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Dry powder aerosols of curcumin-chitosan nanoparticle complex prepared by spray freeze drying and their antimicrobial efficacy against common respiratory bacterial pathogens

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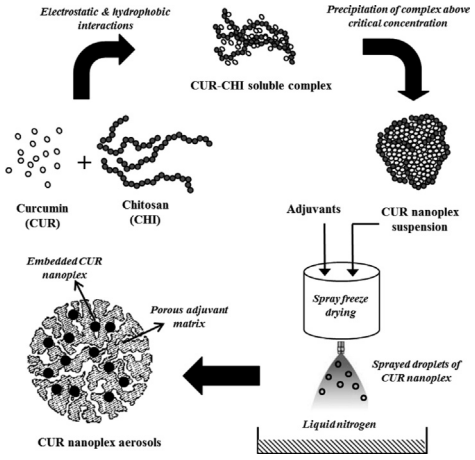
HIGHLIGHTS

- Spray freeze drying produced highly aerosolizable curcumin nanoplex aerosols.
- Aerosol transformation diminished supersaturation generation of curcumin nanoplex.
- Curcumin was released more slowly from aerosols than from aqueous suspension.
- Curcumin nanoplex aerosols maintained activity against respiratory pathogens.
- Curcumin nanoplex aerosols exhibited minimal cytotoxicity towards lung cells.

GRAPHICAL ABSTRACT



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ABSTRACT

While the therapeutic benefits of curcumin delivery to the lung to treat various pulmonary disorders have been established, development of inhaled curcumin formulation that can address its inherently low aqueous solubility remains lacking. Although curcumin nanocapsules prepared by conventional encapsulation methods can improve the dissolution rate, their intricate preparation makes them less attractive for widespread implementation. Recently, our group developed a new class of curcumin nanoparticles in the form of curcumin-chitosan nanoparticle complex (or curcumin nanoplex in short) by a simple, cost-effective, and highly efficient method based on self-assembly drug-polysaccharide complexation. Owing to its nanosize and amorphous state, the curcumin nanoplex possessed high supersaturation generation capability upon dissolution that in turn produced high apparent solubility of curcumin.

Abbreviations: CF, cystic fibrosis; CFU, colony forming unit; CHI, chitosan; COPD, chronic obstructive pulmonary diseases; CUR, curcumin; d_A, aerodynamic diameter; d_G, geometric diameter; DMEM, Dulbecco's modified Eagle's medium; DMSO, dimethyl sulfoxide; DPI, dry powder inhaler; ED, emitted dose; EDTA, ethylenediaminetetraacetic acid; FESEM, field emission scanning electron microscope; FPF, fine particle fraction; GRAS, generally recognized as safe; HPLC, high performance liquid chromatography; ID, internal diameter; IP, induction port; PS, pre-separator; Leu, L-leucine; Man, D-mannitol; MHB, Mueller Hinton broth; MIC, minimum inhibitory concentration; MMAD, mass median aerodynamic diameter; MTT, (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide); n, number of experimental replicates; OD_{600}, optical density at 600 nm; *p-valu*, esignificance parameter in the Student's *t*-test; PET, powder entrainment tube; SFD, spray freeze drying; PCS, photon correlation spectroscopy; PBS, phosphate buffered saline; S_{Final}, nanoplex size after reconstitution; $S_{Initial}$, initial nanoplex size; SEM, scanning electron microscope; UV–vis, ultraviolet visible spectroscopy.

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Pulmonary drug delivery
Aerosols

In the present work, we developed dry powder aerosol formulation of the curcumin nanoplex by spray freeze drying (SFD) using L-leucine and D-mannitol as adjuvants. The curcumin nanoplex aerosols were found to exhibit excellent aerosolization efficiency attributed to their large and low-density morphology, and the presence of L-leucine – a well-established aerosol dispersion enhancer – in their matrix. The aerosols, however, exhibited weaker supersaturation generation capability compared to the aqueous nanoplex suspension due to their slower dissolution rates caused by irreversible aggregations of the nanoplex during SFD. Nevertheless, the curcumin nanoplex aerosols still produced apparent solubility that was approximately 50% higher than the native curcumin's solubility, thus signifying their dissolution enhancement capability. Despite their slower dissolution rate, the curcumin nanoplex aerosols maintained the same antimicrobial activity as the nanoplex suspension against four clinically-derived respiratory bacterial pathogens (i.e. *Pseudomonas aeruginosa*, *Burkholderia cepacia*, *Klebsiella pneumoniae*, and *Stenotrophomonas maltophilia)*. Lastly, the aerosols exhibited minimal cytotoxicity towards the lung epithelium cells just like the native curcumin.

1. Introduction

Curcumin (CUR) – a natural flavonoid isolated from turmeric plants – has been well documented to possess excellent anti-inflammatory [1] and wide-ranging antimicrobial and anticancer activities [2,3]. As persistent inflammation of the airways represents the common symptom in many major lung diseases, such as asthma, cystic fibrosis (CF), chronic obstructive pulmonary disease (COPD), and bronchitis, the anti-inflammatory effects of CUR in various lung diseases have been extensively investigated [4], from which the ability of CUR to alleviate airway inflammations was successfully established [5–7]. In addition to its anti-inflammatory effect, CUR was also shown to be able to partially correct phenotypic lung defects in CF [8], inhibit the growth of bacteria isolated from CF patients [9], and suppress the invasion and metastasis of lung cancer cells [10,11].

These many therapeutic benefits of CUR, however, can only be realized if effective localized delivery of CUR to the lung is achieved. Besides lung disease therapies, pulmonary delivery of CUR is also useful in enhancing the systemic bioavailability of CUR for the treatment of non-lung diseases [12,13] as oral delivery of CUR is ineffective due to the rapid intestinal metabolism of CUR [14]. The pulmonary delivery of CUR in its native form, however, faces a major obstacle in the inherently low aqueous solubility of CUR (∞ 1 μg/mL). The low solubility limits the deliverable dosage when CUR is delivered as nebulized solution, and when CUR is delivered as dry powder aerosols, the low solubility leads to its slow dissolution upon reaching the airways lumen. As a result, pulmonary delivery of CUR in its native form would inevitably result in low bioavailability. Thus, there is a need to develop pulmonary delivery formulation of CUR that can address the issue of its low solubility.

CUR nanoparticles, where CUR is encapsulated or physically dispersed in nanoparticle carriers, have been demonstrated in numerous studies to enhance the dissolution rate of CUR attributed to their large specific surface areas [15]. CUR nanoparticles intended for pulmonary delivery in the form of CUR nanocrystals themselves [13], or CUR encapsulated in polymeric micelles [16], dequalinium vesicles [17], and lipid vesicles [18] have been developed. However, these inhaled CUR nanoparticle formulations had a major drawback in their intricate preparation, where it required multiple steps or heavy use of organic solvents in order to obtain high CUR payloads, or in the case of CUR nanocrystals, its preparation was time and energy-intensive.

Previously, we developed a simple and highly efficient solvent-free preparation method of high-payload CUR nanoparticles in the form of amorphous CUR-chitosan nanoparticle complex (or nanoplex in short) [19]. The CUR nanoplex was prepared by electrostatically driven self-assembly complexation between CUR and the oppositely charged chitosan (CHI). The nanoplex was prepared at high CUR utilization rates (>90%) by simply mixing of the aqueous solutions of CUR and CHI under ambient condition. Importantly, the CUR nanoplex was safe as only Generally Recognized as Safe (GRAS) materials were used in its preparation [19].

In this method, ionized CUR molecules were mixed with oppositely charged CHI molecules to form soluble CUR-CHI complex (Fig. 1). Owing to hydrophobic interactions among the bound CUR molecules, aggregates of the CUR-CHI complex were formed after which they precipitated upon reaching a critical concentration to form the CUR nanoplex. The strong electrostatic interactions between CUR and CHI prevented the former from assembling into ordered crystalline structures during its precipitation, resulting in the formation of amorphous CUR nanoplex. Compared to crystalline nanoparticles, the supersaturation generation afforded by the amorphous state of the nanoplex led to high apparent solubility, hence higher bioavailability [20].

Herein we developed dry powder inhaler (DPI) formulation of the CUR nanoplex by spray freeze drying (SFD), which represents one of the most widely used DPI formulation methods for therapeutic nanoparticles [21]. In SFD, the CUR nanoplex suspension and cryoprotective adjuvants were sprayed into a cryogen (*i.e.* liquid nitrogen), resulting in instantaneous freezing of the sprayed droplets, hence preserving the original size and spherical shape of the droplets (Fig. 1). The frozen droplets subsequently underwent freeze drying, where sublimation of the frozen ice crystals in the interstitial space between the nanoplex took place. As a result, spherical particles with large and low-density morphology, which are ideal for aerosolization by DPI [22], were produced by SFD.

The first objective of the present work was to investigate the effect of adjuvant formulation in SFD, specifically the ratio of L-leucine to D-mannitol, on the aerosolization efficiency of the CUR nanoplex aerosols produced. L-leucine and D-mannitol were used as the adjuvants because L-leucine has been established as an effective aerosol dispersion enhancer attributed to the surface rugosity generated in its presence [23], while D-mannitol as a sugar alcohol is well-known for its cryoprotective properties [24]. Furthermore, D-mannitol was chosen among other cryoprotectants because of its mucolytic property [25] as many lung diseases are characterized by the presence of thick stationary mucus in the airways.

The second objective of the present work was to evaluate the effect of the dry powder transformation by SFD on the supersaturation generation capability of the CUR nanoplex, and how it subsequently affected the (1) antimicrobial efficacy of the dry powder aerosols of the CUR nanoplex against clinically derived respiratory bacterial pathogens (*i.e. Pseudomonas aeruginosa*, *Burkholderia cepacia*, *Klebsiella pneumoniae*, and *Stenotrophomonas maltophilia*), and (2) their *in vitro* cytotoxicity towards lung epithe-