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Steroid-Decorated Antibiotic Microparticles for Inhaled Anti-Infective Therapy

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Abstract

Despite advances in vaccination and antimicrobial therapy, community-acquired pneumonia (CAP) remains as a leading cause of morbidity and mortality worldwide. As the severity of CAP has been linked to the extent of inflammation in the body, adjunctive therapeutic measures aimed at modulating the immune response have therefore become increasingly attractive in recent years. In particular, for CAP patients with underlying medical conditions such as chronic obstructive pulmonary disease (COPD), a steroid-antibiotic combination will no doubt be a useful and timely therapeutic intervention. Unfortunately, no combined steroid-antibiotic dry powder formulation is available commercially or has been reported in the academic literature. The aim of this work was hence to develop a novel steroid-antibiotic dry powder inhaler (DPI) formulation (ciprofloxacin hydrochloride (CIP) and beclomethasone dipropionate (BP)) for inhaled anti-infective therapy. The spray-dried powder was of respirable-size (d_{50} of ~2.3 μm), partially crystalline and had BP preferentially deposited on the particle surface. Favourably, when formulated as a binary mix, both CIP and BP showed much higher drug release and fine particle fractions (of the loaded dose) (FPF) over their singly-delivered counterparts, and had robust activity against the respiratory tract infection-causing bacteria *K. pneumoniae*, *P. aeruginosa* and *S. aureus.*

Keywords: Dry powder inhaler, Community-acquired pneumonia (CAP), Chronic obstructive pulmonary disease (COPD), Antimicrobial, Corticosteroids, Combinatorial therapy, Spray drying

1. Introduction

Community-acquired pneumonia (CAP) is an inflammatory condition of the lungs due to an infection arising from sources external to a hospital or extended-care facility (e.g. nursing home) setting. Although CAP can be brought about by bacteria, viruses or fungi, the most common cause of CAP in adults is still via a bacterial infection. The severity of CAP has been linked to the extent of inflammation in the body, as inflammation is the body's natural response to the invading infection-causing pathogens [1,2]. Despite advances in vaccination and antimicrobial therapy, CAP remains as a leading cause of human morbidity and mortality, and has afflicted the world population with a huge socio-economic cost [1-4].

Chronic obstructive pulmonary disease (COPD) is a pulmonary condition where the airways become chronically inflamed and irreversibly narrowed, hence leading to a shortness of breath. Furthermore, patients with COPD appear to be at a higher risk of developing CAP than patients in the general population [5,6]. Recent studies have reported on the impaired immunological mechanisms in COPD patients brought about by the defective alveolar macrophage phagocytosis of bacteria. This consequently increases the susceptibility of COPD patients to infections [7,8]. Moreover, COPD patients tend to develop persistent bacterial colonization in the lower airways through the inflammatory mechanism, hence leading to exacerbations of COPD and progression of airway obstruction [9,10]. Therefore, CAP patients with COPD frequently experience poorer clinical outcomes than patients without COPD. The former has been reported to exhibit higher 30- and 90-day mortality than the latter [11]. As these risks tend to increase with COPD severity, it is vital for CAP patients with COPD to seek medical intervention early to prevent COPD exacerbations and limit disease progression.

Currently, antibiotic treatment remains the mainstay of CAP therapy. However, patients with obstructive airways disease complicated by CAP could also benefit from the inclusion of inhaled corticosteroids into their treatment regimen in view of the drug's potent immunomodulatory and anti-inflammatory properties [3]. Although the release of inflammatory mediators from alveolar macrophages is useful for eliminating invading pathogens from pulmonary infections, excessive releases are on the contrary, harmful to the lungs. Hence, modulation of the inflammatory response is vital to creating a balance between the beneficial and harmful effects [12-14].

Corticosteroids are the most commonly used physiological inhibitors for inflammation. They interfere with the inflammatory expression and action that are associated with COPD and pneumonia. The inflammatory response in the lung is a complex process that involves the coordinated expression of both pro-inflammatory and anti-inflammatory cytokines [1]. Corticosteroids can switch off genes that encode pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-α), interleukin 1 beta (IL-1β) and interleukin-6 (IL-6), and switch on those that encode anti-inflammatory cytokines such as IL-10 [15-17]. For CAP patients with COPD, the down-regulation of the unwanted excessive cytokine response by corticosteroids has the beneficial effect of reducing excessive pulmonary inflammation and accelerating clinical recovery. In addition, bronchospasms (wheezing), a common condition found in these patients, could also be treated with the corticosteroids [3,18,19].

Understandably, clinicians have therefore applied the use of corticosteroids as an adjunctive therapy (i.e. anti-inflammatory properties) for different infectious diseases as early as the

1950s [20]. The use of corticosteroids in combination with antibiotics has been proven to be effective and safe in several randomized controlled trials (RCTs) in patients with bacterial and tuberculous meningitis [21-23], tuberculous pericarditis [24], septic arthritis [25] and septic shock [26]. In CAP-COPD patients, co-administration of corticosteroids with antibiotics has been shown to be safe and efficacious, and is therefore frequently administered by the physician for treatment [2,3]. On a further note, for smokers who are predisposed to COPD and bacterial infections (i.e. tobacco smoke compromise the anti-bacterial function of leukocytes, including neutrophils, monocytes, T cells and B cells) [6], treatment via the steroid-antibiotic combination will no doubt be a useful and timely therapeutic intervention as well. Unfortunately, no combined steroid-antibiotic dry powder formulation is available commercially or has been reported in the academic literature.

Hence, the objective of this study is to pioneer, via state-of-the-art nanospray drying [27,28], a novel inhalable dry powder formulation incorporating both an antibiotic and a corticosteroid for the treatment of bacterial infections in CAP-COPD patients. A dry powder inhaler (DPI) formulation is favoured over the other aerosol delivery modes (e.g. nebuliser or metered dose inhaler (MDI)) due to the improved formulation stability associated with the powdered drug (as compared to the solution or suspension in the MDI and nebuliser), improved delivery efficiency, portability, ease-of-use and the avoidance of undesired precipitation in solutions (e.g. in the nebuliser) [29-32].

Ciprofloxacin HCl (CIP) and beclomethasone dipropionate (BP) were selected as the model antibiotic and corticosteroid in view of their broad-spectrum activity against both Gram-

negative and Gram-positive bacteria (especially in CAP [4]), and robust anti-inflammatory activity [33], respectively. Among the members of the fluoroquinolone family (a class of broad-spectrum antibiotics), the antibiotic ciprofloxacin has been known for its clinical efficacy and low potential for adverse effects [34-36]. In addition, the efficacy, safety and tolerability of ciprofloxacin DPI in human subjects had been successfully evaluated under Phase I and II clinical trials [36,37]. Phase III efficacy studies are currently underway [38]. Inhaled BP, being a robust anti-inflammatory agent, was previously demonstrated to be an effective treatment for patients with non-asthmatic chronic airflow obstruction [39].

The feasibility of combining the antibiotic and the corticosteroid as an inhalable dry powder formulation for direct concomitant delivery to the lung was investigated. The spray-dried binary formulation that contained CIP and BP (SD-CIP/BP), as well as their single counterparts (SD-CIP and SD-BP) were evaluated for their physicochemical characteristics, aerosol performance and antimicrobial properties.

2. Materials and methods

2.1. Materials

Ciprofloxacin hydrochloride (CIP) and beclomethasone dipropionate (BP) were supplied from Junda Pharmaceutical Co. Ltd. (Changzhou, China). Disodium hydrogen phosphate and phosphoric acid were purchased from Sigma Chemical Co. (Louis, MO, USA). Ultrapure water was used in the experiments. HPLC grade acetonitrile was supplied by Merck (Darmstadt, Germany). The model bacteria used in the study were obtained from the American Type Culture Collection (ATCC) and included *Klebsiella pneumoniae,*

Pseudomonas aeruginosa and *Staphylococcus aureus* obtained from the National University Hospital (Singapore). Mueller-Hinton broth (Oxoid, Bashingstoke, UK) was used as the culture media for the antimicrobial activity test.

2.2. Preparation of Spray-Dried Particles

Powders of ciprofloxacin hydrochloride (SD-CIP), beclomethasone dipropionate (SD-BP), and binary combination powders of beclomethasone dipropionate/ciprofloxacin hydrochloride in a weight ratio of 1:32.5 (SD-CIP/BP) were prepared by spray drying CIP alone, BP alone and CIP with BP from a methanol-water co-solvent feedstock using a B-90 Nano Spray Dryer (Büchi Labortechnik AG, Flawil, Switzerland) [27,28] with operating parameters as detailed in Table 1. All solutions were filtered through a 0.45µm syringe filter (Millipore, Bedford, MA, USA) prior to spray-drying to minimize blockage due to any undissolved particles at the spray mesh. The spray-dried powders were stored in a desiccator at room temperature for further characterization.

2.3. Surface morphology

The morphology of the powder particles was examined by field emission scanning electron microscopy (FESEM, JEOL JSM-6700) at 5kV. Prior to imaging, the samples were dispersed onto carbon sticky tabs and coated with gold for 100 s using a sputter coater (Cressington 208HR, Watford, UK).

2.4. Particle size analysis

The particle size distribution of the spray-dried powders was determined by laser diffraction using Malvern Mastersizer 2000 (Malvern Instruments, UK) using the Scirocco dry dispersion unit. The powders were dispersed in triplicates at 3 bars of pressure using refractive index (RI) of 1.520 for SD-CIP and SD-BP/CIP, and 1.564 for SD-BP.

2.5. Powder crystallinity

Powder crystallinity of the samples was assessed by powder X-ray diffraction (pXRD) at room temperature using an X-ray diffractometer (D8 Advance; Bruker AXS GmbH, Karlsruhe, Germany). Samples were scanned from 2-50^{o} (2θ) at an angular increment of 0.04^{o} and at 1 s per step using Cu K_{α} radiation generated at 35 kV and 40 mA.

2.6. Surface composition evaluation

The surface elemental composition of the powdered samples was measured using X-ray photoelectron spectroscopy (XPS) on a VG ESCALAB 250 spectrometer (Thermo Electron, U.K.) that was equipped with a non-monochromatized Al α X-ray source (1486 eV). Measurements were recorded for a 20 eV pass energy, a 0.1 eV kinetic energy step and a 0.05 s dwelling time. The powders were pressed onto double sided conductive adhesive tapes. Experimental molar percentages of all elements except hydrogen were derived from the XPS peak areas as described elsewhere [40]. Elemental weight percentages (Wt. %) (Table 4) were obtained via multiplying the molar percentages (At. %) with the respective atomic mass.

2.7. *In vitro* aerosol performance

The aerosol performance of the spray-dried powders was assessed using a multi-stage liquid impinger (MSLI, Copley Scientific, Nottingham, UK) coupled with a United State Pharmacopoeia (USP) stainless steel throat. The method followed the procedure specified for DPIs in the British Pharmacopoeia [41]. Prior to testing, 20 mL of water-methanol co-solvent in the ratio 1:3.5 (v/v) was added across all four stages of the MSLI. Approximately 20 ± 2 mg of powder from each formulation was filled into a hydroxypropyl methylcellulose (HPMC) capsule (size 3, Capsugel®, NJ, USA), loaded into the high efficiency Aerolizer® inhaler (Novartis Pharmaceuticals, Basel, Switzerland) [42] and pierced. The powder was then

dispersed for 4 s at 60 L/min and 2.4 s at 100L/min. The flow through the MSLI was measured using a calibrated flow meter (TSI Model 4040C, TSI Instrument Ltd., Buckinghamshire, UK), controlled by a high capacity vacuum pump (Model HCP5, Copley Scientific, Nottingham, UK) and a critical flow controller (TPK 2000, Copley Scientific, Nottingham, UK). After dispersion, the device, capsule, throat and each stage of the MSLI were washed separately and thoroughly using water-methanol co-solvent. The solutions were then assayed by high performance liquid chromatography (HPLC) after appropriate sample dilutions. Each dispersion test was performed in triplicates to obtain mean values. Dispersion was carried out in a walk-in environmental chamber (Synersys, Singapore) maintained at 25 ^{o}C and 40% RH. In this study, fine particle fraction (FPF) represents the mass fraction of drug particles smaller than 5 μm in the aerosol cloud relative to the total mass recovered and was obtained by interpolation to the cumulative percent undersize at 5 μm. FPF(emitted) was obtained when the fine particle dose was expressed relative to the emitted dose.

2.8. Drug content quantification

Drug contents in the spray-dried powders and in the aliquots collected from the MSLI were analyzed via HPLC (1100 series, Agilent Technologies, CA, USA). For CIP assays, a 20 μL aliquot sample was injected into the HPLC system equipped with a Zorbax SB C-18 column (4.6 mm x 150 mm, 3.5 μm) (Agilent Technologies, CA, USA) as the stationary phase (column temperature 25^{o}C), and a mixture of 0.025M disodium hydrogen phosphate buffer (adjusted to pH 3.0 with phosphoric acid) and acetonitrile (80:20, v/v) as the mobile phase, at a flow rate of 1 mL/min and an UV detection wavelength of 293 nm. For the assay of BP, the mobile phase consisted of acetonitrile/water (60:40, v/v) delivered at 1.5 mL/min under a UV detection wavelength of 250 nm. The retention time of CIP and BP was 2.3 min and 4.7 min, respectively.

2.9. Powder dissolution

Two hundred milliliter dissolution medium (0.2% w/v sodium dodecyl sulphate solution) at 37.0±0.5°C was passed through a 25 mm i.d. flow-through cell (Millipore Swinnex filter holders, USA, utilizing a Pall HT Tuffryn 0.2 μm membrane disc filters, USA) containing 20 mg of sample, and re-circulated in a closed-loop configuration at 1.6 mL/min using a peristaltic pump (pump speed stability of ±0.5%) (Gilson MiniPuls3, USA) [43,44]. At regular time intervals, 0.7 mL samples were withdrawn, diluted with 0.5 mL ethanol and then analyzed for their concentrations using the previously described HPLC methods. Studies were carried out in triplicate.

2.10. Determination of antimicrobial minimum inhibitory concentration (MIC)

The in vitro activity of SD-BP, SD-CIP and SD-BP/CIP was tested against bacterial type strains of *Klebsiella pneumoniae* ATCC 12885, *Pseudomonas aeruginosa* ATCC 90207 and *Staphylococcus aureus* ATCC 4330. The microdilution test was performed in accordance to the Clinical and Laboratory Standards Institute (CLSI) procedures [45] using a final bacterial inoculum of approximately 5 $x10^5$ (colony forming units) CFU/ml in a sterile 96-well plate bearing two-fold drug dilutions. The MIC was defined as the lowest drug concentration that inhibited the visible growth of microorganisms after an overnight incubation. The MIC assays were carried out in duplicate for each strain.

2.11. Statistical analysis

Statistical significance was carried out using one-way analysis of variance with Tukey's post-hoc analysis at a p-value of 0.05 using Minitab.

3. Results and discussion

3.1. Preparation of spray-dried particles

Spray-dried powders of corticosteroid alone (SD-BP), antibiotic alone (SD-CIP) and binary combination of corticosteroid and antibiotic (SD-BP/CIP) were successfully prepared and collected at yields of more than 80% (Table 1) using the Nano Spray Dryer B-90 [28]. High product yield is readily achieved on the Nano Spray Dryer B-90, with published reports listing it as between 70% and 90% [27,46,47].

CIP is a water-soluble antibiotic whereas BP is a poorly-water soluble steroid that can only be dissolved in organic solvents such as methanol, chloroform or acetone. In order to co-spray dry both hydrophilic and hydrophobic APIs simultaneously for the production of particles with uniform drug composition, co-solvent selection and ratio are both very important aspects. In this work, a water-methanol mix was selected as the co-solvent system for BP and CIP in view of their mutual miscibility and low toxicity. The water-methanol co-solvent volume ratio was adjusted to 1:3.5 to ensure good solubility of both APIs in the co-solvent system with no precipitation.

Unlike oral corticosteroids which must travel throughout the patient's body to reach the lung, inhaled corticosteroids are typically used in much smaller doses (i.e. a few hundred micrograms) as there is direct lung-targeting [48]. Furthermore, the maximum daily dose of inhaled BP has been recommended to be at approximately 1 mg to avoid significant reduction in plasma cortisol concentrations [49,50].

Antibiotics are widely used to treat bacterial infections by either killing (bactericidal) or inhibiting the growth (bacteriostatic) of bacteria. It is usually used at high concentrations to ensure good tissue penetration and maximum bacterial kill [51,52]. Currently, Cipro Inhale®, an inhaled antibiotic dry powder formulation undergoing Phase III clinical trial, has been found to be safe and well-tolerated (via Phase I and II trials), when applied at a single inhaled dose of 32.5 mg [36,37].

In this work, the binary dry powder formulation containing BP and CIP was prepared at 1: 32.5 weight ratio (adapted from reported clinical dosing). Although the weight ratio of BP was as low as 3% in the binary formulation, it was still well-preserved (as close as 100% in comparison to the feed, Table 2) in the final spray-dried powder.

3.2. Surface morphology and particle size

The surface morphologies of the single and binary spray-dried particles are shown in Fig. 1. All the spray-dried particles were generally spherical in geometry, with the SD-CIP having a smoother surface (Fig. 1c and d) than the SD-BP (i.e. slightly corrugated surface) (Fig. 1a and b). The surface morphology of SD-BP/CIP (Fig. 1e and f) was found to be closer to that of SD-BP, whereby a slightly corrugated surface was obtained. This observation was not surprising, even though CIP was the major component of the powder (i.e. 97%. w/w CIP vs 3% w/w BP). The final distribution of each solute component in the spray-dried particle would very much depend on the solutes' surface activities (which leads to the preferential adsorption of components on the sprayed droplet surface) and diffusivities in the spray solution. In the present binary co-solvent system (i.e. methanol-water), the saturation of

hydrophobic API (BP) occurred much faster than that of the hydrophilic API (CIP) due to the preferential evaporation of methanol over water and the associated changes in methanol-water ratio during the drying process. This then led to the accumulation and precipitation of the hydrophobic component at the liquid-air interface. Consequently, more hydrophobic component is found on the surface of the dried particles [53-55]. Thus, this explains the similar surface morphologies of both SD-CIP/BP and SD-BP. The XPS results discussed under Section 3.4 further supports this finding.

As all the single and binary spray-dried powders were prepared under the same spray-drying conditions, they were thus found to have comparable particle size and span (Table 3). The particle size analysis revealed that all the spray-dried powders were within the respirable size range, with a d_{50} of 2.21 ± 0.07 µm, 2.36 ± 0.02 µm and 2.36 ± 0.01 µm for SD-BP, SD-CIP and SD-BP/CIP, respectively.

3.3. Powder crystallinity

The diffractogram of the raw materials, single spray-dried powders and co-spray-dried powder is shown in Fig. 2. The sharp peaks found in the diffractograms of Raw-CIP and Raw-BP indicated that both starting materials were crystalline powders. The XRD pattern of SD-BP showed only a single diffused broad peak, indicating its amorphicity. In comparison, reduced crystallinity was observed for SD-CIP. This observation was not surprising as we have previously reported on the reduced crystallinity of SD-CIP on the Nano Spray Dryer B90 [46]. The XRD pattern of SD-BP/CIP appeared to be very similar to that of SD-CIP due to the high weight ratio of CIP in the formulation.

3.4. Surface composition evaluation

Table 4 illustrates the experimentally measured surface composition of the raw materials and spray-dried powders that was derived from the XPS peak areas. Generally, the experimentally measured surface elemental composition of the raw materials (i.e. Raw-BP and Raw-CIP) was comparable to their spray-dried counterparts (i.e. SD-BP and SD-CIP). This implies that the possibility of surface composition alteration via spray-drying process was minimal. On the other hand, the experimentally measured surface composition of the binary spray-dried powder, SD-BP/CIP, did not follow the trends of either SD-BP or SD-CIP. Hence, neither BP nor CIP solely resided on the surface of SD-BP/CIP. In order to further evaluate the surface composition of binary SD-BP/CIP, the expected elemental weight percentages ($Wt._{expected}$ %) of the binary SD-BP/CIP was calculated (by assuming that both BP and CIP were distributed evenly on the particle surface). The expected elemental weight percentages could be calculated from the elemental weight percentages that were measured experimentally on the raw materials (Table 5). For example, the expected elemental weight percentage of carbon in SD-BP/CIP was the summation of the products obtained via multiplying the Wt.% of carbon in Raw-BP with weight proportion of BP (*i.e.* 3% w/w) in SD-BP/CIP and Wt.% of carbon in Raw-CIP with the weight proportion of CIP in SD-BP/CIP (*i.e.* 97% w/w). The experimentally measured elemental weight percentages (Wt. %) of the binary SD-BP/CIP were then compared with the expected elemental weight percentages ($Wt._{expected}$ %). Any difference between the Wt. % and $Wt._{expected}$ % suggested an over- or under-abundance of certain elements and hence the types of molecules on the particle surface. From Table 5, the $Wt._{expected}$ % of the binary SD-BP/CIP was comparable to the Wt.% of Raw-CIP and SD-CIP. It was as expected for an assumed even distribution of BP and CIP on the particle surface. BP

being a very minor component (just 3% weight ratio), would be considered negligible when compared to the presence of CIP (97% weight ratio) on the surface.

Nitrogen and fluorine are two elements that are specific to CIP ($C_{17}H_{18}FN_3O_3$.HCl) but not BP ($C_{28}H_{37}ClO_7$), so a lower C/N, C/F, O/N, O/F, Cl/N and Cl/F experimental ratio in SD-BP/CIP over that of SD-CIP could possibly suggest that CIP resided less preferentially on the surface of SD-BP/CIP as compared to BP (Table 6). However, due to the large weight ratio of CIP (97%) in SD-BP/CIP, some of the CIP molecules could still be found on the particle surface of SD-BP/CIP. The enrichment of BP on the particle surface could also be inferred from the C/O ratios. SD-BP has a higher C/O ratio than SD-CIP and the high C/O ratio obtained for SD-BP/CIP again implies that BP preferentially resides on the surface of the particle.

The enrichment of BP on the particle surface is an expected outcome, as during spray drying, the more volatile solvent (i.e. methanol) in a mixed methanol-water droplet would evaporate much faster than water. Due to the low solubility of BP in the remaining aqueous phase, precipitation of the compound onto the surface of the alcohol-deficient droplets and hence the dried particle, is hence encouraged. Similarly, Kumon *et. al.* [53] had previously shown that hydrophobic drugs (rather than the hydrophilic excipient) tend to reside on the particle surface when spray-dried from an alcohol-water mix.

3.5. *In vitro* aerosol performance

In this study, the aerosol performance of the spray-dried powders was studied under two levels of realistic inspiratory effort: low (60 L/min) and high (100 L/min). The lower air flow of 60 L/min is likely to be generated by patients with compromised lung functions (e.g. pneumonia [46]) while the higher air flow of 100 L/min through the Aerolizer® inhaler represents a comfortable inspiratory effort readily achieved by patients with healthy lung functions [53,56,57].

The aerosol performance results of the spray-dried powders are shown in Fig. 3 and Table 7. Generally, SD-CIP was found to perform poorer, with FPFs of ~17.8% at 60L/min and ~25.1% at 100L/min as compared to SD-BP which had FPFs of ~ 32.0% at 60L/min and ~41.7% at 100L/min. This might be due to SD-CIP's higher agglomeration propensity in the aerosol (evident via the high powder deposition throughput on Stage 1) (Fig. 3b). However, agglomerate break-up was achievable when the flow rate was increased from 60 to 100L/min (Fig. 3e). For SD-CIP, powder deposition on Stage 1 decreased from ~24% to ~19.5% with an increase in flow rate. In addition, increasing the flow rate from 60 to 100L/min also has the advantage of improving the emitted fraction (i.e. inferred via an improved FPF but similar FPF(emitted)) (Table 7).

Interestingly, the MSLI deposition profile of binary SD-BP/CIP followed that of SD-BP rather than SD-CIP, even though SD-CIP was the major component in the formulation (wt. ratio of 1:32). Possibly, this could be due to the distribution of BP on the particle surface of SD-BP/CIP, which in a way reinforces the earlier findings from the XPS and SEM analyses.

For the binary SD-BP/CIP, there was concomitant deposition observed across all stages (including the throat, inhaler and capsule) at 60 and 100L/min, suggesting uniformity in the powder and/or aerosol (Fig. 3c & f). From Table 7, it also does seem that formulating the drugs into a binary mix has the advantage of improving their delivery more effectively to the patient. FPF and FPF(emitted) for SD-BP and SD-CIP were generally much lower than BP and CIP in SD-BP/CIP. For CIP in particular, the improvement was significant when it was co-delivered with BP at 60 and 100L/min ($p < 0.05$).

3.6. *In vitro* drug release

BP, being a Biopharmaceutical Classification System (BCS) class II drug (i.e. low solubility, high permeability) is limited by dissolution-rate limited kinetics[58]. Therefore, any improvements to the drug's pulmonary solubility would be beneficial to the absorption process in the lungs [59].

Fig. 4 shows that when BP (a poorly water soluble drug) was formulated into a binary mix with the water soluble CIP, its dissolution rate and extent were drastically improved over the single species ($p < 0.05$). Hence, this formulation approach does seem to be highly effective in 'lifting' the performance of the poorer-performing species to almost the same extent as the robust species. Drug release of CIP in the binary mix was comparable to that of the single species. The release mechanism in this case was analogous to an 'API-API solid dispersion system' whereby the poorly water-soluble BP was dispersed into the more water soluble CIP matrix, hence enhancing the wettability and solubility of the former (Figure 5) [60].

3.7. Antibacterial activity of spray-dried powders

K. pneumoniae, *P. aeruginosa* and *S. aureus* are common etiologic agents for CAP [4]. Table 8 shows the antibacterial activity of the spray-dried powders with MICs of SD-BP, SD-CIP and SD-BP/CIP determined via the microdilution method on type strains of *K. pneumoniae*, *P. aeruginosa* and *S. aureus.* As expected, the MIC of SD-BP was not determinable as BP is a typical corticosteroid that does not exhibit any antibacterial properties. The MICs of SD-BP/CIP on the various bacterial species were found to be comparable to the MICs of SD-CIP, suggesting the antibacterial properties of CIP in the binary mix was adequately retained.

4. Conclusions

A novel steroid-antibiotic DPI formulation (SD-CIP/BP) was successfully pioneered via nanospray drying. The powder obtained was of respirable-size (d_{50} of ~2.3 μm), partially crystalline (potentially improved storage stability) and had BP preferentially deposited on the particle surfaces. When dispersed via an Aerolizer at 60L/min and 100L/min, the powder showed concomitant and uniform *in vitro* deposition profile across all impaction stages, with obtained FPFs of ~36-46%. Favourably, when formulated as a binary mix, both CIP and BP showed much higher drug release and FPFs over their singly-delivered counterparts, and was also active against the respiratory tract infection-causing bacteria *K. pneumoniae*, *P. aeruginosa* and *S. aureus*. Co-spray-dried powder of BP and CIP (SD-BP/CIP) may hence be a novel and useful anti-inflammatory and antimicrobial formulation for CAP patients with COPD complications.

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Table 1. Spray drying parameters

Parameters	
Spray mesh size (µm)	7
Feed concentration (w/v %)	0.7
Co-solvent (water-methanol) ratio (v/v)	1:3.5
Nitrogen flow rate (L/min)	120
Relative spray rate (mL/h)	4
Inlet Temperature (^{o}C)	75
Outlet Temperature (^{o}C)	38-42
Yield (%)	80-90

Table 2. Composition of the spray-dried particles. Drug contents measured by HPLC.

Formulation	Drug Content (w/w %)				% of Ideal (Relative to feed)	
	Feed		Actual			
	BP	CIP	BP	CIP	BP	CIP
SD-BP	100.0	-	99.7 ± 0.5	-	99.7 ± 0.5	
SD-CIP	-	100.0	-	99.3 ± 0.7		99.3 ± 0.7
SD-BP/CIP	3.0	97.0	2.9 ± 0.1	97.7 ± 0.8	96.7 ± 3.3	100.7 ± 0.8

Mean ± standard deviation (n=3)

Table 3. Volume particle size distribution of spray-dried powder.

	SD-BP (µm)	SD-CIP (µm)	SD-BP/CIP (µm)
d_{10}	1.01 ± 0.01	0.93 ± 0.01	0.88 ± 0.06
d_{50}	2.21 ± 0.07	2.36 ± 0.02	2.36 ± 0.01
d_{90}	4.48 ± 0.19	5.43 ± 0.29	5.37 ± 0.29
Span	1.57 ± 0.04	1.91 ± 0.13	1.91 ± 0.16

Mean ± standard deviation, n=3

d_{10} - volume diameter under which 10% of the sample resides.

d_{50} - volume median diameter

d_{90} - volume diameter under which 90% of the sample resides.

Span = $(d_{90} - d_{10})/d_{50}$

Table 4. Surface composition of the raw materials and spray-dried powders.

Sample	Element	At. %	Wt. %
Raw BP ($C_{28}H_{37}ClO_7$)	Carbon	73.9	60.5
	Oxygen	17.9	19.6
	Chlorine	8.2	19.9
Raw CIP ($C_{17}H_{18}FN_3O_3$.HCl)	Carbon	63.9	49.7
	Oxygen	11.3	11.8
	Chlorine	10.7	24.7
	Nitrogen	10.7	9.7
	Fluorine	3.4	4.1
SD-BP ($C_{28}H_{37}ClO_7$)	Carbon	72.4	59.7
	Oxygen	20.1	22.1
	Chlorine	7.5	18.2
SD-CIP ($C_{17}H_{18}FN_3O_3$.HCl)	Carbon	64.0	52.1
	Oxygen	12.6	13.5
	Chlorine	8.6	20.3
	Nitrogen	10.5	9.8
	Fluorine	3.4	4.3
SD-BP/CIP	Carbon	53.6	42.4
($C_{28}H_{37}ClO_7$)	Oxygen	35.6	37.5
($C_{17}H_{18}FN_3O_3$.HCl)	Chlorine	7.0	16.3
	Nitrogen	3.0	2.8
	Fluorine	0.9	1.1

Table 5. Calculation of expected elemental percentage in SD-BP/CIP

Sample	Drug component	Wt. proportion (%)[a]	Theoretical elemental Wt.%[b]		Expected elemental Wt.%[c]		Experimental elemental Wt.%[d]	
SD-BP/CIP	BP	3	Carbon	1.8	Carbon	50.0	Carbon	42.4
			Oxygen	0.6	Oxygen	12.0	Oxygen	37.5
			Chlorine	0.6	Nitrogen	9.4	Nitrogen	2.7
					Chlorine	24.6	Chlorine	16.3
	CIP	97	Carbon	48.2	Fluorine	4.1	Fluorine	1.1
			Oxygen	11.4	Total	100	Total	100
			Nitrogen	9.4				
			Chlorine	24.0				
			Fluorine	4.0				

[a]Weight proportion (%) = [weight ratio of BP or CIP in feed/(total weight ratio in feed)]x100; weight ratio of BP:CIP in feed = 1:32.5

[b]Theroretical elemental Wt.% = Wt.% (raw materials) x weight proportion

[c]Expected elemental Wt.% = summation of theoretical elemental Wt. % (e.g for carbon, expected elemental Wt.% = 1.8 + 48.2 = 50.0); assuming both BP and CIP were distributed evenly on particle surface

[d]Experimental elemental Wt.% = At.% x atomic weight of the element

Table 6. Various elemental ratios of spray-dried powders.

Ratio	SD-BP	SD-CIP	SD-BP/CIP
Carbon/oxygen	1:0.37	1:0.26	1:0.88
Carbon/nitrogen	-	1:0.19	1:0.07
Carbon/fluorine	-	1:0.08	1:0.03
Oxygen/nitrogen	-	1:0.73	1:0.07
Oxygen/fluorine	-	1:0.32	1:0.03
Chlorine/nitrogen	-	1:0.48	1:0.17
Chlorine/fluorine	-	1:0.21	1:0.07

Table 7. Deposition parameters (mean ± standard deviation, n = 3) of different formulations measured by MSLI at 60 and 100 L/min.

	60L/min				100L/min			
	SD-BP	SD-CIP	SD-BP/CIP		SD-BP	SD-CIP	SD-BP/CIP	
			BP	CIP			BP	CIP
FPF[a] (%)	32.0 ± 1.2	17.8 ± 1.0	36.4 ± 1.3	38.3 ± 0.8*	41.7 ± 0.3	25.1 ± 4.0	44.5 ± 2.1	45.9 ± 1.5*
FPF (emitted)[b] (%)	47.6 ± 1.2	21.6 ± 2.0	49.0 ± 2.5	51.3 ± 1.9	48.5 ± 0.2	33.8 ± 3.7	50.0 ± 0.7	50.6 ± 0.2

[a] FPF - fine particle fraction

[b] FPF (emitted) - emitted fine particle fraction

* significant difference from CIP in SD-CIP ($p < 0.05$)

Table 8. MIC values of the spray-dried powders.

Bacteria species and strain	MIC (µg/ml)		
	SD-BP	SD-CIP	SD-BP/CIP
Klebsiella pneumoniae ATCC 12885	ND[a]	1 - 2	2
Pseudomonas aeruginosa ATCC 90207	ND	2	1
Staphylococcus aureus ATCC 4330	ND	1 - 2	2

[a] ND - not determined due to the absence of inhibition

Figure caption

Fig. 1 FESEM images of spray-dried powders. (a) SD-BP, (b) SD-BP (close-up view), (c) SD-CIP, (d) SD-CIP (close-up view), (e) SD-BP/CIP and (f) SD-BP/CIP (close-up view).

Fig. 2 XRD patterns of Raw-CIP, Raw-BP, SD-BP, SD-CIP and SD-BP/CIP.

Fig. 3 In vitro deposition of (a) SD-BP, (b) SD-CIP, (c) SD-BP/CIP at 60L/min and (d) SD-BP, (e) SD-CIP and (f) SD-BP/CIP at 100L/min. Data presented as mean ± standard deviation (n=3). S1-S4 denote impactor stages, followed by their corresponding lower aerodynamic cut-off diameter in parentheses.

Fig. 4 In vitro dissolution rate profiles of CIP and BP in individual and combined formulations.

Fig. 5 Schematic diagram illustrating release mechanism.

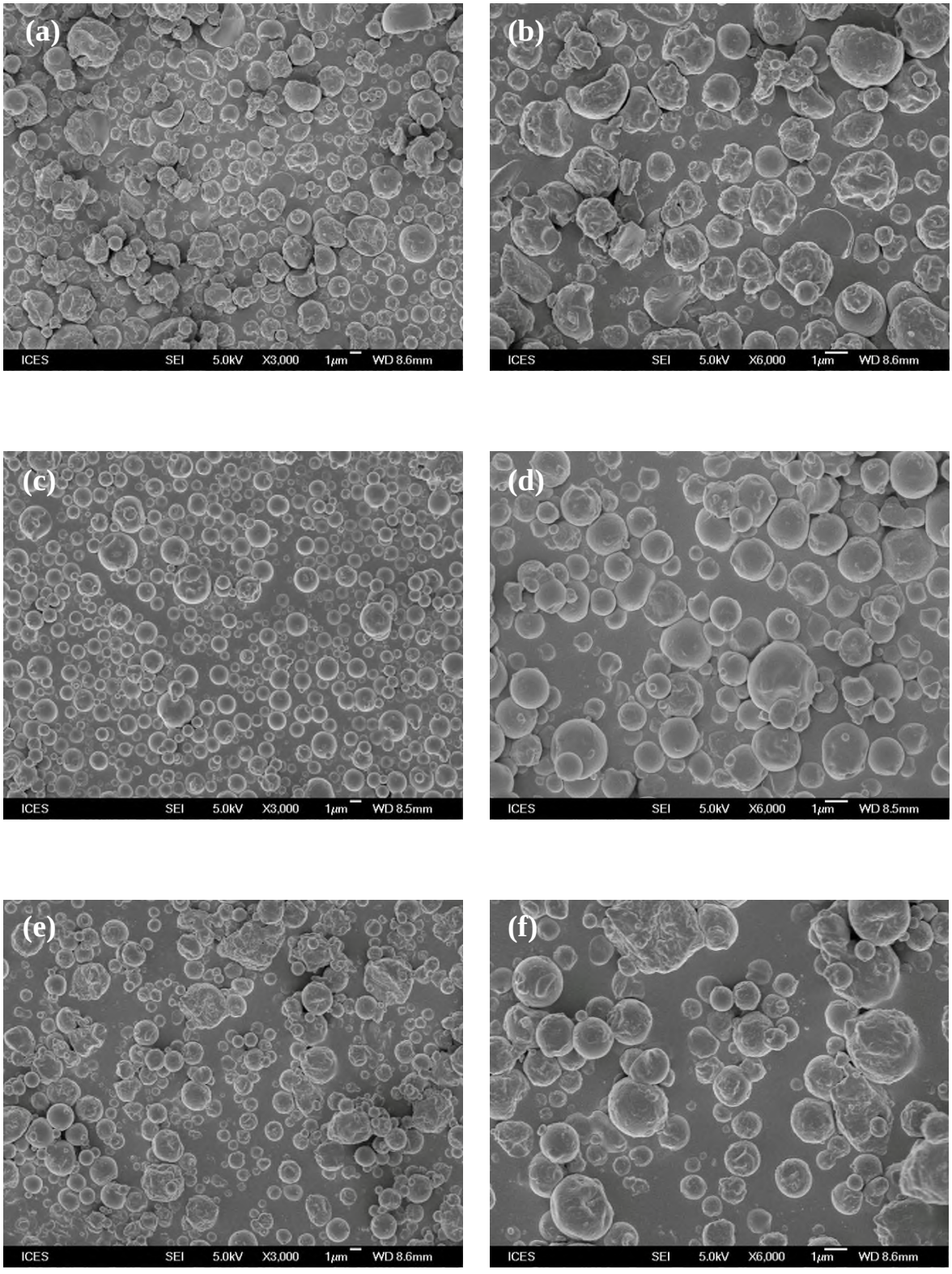

(a)
ICES SEI 5.0kV X3,000 1μm WD 8.6mm
(b)
ICES SEI 5.0kV X6,000 1μm WD 8.6mm
(c)
ICES SEI 5.0kV X3,000 1μm WD 8.5mm
(d)
ICES SEI 5.0kV X6,000 1μm WD 8.5mm
(e)
ICES SEI 5.0kV X3,000 1μm WD 8.6mm
(f)
ICES SEI 5.0kV X6,000 1μm WD 8.6mm

Fig. 1

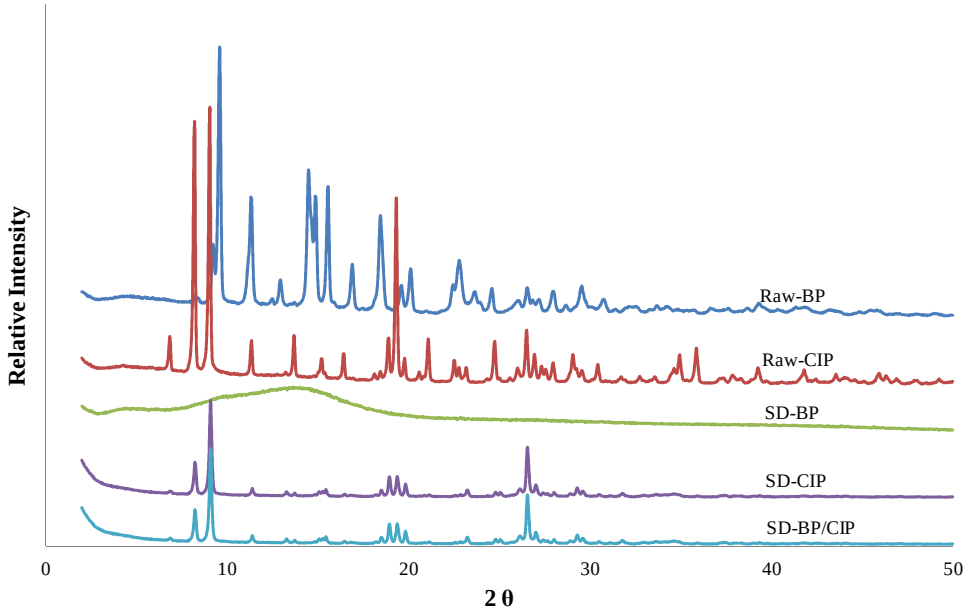

Relative Intensity
Raw-BP
Raw-CIP
SD-BP
SD-CIP
SD-BP/CIP
0
10
20
30
40
50
2 θ

Fig. 2

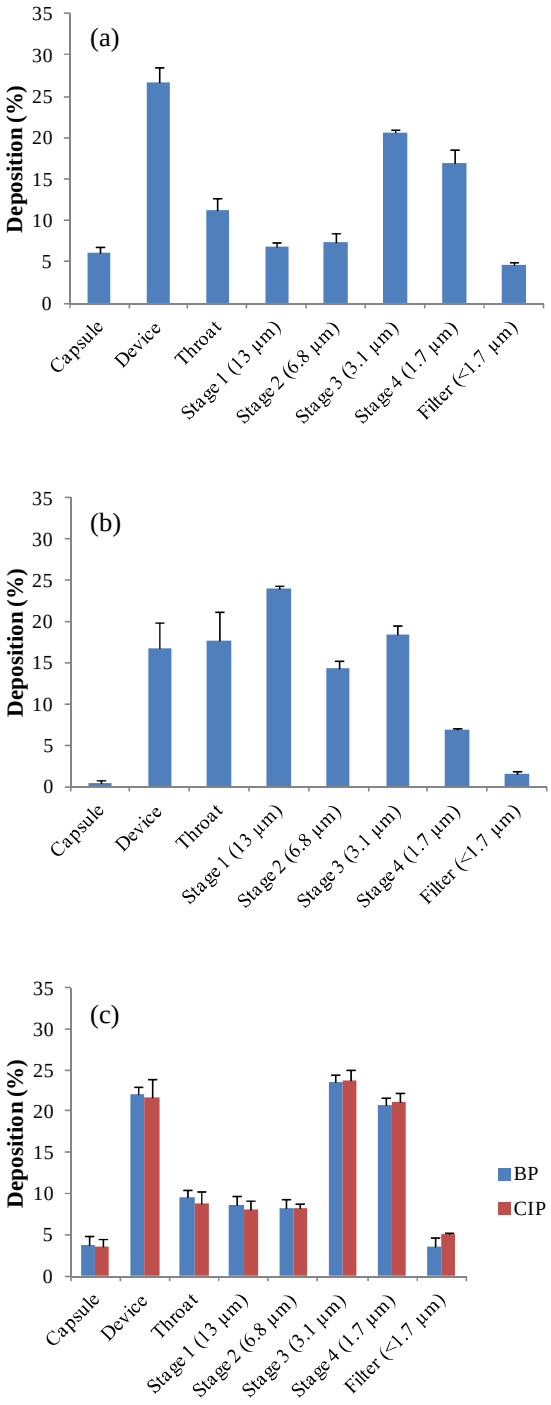

(a)
Deposition (%)
35
30
25
20
15
10
5
0
Capsule
Device
Throat
Stage 1 (13 µm)
Stage 2 (6.8 µm)
Stage 3 (3.1 µm)
Stage 4 (1.7 µm)
Filter (<1.7 µm)
(b)
Deposition (%)
Capsule
Device
Throat
Stage 1 (13 µm)
Stage 2 (6.8 µm)
Stage 3 (3.1 µm)
Stage 4 (1.7 µm)
Filter (<1.7 µm)
(c)
Deposition (%)
BP
CIP
Capsule
Device
Throat
Stage 1 (13 µm)
Stage 2 (6.8 µm)
Stage 3 (3.1 µm)
Stage 4 (1.7 µm)
Filter (<1.7 µm)

Fig. 3

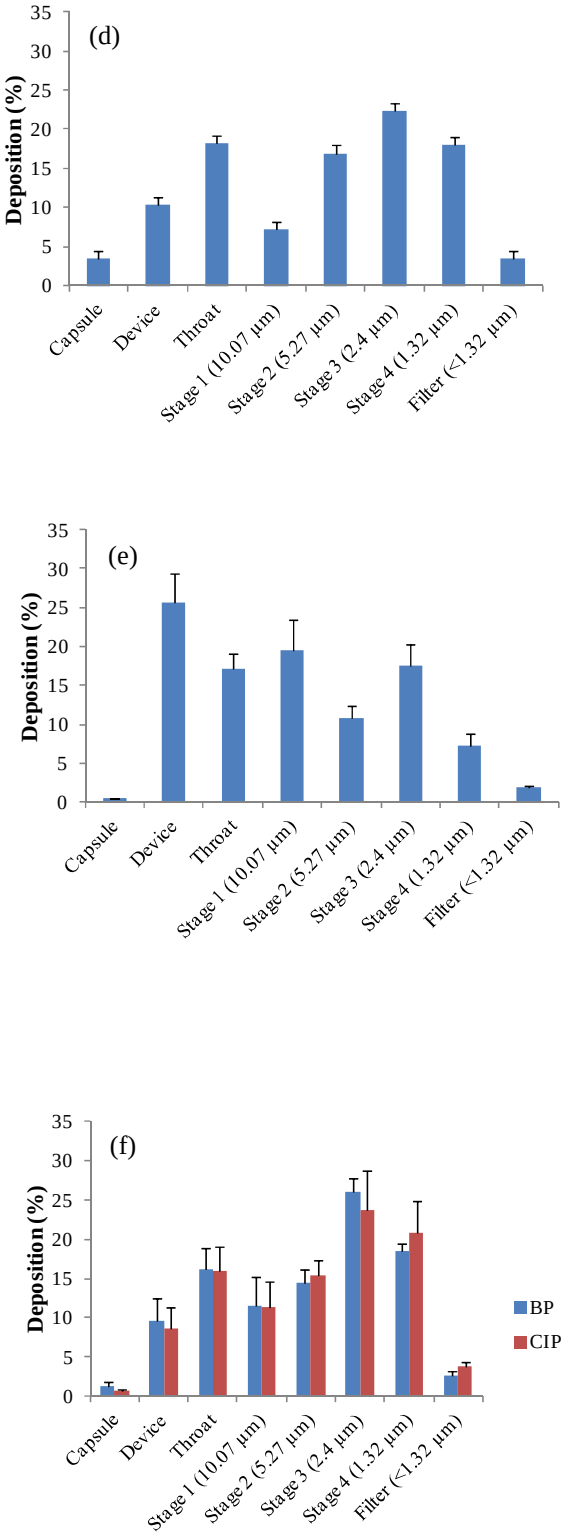
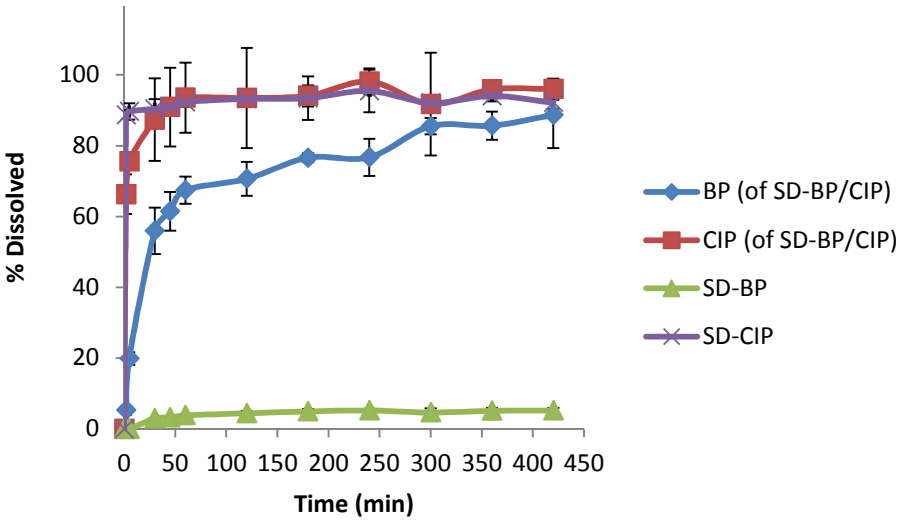

(d)
Deposition (%)
35
30
25
20
15
10
5
0
Capsule
Device
Throat
Stage 1 (10.07 µm)
Stage 2 (5.27 µm)
Stage 3 (2.4 µm)
Stage 4 (1.32 µm)
Filter (<1.32 µm)
(e)
Deposition (%)
35
30
25
20
15
10
5
0
Capsule
Device
Throat
Stage 1 (10.07 µm)
Stage 2 (5.27 µm)
Stage 3 (2.4 µm)
Stage 4 (1.32 µm)
Filter (<1.32 µm)
(f)
Deposition (%)
35
30
25
20
15
10
5
0
BP
CIP
Capsule
Device
Throat
Stage 1 (10.07 µm)
Stage 2 (5.27 µm)
Stage 3 (2.4 µm)
Stage 4 (1.32 µm)
Filter (<1.32 µm)

Fig. 3 (cont'd)


% Dissolved
100
80
60
40
20
0
0
50
100
150
200
250
300
350
400
450
Time (min)
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CIP (of SD-BP/CIP)
SD-BP
SD-CIP

Fig. 4

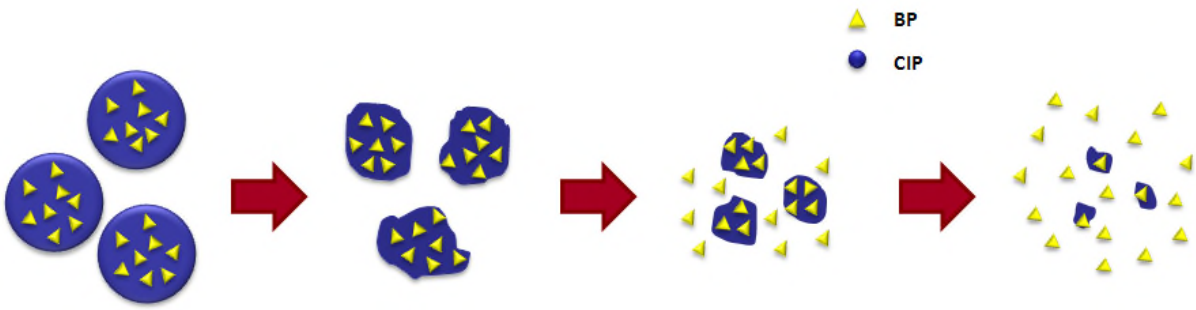

Fig. 5