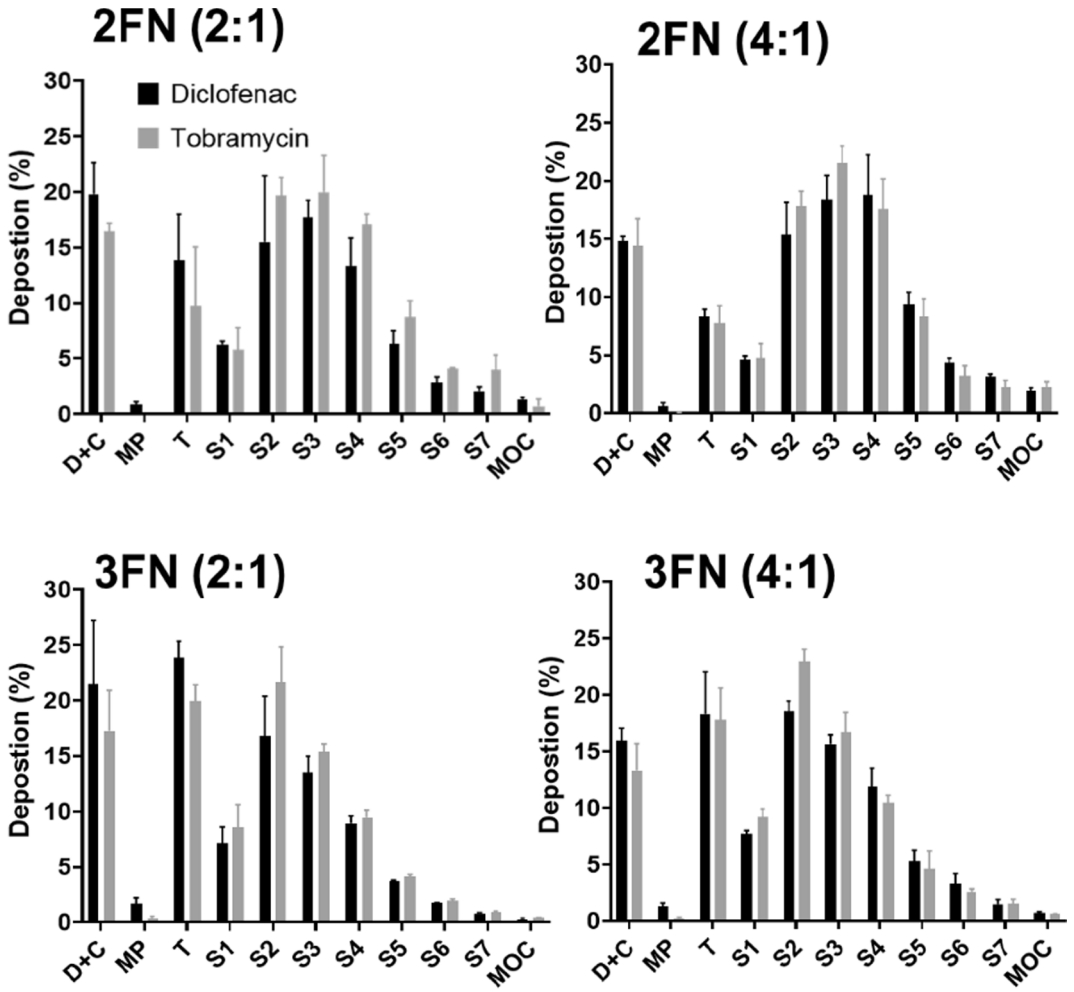

Fig. 5. Next-generation impactor stage deposition of co-spray dried tobramycin and diclofenac powders prepared by using two-fluid **A.** 2FN (2:1), **B.** 2FN (4:1) and three-fluid nozzles **C.** 3FN (2:1) and **D.** 3FN (4:1). (D + C = Device + capsule; MP = Mouthpiece; T = Throat; MOC-micro-orifice collector). (Mean ± SD, $n = 3$).

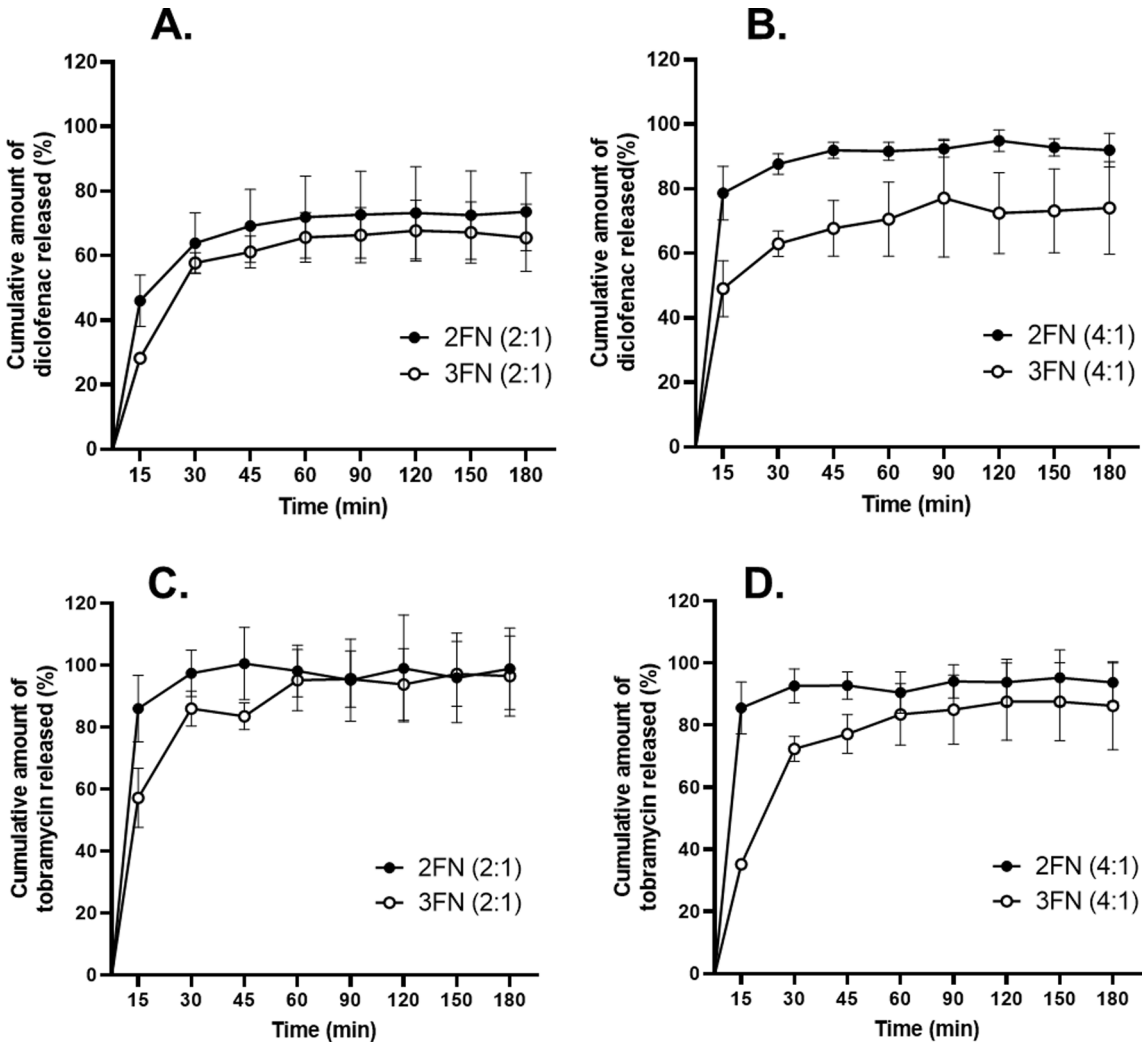

Fig. 6. Dissolution profiles of co-spray dried formulations of tobramycin and diclofenac at various ratios using either two-fluid or three-fluid nozzles. Cumulative release of diclofenac at various time intervals for **A**. 2FN or 3FN (2:1) and **B**. 2FN or 3FN (4:1). Cumulative release of tobramycin at various time intervals for **C**. 2FN or 3FN (2:1) and **D**. 2FN or 3FN (4:1). Data are expressed as mean ± SD ($n = 3$).

Table 7
Comparison of similarity (f_2) factor in the dissolution profiles of drugs released from the co-spray dried powders.

	2FN (2:1) *vs* 3FN (2:1)	2FN (4:1) *vs* 3FN (4:1)	2FN (2:1) *vs* 2FN (4:1)	3FN (2:1) *vs* 3FN (4:1)
Diclofenac	50.58	32.61*	31.91*	47.37*
Tobramycin	44.73*	34.02*	57.95	44.95*

*Represents the differences in dissolution profiles between formulations. Note: f_2 values between 50 and 100 are considered similar or equivalent.

and diclofenac content. A rapid burst release of tobramycin (80% within 15 min) was observed for 2FN formulations. In contrast, 3FN formulations showed comparatively slower (57% for 2FN and 35% for 3FN − 15 min) and sustained release of tobramycin (90% for 2FN and 80% for 3FN- 3 h).

4. Discussion

A series of excipients-free DPI formulations of tobramycin and diclofenac intended for combination therapy for CF were prepared using a mini spray dryer in conjunction with 2 different nozzles including a traditional 2FN and 3FN. The powder characteristics were tested and compared for their aerosolization performance. While excipients free spray-dried tobramycin has been previously studied (Pilcer et al., 2009), spray-dried preparations of diclofenac alone or its combination with tobramycin with different spray drying nozzles are yet to be explored. Herein, we prepared excipients-free inhalable particles with a maximum drug loading per particle to achieve higher local concentrations in the lungs and decrease the amount of inhalable dry powder that could be required to achieve therapeutic effects.

The spray drying yield was relatively lower when co-spray dried at a higher concentration of tobramycin. The yield was particularly low for 3FN formulation as compared to 2FN at 4:1. This discrepancy could be related to the differences in the drying kinetics of the solvents' mixtures when fed from different nozzles. Our observation of low yield from 3FN was consistent with Leng et al. who showed a higher yield for 2FN when compared to 3FN (Leng et al., 2018). It is well known that low-density particles (tapped density of 0.4–0.1 g/cm^3) are known to have better aerosolization performance that leads to higher lung deposition (Chvatal et al., 2019). The density of the co-spray dried formulations was found to be significantly influenced by the type of nozzles. The 2FN formulations had lower tapped density (within 0.4–0.1 g/cm^3) than 3FN suggesting the potential of 2FN formulations for better flowability and lung deposition upon aerosolization. The low density in the 2FN formulation is more likely attributed to the difference in feed distribution patterns in the nozzles. It is possible that the solvent feed from 2FN is evenly dispersed to form smaller dry particles, whereas in the 3FN formulations

a diclofenac 'shell' was formed on the tobramycin droplets, potentially delaying water evaporation, and forming larger particles. The co-spray dried particles showed good recovery of both drugs closer to their theoretical values, suggesting a minimal loss in the spray drying process. The high recovery of the drugs from co-spray powders could be attributed to the use of highly miscible solvents (ethanol and water) in the feed solutions for both nozzles.

As indicated from the laser diffraction data, the nozzle types influenced the particle size distribution of the powder. The 3FN generated larger particles than 2FN which could be related to a high vapour pressure created in the inner aqueous core fluid (tobramycin solution) of the formed droplets. Similar observations for 3FN forming larger particles as compared to 2FN were reported in previous studies (Leng et al., 2018; Maria Leena et al., 2020; Sunderland et al., 2015). After atomization of feed from 2FN, the stream of solvent droplets rapidly shrinks due to instantaneous evaporation of drugs to form dried particles. When binary mixtures of organic and aqueous solvents were used in the feed solution, the solvents carrying hydrophobic drugs tend to remain on the particle surface (Chan et al., 2013). In the case of 3FN, the nozzle was designed in such a way that the inner 100% v/v aqueous feed (tobramycin) was covered by the outer feed of 20% v/v solution of ethanol (diclofenac) that mixed at the tip of the nozzles, forming droplets that rapidly evaporated to form dry particles. This process tends to disperse the hydrophobic composites (diclofenac) over the surface of the hydrophilic compounds (tobramycin). Higher surface enrichment with diclofenac during the spray-drying process in conjunction with 3FN can be explained by its Peclet number (Vehring et al., 2007). A Peclet number is defined as a ratio of the solvent evaporation rate to the diffusivity of the solute. Generally, when solvent evaporation is faster than solute diffusivity, the solute tends to concentrate on the outer surface (Bhujbal et al., 2021). Usually, the addition of ethanol increases the Peclet number in the ethanol-water co-solvent system which results in faster evaporation and lower diffusion of a solute into the core allowing solute to be enriched on the surface of the particles. When two solvents met and form droplets in 3FN, the evaporation on the droplet surface is initiated at a much faster rate as 20% v/v ethanol with diclofenac feed supplied from the outer channel is dominant on the surface. During the drying process, the diclofenac concentration on the droplet surface may exceed the solubility or reach the supersaturation point which reduces the diffusivity of the diclofenac to the core thereby facilitating the accumulation of diclofenac on the outer surface of particles (Littringer et al., 2013). To confirm the spatial distribution of hydrophobic diclofenac on the surface of co-spray dried particles, an elemental analysis of sodium and chlorine was carried out. As predicted, the 3FN formulation showed a relatively higher surface intensity of sodium and chlorine than 2FN, which suggested that diclofenac was more likely to distribute on the surface of the 3FN formulation. In contrast, the low sodium and chlorine counts on the surface of 2FN formulations suggested that diclofenac appears to be evenly distributed with the tobramycin in the particles.

DSC thermograms of co-spray dried formulations (except diclofenac and 2FN) showed broad endothermal peaks suggesting the possibility of the formation of amorphous material, while evidence of a partial crystallinity was observed when diclofenac was dominant in the 3FN (2:1) compared to 3FN (4:1) powders. The XRD analysis further confirmed the formation of amorphous particles for the 2FN formulations and almost negligible crystallinity for the 3FN formulations. The change in the thermal activity of the formulations could be contributed by the change of drug degradation peaks from ~ 301 °C to ~ 255–280 °C, and the loss of the diclofenac endothermic melting peak at 290 °C in all co-spray dried formulations further suggested the potential of drug interactions. 2FN formulations showed larger endothermic peaks compared to 3FN, possibly related to the difference in the distribution patterns of hydrophobic diclofenac on the surface of the particles. Moisture sorption analysis of powders suggested that all spray-dried powders were hygroscopic. The individually spray-dried tobramycin showed a sudden decrease in mass (~1%) at 50% RH due to recrystallisation of the amorphous drug followed by a steady increase in its mass. Similarly, a decrease in mass at 60% RH was observed with co-spray dried formulations with a higher amount of tobramycin to diclofenac (4:1) demonstrating enhanced moisture stability of powder when co-spray dried with hydrophobic diclofenac. The mass decrease was likely caused by the lyotropic-induced phase transition of tobramycin from a rubbery metastable amorphous state to a crystalline phase leading to the expulsion of plasticizing water (Adi et al., 2008; Li et al., 2014). On the other hand, no such changes were observed for co-spray dried tobramycin and diclofenac at a 2:1 ratio, suggesting the formation of a stable powder. Thus, co-spray dried powders of tobramycin and diclofenac appear to enhance the moisture stability relative to individually spray-dried drug powders.

In cystic fibrosis, the bacterial loads and local inflammation are primarily dominant in the lower respiratory tract. Therefore, it is important to ensure that a high dose of the anti-inflammatory drug and antibiotic reaches the lungs to achieve maximum therapeutic benefit. For an optimum dry powder aerosol formulation, a high FPF is desirable as it guides the proportion of drugs deposited in the lungs. Aerosolization of 2FN formulations showed a higher FPF for both the drugs than 3FN formulations suggesting their suitability for deep lung delivery. This could be attributed to the smaller physical size of the 2FN formulations. Interestingly, the co-deposition profiles of both drugs were similar in all the stages for all formulations suggesting the homogenous dispersion of the drugs in the powder. This observation can be contributed by the use of compatible solvents (water and ethanol) in all formulations where the drugs are likely to be molecularly dispersed in the solvents, ensuring a uniform distribution of diclofenac and tobramycin within the particles. This result was further confirmed by Edx analysis of 2FN powders which showed an even pattern of diclofenac distribution within the tobramycin particles. In contrast, in the case of 3FN formulations diclofenac was mostly distributed on the surface. The similar MMAD profiles for both the drugs (diclofenac and tobramycin) within the same formulation ensure that deposition of the drugs is likely within the same region in the lungs following aerosolization.

Following impaction, dry powders deposited within the lungs are required to undergo complex drug absorption processes that include wetting, dissolution, and diffusion. Since both hydrophobic (diclofenac) and hydrophilic (tobramycin) drugs were co-spray dried, the formation of microparticles with different surface compositions (as proven from Edx data) was expected. As diclofenac is hydrophobic, slow dissolution of the drug was expected. While no standardised dissolution protocols are available for the inhalable formulation, the standard Franz cells as previously reported appear to be the most relevant existing model for drug release/diffusion assay (Chan et al., 2013; Parlati et al., 2009). Both diclofenac content and nozzle types contributed to the observed drug dissolution patterns from co-spray dried formulations. As compared to 2FN, both drugs dissolved at a slower rate than 3FN formulations. The slow dissolution rate of the 3FN formulations can be explained by the presence of larger particle size, partial crystallinity and higher surface enrichment of the hydrophobic diclofenac which led to slow wetting and dissolution. The initial burst effect of tobramycin from 2FN formulations could be a result of high surface coverage of the tobramycin as demonstrated from EdX analysis in the microparticles. Furthermore, the rapid drug dissolution rate presented by the 2FN formulations can be attributed to their amorphous particle morphology, small particle size and larger surface area for hydration.

5. Conclusions

This study examined the feasibility of formulating excipients free respirable co-spray dried powders of tobramycin and diclofenac, using traditional 2FN and newer 3FN, with their potential use as a combination therapy in CF. Among all the tested formulations, the 2FN formulations showed a superior aerosolization performance, with a higher FPF

value for both tobramycin and diclofenac, likely due to smaller particle size. Particle surface enrichment in 2FN with hydrophobic diclofenac and nozzle types influenced the drug release rate with a more rapid and burst release rate of drugs. As evidenced from Edx analysis, diclofenac was most likely distributed on the surface of 3FN formulations as compared to 2FN. Furthermore, diclofenac enrichment within the particles reduced moisture uptake and prevented drug recrystallisation at a higher RH suggesting enhanced stability of co-spray dried tobramycin and diclofenac powders at 2:1. Taken together, 2FN formulations at tobramycin and diclofenac at 2:1 appear to be the best formulations with respect to yield, drug deposition homogeneity, moisture stability, aerodynamic performance and drug release. Collectively, the obtained data further highlights the importance of selecting optimal nozzle and drug ratios (especially when co-spray drying hydrophilic and hydrophobic drugs) for the rationale design of inhalable formulations.

CRediT authorship contribution statement

Nirmal Marasini: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Zara Sheikh:** Investigation, Formal analysis, Writing – review & editing. **Chun Y.J. Wong:** Investigation, Formal analysis, Writing – review & editing. **Maryam Hosseini:** Investigation, Formal analysis, Writing – review & editing. **Patrick T. Spicer:** Formal analysis, Resources, Writing – review & editing. **Paul Young:** Resources, Software, Supervision, Project administration, Writing – review & editing. **Hui Xin Ong:** Conceptualization, Formal analysis, Resources, Software, Supervision, Project administration, Writing – review & editing. **Daniela Traini:** Conceptualization, Funding acquisition, Formal analysis, Resources, Software, Supervision, Project administration, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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