NanoClusters Enhance Drug Delivery in Mechanical Ventilation

By

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ABSTRACT

The overall goal of this thesis was to develop a dry powder delivery system for patients on mechanical ventilation. The studies were divided into two parts: the formulation development and the device design. The pulmonary system is an attractive route for drug delivery since the lungs have a large accessible surface area for treatment or drug absorption. For ventilated patients, inhaled drugs have to successfully navigate ventilator tubing and an endotracheal tube. Agglomerates of drug nanoparticles (also known as 'NanoClusters') are fine dry powder aerosols that were hypothesized to enable drug delivery through ventilator circuits.

This Thesis systematically investigated formulations of NanoClusters and their aerosol performance in a conventional inhaler and a device designed for use during mechanical ventilation. These engineered powders of budesonide (NC-Bud) were delivered via a Monodose® inhaler or a novel device through commercial endotracheal tubes, and analyzed by cascade impaction. NC-Bud had a higher efficiency of aerosol delivery compared to micronized stock budesonide. The delivery efficiency was independent of ventilator parameters such as inspiration patterns, inspiration volumes, and inspiration flow rates. A novel device designed to fit directly to the ventilator and endotracheal tubing connections and the Monodose® inhaler showed the same efficiency of drug delivery. The new device combined with NanoCluster formulation technology, therefore, allowed convenient and efficient drug delivery through endotracheal tubes.

Furthermore, itraconazole (ITZ), a triazole antifungal agent, was formulated as a NanoCluster powder via milling (top-down process) or precipitation (bottom-up process) without using any excipients. ITZ NanoClusters prepared by wet milling showed better aerosol performance compared to micronized stock ITZ and ITZ NanoClusters prepared by precipitation.

ITZ NanoClusters prepared by precipitation methods also showed an amorphous state while milled ITZ NanoClusters maintained the crystalline character. Overall, NanoClusters prepared by various processes represent a potential engineered drug particle approach for inhalation therapy since they provide effective aerosol properties and stability due to the crystalline state of the drug powders. Future work will continue to explore formulation and delivery performance *in vitro* and *in vivo*.

Dedicated to:

My family,

whose boundless faith and encouragement

have enabled my success.

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Chapter 1

Introduction

1.1 Thesis goal, outline and specific aims

The overall goal of this work was to develop a dry powder delivery system for patients on mechanical ventilation. The studies were divided into two parts: the formulation development and the device design. NanoCluster technology was used for formulating inhaled drug powder. Budesonide, a potent glucocorticoid, was selected as a drug model. Inhaled budesonide has been used for treatment of asthma and COPD for a long time[1-3]. Budesonide could be a potent therapy for ventilated patients if delivered effectively. Because the endotracheal tube is a great barrier to successful delivery, in chapter 2, agglomerates of budesonide (Bud) nanoparticles (also known as 'NanoClusters') were formulated and investigated when delivered through different kinds of tubes with negative pressure. Formulas with or without excipients were prepared and compared with micronizied stock budesonide and with the Pulmicort® Flexhaler powder (AstraZeneca LP, Wilmington, Delaware).

The optimized formulation was tested on a mechanical ventilation system (chapter 3). The effect of ventilator parameters on drug powder performance was investigated. Furthermore, a novel device was designed to fit with the ventilator circuit. The performance of the novel device was compared to a modified Monodose® inhaler. The novel device was preferred to the Monodose® inhaler due to the convenience of connecting with the ventilator and endotracheal tubing while maintaining efficient aerosol delivery. NanoCluster technology combined with a new device may provide simple and effective drug delivery to ventilated patients.

The success of NanoCluster technology on budesonide (drug model) led to a formulation of itraconazole NanoClusters (chapter 4). Itraconazole (ITZ) is a triazole antifungal agent that is used to treat fungal infections. It works by slowing the growth of fungi that cause infection. For treatment of fungal infections in the lungs, ITZ capsules are taken orally. However, ITZ can

show low and variable oral absorption due in part to variability in gastric conditions. A study reported the lung tissue concentration increased nearly 10 times when ITZ was administered by inhalation compared to the oral route[4]. Therefore, NanoCluster technology may offer effective drug formulation of ITZ for direct administration to the lungs of mechanically ventilated patients.

1.2 Introduction

Inhalation therapy has been used for treatment of respiratory disorders for a long time[5,6]. In ancient times, patients inhaled a variety of substances released from plants for medicinal proposes such as volatile gases from pines, vapors from heated *Hyosycamus muticus,* and smoke containing atropine from *Atropa belladonna* leaves[7]. For over half a century, inhalation therapy has been employed in mechanically ventilated patients. Although the ventilator was not originally designed for aerosolized medicine, various inhalers have been designed for use in ventilator-supported patients, especially drugs with poor oral bioavailability or local effect in lungs. Despite periodic successes, aerosolized therapies are often not successful due to ineffective and inconsistent aerosol delivery. The major barriers to success are aerosol-generating devices appropriate for ventilator circuits and poor drug formulations that are not conducted through the ventilator circuit and endotracheal tube. To overcome these barriers and optimize aerosol delivery in patients, new aerosol technologies, formulations and devices are being designed.

In the critical care setting, inhaled drugs are routinely delivered to ventilated patients in the form of wet aerosols via nebulizers or pressurized metered-dose inhalers (pMDI). These wet aerosols suffer from rain out, condensing in the ventilator circuit and the endotracheal tube

leading to poor delivery[8]. Thus, dry powder aerosols are being investigated as alternative formulations. In general, dry powder formulations are delivered direct-to-mouth via dry powder inhalers (DPIs). None of the DPIs on the market, however, have been designed for use with ventilator systems. The design of inhalers is important for developing drug delivery systems appropriate for mechanical ventilation. Such DPIs should be designed to optimize drug delivery with typical mechanical ventilation settings. Sufficient shear forces should be generated to break up agglomerated powders. Moreover, the design of the inhaler should allow direct connection with minimal adapters for easier use. In the development of new drug delivery technologies for patients on mechanical ventilation, both particle engineering and inhaler designs play a significant role in the delivery of dry powders. This review will summarize the state of the art for aerosol delivery to patients on mechanical ventilation with special emphasis on emerging opportunities for dry powder aerosols.

1.3 The lungs as a delivery target

The respiratory tract is divided into the upper and the lower airways. The upper airway begins at the nasal or mouth cavity and travels into the larynx, and the lower airway begins at the larynx and travels to the alveoli (Figure 1.1). When inspiration occurs, air passes through the conducting zone (from the trachea down to the terminal bronchioles, the smallest airways without alveoli) to the respiratory zone with respiratory bronchioles and alveoli, where gas exchanges through alveolar walls[9].

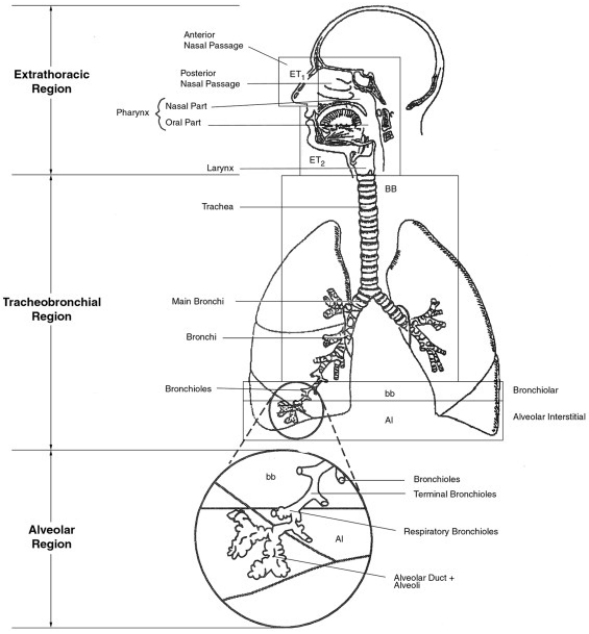

Figure 1.1 Structure of the respiratory system (reprint with permission).[10]

From the trachea, a series of branching airways become more numerous and smaller in diameter down to the alveolar region. The branching pattern of the lungs produces a large surface area within a small volume. The diameter of the airways decreases from about 1.8 cm in the trachea to about 300 μm in alveolar sacs with a corresponding increase in the surface area of the airways[11]. The total surface area of the lungs is approximately 140 square meters in an adult human[12]. There are around 500 million alveoli in the human lungs with a large surface of more than 100 square meters[6,13]. Thus, the lungs generate large surfaces for gas diffusion although their volume is only about 4 liters. Lung epithelium becomes thinner as the airways decrease in diameter, reaching a thickness less than 0.1 μm in the alveoli region[6,14]. The large surface area combined with the thin epithelium provides advantages in both local and systemic delivery.

1.3.1 The lungs as a target for local drug delivery

Inhaled medications have been available for treatment of diseases such as asthma and chronic obstructive pulmonary disease (COPD) for many years. More than 65 inhaled products of more than 20 active pharmaceutical ingredients (API) are on the market[15-17]. For treatment of respiratory diseases, inhalation has many advantages over the systemic route. Pulmonary drug delivery can provide high drug concentrations at the site of disease with minimized systemic exposure and side effects compared to the oral route. If attempting to deliver drugs systemically, inhalation can also eliminate barriers such as poor gastrointestinal absorption and first-pass metabolism.

The efficiency of drug treatment, however, depends on the distribution of drug aerosols within the lungs. For instance, a study showed that histamine aerosols deposited in the conducting airways increase airways obstructions more than histamine aerosols deposited diffusely, because histamine receptors were predominantly in the central airways[18]. Similarly, other studies suggested that the site of drug deposition was important to maximize the therapeutic response[15,19,20]. For example, β_2-agonist deposited in the conducting airways is more important in moderating the bronchodilator response than β_2-agonist deposited into the alveolar region[19]. Ipratropium bromide (an anticholinergic drug used for the treatment of chronic obstructive pulmonary disease and acute asthma) needs to be delivered to the conducting airways to produce therapeutic effects due to the presence of receptors in the smooth muscle of the bronchi[21,22]. Inhaled corticosteroids act through the glucocorticoid receptor in bronchial epithelial cells[20,23], so the target area of corticosteroid deposition is also in the conducting airways. A large portion of corticosteroid deposited in the alveolar region may not achieve the

desired efficacy. On the other hand, some lung infections require the antibiotic to be evenly distributed throughout the lungs or primarily in the peripheral region[15]. Although the target site for most respiratory diseases is in the conducting airways, the optimal site of deposition for aerosolized antibiotics depends on the indication.

1.3.2 The lungs as a target for systemic drug delivery

Besides local diseases, inhalation has been recently studied for treatment of systemic diseases such as diabetes[24], migraines[25,26] and seizures[27]. Pulmonary drug delivery offers several advantages over other routes of administration for delivery of therapeutic drugs into the systemic circulation. It has no 1st pass effect when drugs are administered via pulmonary drug delivery. As discussed earlier, the lungs have a large absorptive surface area and a highly permeable membrane of thin epithelium in the alveolar region. The alveolar membrane is an ideal site for exchange between the blood compartment and the external environment. Pulmonary drug delivery can provide rapid absorption and increased bioavailability of drug molecules compared to oral administration. It is suitable for a wide range of substances from small molecules to large proteins, especially for large molecules with low absorption rates[14].

Generally, macromolecules such as proteins cannot be administered orally because they are digested and not absorbed into the bloodstream due to enzyme activity and the absence of an appropriate transcellular transport pathway. Administration of these kinds of molecules is primarily intravenous or subcutaneous injection. Inhalation, however, can increase bioavailability of peptides and proteins 10 to 200 times compared to other routes[28]. Inhaled insulin (Exubera) was approved by the FDA and the European Medicines Agency[29]. Inhaled insulin showed similar pharmacokinetic and intra-individual variability as injectable insulin[30]. It

provided noninvasive "needle-free" administration, especially for patients with a true needle phobia. In clinical trials, patients were more satisfied with inhaled insulin than with subcutaneous insulin[31,32]. Although Exubera was discontinued, other manufacturers of inhaled insulin are still in clinical trials[24].

For small molecules, pulmonary delivery can provide very high bioavailability and more rapid onset of drug action compared to the oral route. This is suitable for patients who need a sudden response because some drugs, particularly hydrophobic molecules, can be absorbed within seconds after inhalation[28]. Inhaled ergotamine tartrate has been studied for treatment of migraine for many years[33]. The drug itself had been used for the treatment of migraine since the early twentieth century[34]. Presently, inhaled dihydroergotamine mesylate (DHE) is being developed. The product, called Levadex, is waiting for FDA approval. Researchers mentioned that inhaled DHE should be suitable for patients who were not responding well to oral triptans (serotonin receptor agonists) or patients who needed a long-acting medication[25]. Intrapulmonary delivery is also being developed for seizure protection. Inhaled propofol hemisuccinate (PHS), a prodrug of propofol, has been administered in the form of a nebulized solution. Inhaled PHS was suggested to provide rapid onset for seizure protection and abort seizure clusters without causing prolonged sedation[27].

1.3.3 Drug Absorption, Metabolism and Clearance from the Lung

Drug absorption is important for both locally-acting and systemically-acting drugs. Drug absorption may determine the residence time of the drug in the lungs, as well as the onset of systemic effects. Drug absorption also affects the therapeutic profile for systemic drugs. The absorption of pulmonary drugs depends on the drug type and site of drug deposition. Small

hydrophobic molecules, for example, are absorbed within seconds throughout the lungs by passive diffusion whereas small hydrophilic molecules are absorbed by specific transporters or via the tight junctions[14].

After deposition in the tracheobronchial airways or alveolar regions, the inhaled drugs encounter mucociliary escalator (mucus and ciliated cells) or lung surfactant, respectively[6]. For macromolecules such as peptides or proteins, mucus is a barrier to molecule access to the epithelium. Similarly, lung surfactant in alveolar regions may also cause a barrier for absorption by inducing aggregation of large molecules, resulting in engulfment and digestion by airspace macrophages[14]. However, lung surfactant was found to enhance the solubility of small molecules such as glucocorticosteroids[35] and cationic compounds (due to the negatively charged phosphatidylglycerols[36]).

After passing mucus or surfactant layers, drugs have to diffuse through the 0.01-10 μm thick lining fluid to get to epithelium that vary from monolayer columnar cells (60 μm) in the bronchi to monolayer squamous cells in the alveoli (0.2 μm). If absorbed into the bloodstream, the drugs pass through the basement membrane and the vascular endothelium[37,38].

The metabolism of several inhaled compounds occurs in lung tissue. This tissue consists of several cell types with different expression levels of metabolizing enzymes. CYP3A5 (the cytochrome P450 (CYP) families) are important detoxification enzymes in the lung tissue[39]. These cytochrome families have high expression levels in hepatocytes and enterocytes, but low expression in the lungs. The low metabolic activity combined with rapid absorption can result in high bioavailability for many small molecules; however, the formation of local metabolites should not be ignored since local metabolites may have therapeutic or toxic effects. In contrast,

local metabolism in the lungs can also have advantages for some drugs such as prodrugs and budesonide. Metabolic enzymes can activate prodrugs. For budesonide, the conjugation between budesonide and fatty acids in the lungs has been reported to lower the elimination rate, resulting in longer retention time and duration of effect in the lungs[40,41].

Inhaled particles can be eliminated by cough, mucociliary clearance, or alveolar macrophages, depending on the size of particles and the site of deposition. According to one publication, insoluble particles larger than 6 μm in geometric diameter are normally eliminated after deposition in the airways by mucociliary clearance[42] whereas smaller particles can penetrate the mucus and enter the bronchial epithelium. In patients for whom mucociliary clearance decreases, coughing is more important for removal of particulates from the airways. For example, patients with COPD showed around 60% of particles deposited in central airways were eliminated by coughing compared to 8% in healthy people. The total clearance was similar in both groups[43]. Finally, slowly dissolving particles in the size range of 1.5-3 μm deposited in the alveolar region were found to be phagocytosed by alveolar macrophages[44].

1.4 Particle behavior in the human respiratory tract and deposition

In general, particles are under the influence of mechanical and electrical forces but only charged particles are affected by electrical forces. Since pharmaceutical particles do not usually maintain charge, the transport of the particles in the human lungs is primarily governed by mechanical forces. Mechanical forces on inhaled particles cause the trajectories of inhaled particles to differ from airstream lines. When inhaled particles are carried with inspired air through the respiratory tract, the particles are displaced off airstream lines and deposited on the

surfaces of the respiratory tract. Drugs are deposited in the respiratory tract by three main mechanisms: inertial impaction, gravitational sedimentation and Brownian diffusion[45].

Inertial impaction is a velocity-dependent transport phenomenon. It occurs mainly in the oropharynx and large conducting airways in which the airflow velocity is maximized. When particles with a mass median aerodynamic diameter (MMAD) of more than 5 μm pass through these regions, the particles are transported toward surfaces of the respiratory tract by inertial impaction[46]. This mechanism increases with particle velocity, diameter and density. On the other hand, gravitational sedimentation and Brownian diffusion are time-dependent transport phenomena. Brownian diffusion mainly occurs in small airways where the residence time of the air is maximized (i.e. during breath hold). Ultrafine particles (particles smaller than 0.1 μm in diameter) are, in theory, deposited solely due to diffusion. This random displacement increases with decreasing particle diameter and is independent of the particle density. Density is more important for particles transported by sedimentation. Gravitational sedimentation increases with the size and the density of the particles[45].

The size, shape and density are important aspects of inhaled particles since they can affect transport properties, as well as the deposition of particles in the lungs. For example, a small heavy sphere can have transport properties identical to a large light sphere, resulting in the same deposition. Since the velocity of particles is determined by their size, shape, and density, particles with the same aerodynamic behavior may exhibit the same transport properties. Thus, the particle size is normally defined by the aerodynamic diameter (d_a). By considering a spherical particle settling under gravity through air, the aerodynamic diameter of the particle is

the diameter of a spherical particle with density of 1 g/cm^3 that has the same settling velocity. The aerodynamic diameter can be calculated using the following equation[45,47]:

$$d_a = \sqrt{\frac{\rho}{\rho_0}} d_g \qquad \text{(Eq.1.1)}$$

where ρ is the mass density of the particle, ρ_0 is the unit density (1 g/cm^3) and d_g is the geometric diameter.

The deposition of particles in the lungs can be divided into four regions: extrathoracic, upper bronchial, lower bronchial, and alveolar[48]. Particles larger than 5 μm tend to deposit in the mouth and upper airways whereas smaller particles (d_a = 1 - 5 μm) deposit deeper in the lungs. For deposition in the alveolar region, particles in the size range of 1 to 3 μm are desired. Although particles less than 100 nm have been shown to deposit in the alveolar region, most very small particles (d_a < 1 μm) are exhaled. One study showed that particles smaller than 1 μm can be exhaled up to 80% without deposition[49]. To optimize deposition in the lungs, particles should be small enough to avoid deposition in the mouth, throat and upper airways, but they should not be too small, to avoid exhalation[6].

1.5 Inhalers

Inhalation devices can be divided into three major categories: nebulizers, meter dose inhalers (MDIs), and dry powder inhalers (DPIs). The first nebulizer, called a glass nebulizer, was introduced in the late nineteenth century. The refinement of the glass nebulizer led to the hand-bulb nebulizer in 1938 and then to the first MDI in 1956. DPIs were introduced in 1967, concurrent with the environmental concerns of chlorofluorocarbon (CFC) propellants used in MDIs. DPIs addressed inhalation coordination difficulties associated with the MDIs. DPIs also

faced problems, however, such as individual variation due to the inspiration ability of patients. Nebulizers, MDIs, and DPIs have been continuously refined leading to modern inhalation devices. The details of the evolution of early pulmonary delivery devices have been published[51].

Nebulizers generate drug aerosols through the nebulization of suspensions or liquid droplets, typically water with excipients or co-solvents. Currently nebulizers can be categorized into three main types: compressor (air jet), ultrasonic, and vibrating mesh. Compressor nebulizers are air-driven devices. The liquid stream is broken into aerosol droplets by the compressed air pressure. Droplets in the size range of 1-5 μm can reach the lower airways while droplets larger than 5 μm are more likely to be filtered out by impaction within the device or recirculated in the nebulizer reservoir. This recycling of large droplets can increase drug concentration in solution[52]. Moreover, the drug lost in the nebulizer and the mask or mouthpiece can lower efficiency or cause inter-patient variability.

Ultrasonic nebulizers have a piezoelectric crystal for generating high frequency vibrations. The vibrations are transmitted through liquid medicine to generate fine aerosols delivered to patients. The aerosol from these nebulizers may be larger than the aerosols from compressor nebulizers. Ultrasonic nebulizers have limited use with suspensions or formulations with high viscosity. Ultrasonic nebulizers, however, have advantages of silent operation, less time of nebulization and higher dosing efficiency.

Vibrating mesh nebulizers have recently been developed to overcome many disadvantages of conventional compressor and ultrasonic nebulizers. Vibrating mesh technology uses the piezoelectric crystal to vibrate a mesh plate in contact with the drug. Aerosols are generated as a fine mist when liquid medication passes through the holes of the vibrating metal grid. The pore size of the mesh and output rate can be adjusted to optimize performance for

different drugs. These devices also have quiet operation, high nebulization rates, and have been optimized for small volume formulations[53]. Consequently, the treatment time is also decreased.

Pressurized metered-dose inhalers (pMDIs) are a convenient and accepted mode of for delivering drug medications to patients with asthma and COPD worldwide. Approximately 500 million pMDIs are produced each year since they are cheaper and more portable than other devices. pMDIs can be used to deliver both solutions and suspension formulations by actuating the device to release a metered dose of propellant-drug aerosols through a valve system[54].

The key components of pMDI consist of propellant, formulation, metering valve and actuator. The particle size distribution of the aerosols depends on the physicochemical properties of the formulation and the device design[55]. Drugs are dissolved or suspended in a liquid propellant and surfactants such as oleic acid may be added to disperse drug particles in suspension and lubricate the valve mechanism in the CFC-containing pMDIs. The high concentration of non-volatile surfactants in some formulations may increase the droplet size of the aerosols because they slow down evaporation of volatile propellants. Propellants used in the pMDIs have been changed from chlorofluorocarbons (CFCs) to hydrofluoroalkanes (HFAs) due to environmental concerns. Some surfactants commonly used in CFC pMDIs are insoluble in HFAs. In this case, ethanol can be used as a low-volatility co-solvent in HFA formulations to solubilize the surfactants or solubilize the drug itself. Metering valves function to deliver a reproducible amount of drug formulations. Spray actuators combined with the stem of the metering valve function to spray formulations via the actuator nozzle. The compatibility of the formulation with the metering valve and the design of the actuator are important since variable concentration of suspension at the inlet can cause a variation in drug dosing. Aerosol particle size also depends on the nozzle diameter. Moreover, the pMDIs require coordination between

actuation and inhalation to optimize drug deposition in the lungs. This requirement can cause variability in pMDIs due to patient misuse[56].

Dry powder inhalers (DPIs) are breath-actuated devices that have emerged as the most popular inhalation device. There are over 20 DPIs on the market and more than 25 in development[58]. DPIs can be categorized into three types: single-unit dose devices, multi-dose reservoir devices, and multi-unit dose devices. In single-unit dose devices such as the Handihaler™, each dose is loaded into a capsule, which is inserted into the device before use. After use, the capsule has to be removed before a new one can be placed in the device. Because of the capsule replacement for each dose, Single-unit dose devices are inconvenient for patients compared to multi-dose DPIs. Multi-dose DPIs can be multi-dose reservoir devices, which contain drug powders that are released with each actuation, or multi-unit dose devices which contain multiple capsules or blisters. Multi-unit dose devices can provide more reproducible doses compared to the multi-dose reservoir devices[58].

In general, DPIs are passive devices in which the delivery efficiency depends on inspiration efforts of patients. The DPIs were designed to induce sufficient turbulence airflow to deagglomerate drug particles. DPI formulations can contain the drug mixed with excipients or the drug itself. Lung deposition from these devices has been reported to vary from 12 to 40%[58]. Since the drug delivery efficiency of DPIs depends on turbulent airflow to aerosolize drug formulations, the device design, drug formulation, and inspiration flow pattern are all important.

1.6 Relationship of drug formulations and DPIs

The size of wet aerosols is affected by the device and formulation properties such as surface tension. Similarly, DPIs disperse powders and this process is significantly impacted by

the formulation. Nebulizers and pMDIs use liquid formulation to generate wet aerosols whereas DPIs generate dry powder aerosols via deagglomerating of drug formulations. Although the devices produce different kinds of aerosols, effective aerosols should have aerodynamic diameters of 1-5 μm to deposit in the lungs or 1-3 μm to deposit in the alveolar region.

Particles in dry powder formulations can be engineered in many ways such as deceasing particle density, lowering surface energy, and changing the morphology of the particles. For example, porous particles are designed to decrease aerodynamic diameters of the particles. Because of their low density and large geometric diameter, these particles can have aerodynamic diameters similar to smaller and denser particles but with less surface energy. Engineered particles can have rough surfaces to increase the air drag force. For instance, non-porous corrugated particles showed enhance aerosol performance over smooth spherical particles. The inhaler device and airflow also showed diminished influence on the dispersion behavior of these particles compared to smooth particles[59]. These particles have less contact area between the particles, resulting in reduced cohesion of the drug powder. The aerosol performance can also be enhanced by using fine carriers such as lactose or other excipients (e.g. a lubricant). Fine lactose carriers were found to enhance the powder flow and also increase the powder bulk for capsule filling[60].

The design of the device is also important to control the size range of particles emitted from the DPI[61]. The device design contributes to deagglomeration and dispersion of drug powders. Deagglomeration of powders tends to increase as shear force increases. Although the shear force may be increased by increasing flow rate, the impaction of drug powders may be increased at high flow velocity, as well, resulting in loss of drug powders in the mouth, throat and upper airways.

The resistance of devices affects the turbulence of air through the devices. Normally, high resistance DPIs will generate higher turbulence, leading to higher fine particle fraction (FPF) of drug formulations[62]. DPIs with similar resistance, however, may also have different dispersion efficiency because of the different turbulence patterns in the DPI. The turbulence pattern of the device is caused by the internal geometry of the device such as the dimensions and shape of the air channels. For example, a study showed an important role of grid structure on inhaler performance. They suggested that the inhaler grid directly affected the amount of powder retention within the device by affecting the frequency of contact between the particle and the mouthpiece[63]. The comparison of two DPIs, which have similarly low resistances of 0.03 – 0.04 $cmH2O^{1/2}$/L/min, showed the FPF using the Dinkihaler® were higher than with the Rotahaler® due to the higher dispersion efficiency of Dinkihaler®[61]. In addition, different formulations can show different performance even when employing the same device. Thus, particle engineering normally occurs concurrently with the development of devices. Most of the DPIs on the market have been designed for use with particular drug formulations.

1.7 Drug delivery in mechanical ventilation

In the past, aerosol delivery in patients on mechanical ventilation was difficult due to the very poor efficiency of drug delivery through a ventilator circuit and an endotracheal tube. Moreover, lack of understanding and the dated technology of ventilators contributed to unsuccessful aerosol delivery[64]. Although various kinds of inhalers have been developed for delivering drug aerosols into ambulatory patients, only nebulizers and pMDIs have been routinely used for clinical therapy during mechanical ventilation. Drugs lost in the ventilator circuit and the endotracheal tube are still major barriers to the success of aerosol drug delivery

during mechanical ventilation[65]. For example, *in vitro* studies of a bronchodilator drug delivered by jet nebulizers showed only a very low quantity of inhaled drugs deposited on the filter at the end of an endotracheal tube[66]. Drug dosing is also highly variable[67]. Inhalation therapy is still common during mechanical ventilation, meanwhile new inhalers and connections are being developed[68,69]. Furthermore, factors that affect the efficiency of drug delivery in these patients have been investigated.

1.7.1 Ventilator circuit-related factors influencing aerosol delivery during mechanical ventilation

In general, patients who have a severe breathing problem have to be on mechanical ventilation. A ventilator provides mechanical assistance or replaces spontaneous breathing for patients. It controls the respiratory system of patients on mechanical ventilation using different parameters, which can play a role on aerosol drug delivery to these patients. Thus, to improve drug delivery efficiency to ventilated patients, some parameters such as inspiration patterns, inspiration volume, volumetric flow rate, humidity and tubing are normally investigated.

1.7.1.1 Inspiration patterns

A mechanical ventilator can control phases of breathing in either mandatory or spontaneous modes that can be categorized into three types: continuous mandatory ventilation (CMV), intermittent mandatory ventilation (IMV), and spontaneous mode. The CMV mode is a full ventilation support mode that is selected for patients with respiratory failure, especially those who have a problem of inadequate alveolar ventilation. Mechanical ventilation can be applied in patients by using assisted or controlled modes of support. The assisted mode offers many advantages over controlled mode such as reducing the need for sedation and paralysis,

decreasing the risk of barotrauma, and preventing muscle atrophy[70]. During assisted ventilation, a ventilator pump influences patient breathing. The control of breathing depends on both ventilator settings and the patient's ventilatory demand and respiratory system mechanics. Therefore, the interplay between the ventilator and patient's control over their breathing may lead to patient-ventilator asynchrony[71]. Patient-ventilator asynchrony occurs when there is a mismatch between the inspiration of the patient and mechanically assisted breath, which prevents the ventilator from aiding breathing and may cause injury to the patients[72].

A primary goal of mechanical ventilation is not only to improve gas exchange but also to reduce the work of breathing (WOB). Improper ventilator settings can increase WOB which can lead to muscle fatigue and hypercapnia[73]. Normally, inspiration patterns on a ventilator can be categorized into three kinds of waveforms; sine, ramp (decelerating), and square waveforms. A sine waveform provides a continuous increase and decrease of flow rate in the form of a sine wave. A ramp waveform generates the highest inspiration flow rate at the beginning of the cycle, and then the flow rate gradually decreases along the cycle. A square waveform provides a constant flow rate throughout an inspiration cycle[74]. A suitable waveform depends on the mode of the ventilator setting. A square waveform, for example, is traditionally applied for volume-limited breaths during ACV. The decelerating ramp waveform is also often used in volume-limited modes. The ramp waveform was recommended for patients with COPD requiring intermittent positive-pressure ventilation (IPPV)[75]. The ramp waveform showed the lowest WOB compared to sine and square waveforms. It also improved lung mechanics and optimized gas exchange. In patients with normal respiratory mechanics, inspiration patterns have no major effect on gas exchange and WOB. Nevertheless, in patients with a more severe lung injury, the

ramp waveform may be more effective since it may allow more time for gas distribution in the alveoli[70].

In terms of drug delivery, a study showed that inspiration pattern caused statistically significant differences in nebulizer performance[76]. The square waveform produced higher output efficiencies and a constant output particle size over a breathing cycle. A simulation of different waveforms, however, suggested that the square waveform produced slight over predictions of output compared to real nebulizer patterns. The sine waveform showed the same results as nebulizer patterns in both the bench tests and in simulations. This suggested that the sine waveform was more suitable for testing drug delivery during nebulization. The sine waveform is commonly utilized in respiratory therapy under mechanical ventilation. During inspiration at rest, the inspiration breathing is theoretically a sine function over time[73].

1.7.1.2 Inspiration volume

Patients with respiratory diseases such as COPD normally have lower inspiratory capacity compared to healthy people[77]. A study reported that setting the tidal volume at greater than 500 mL in an adult model improved aerosol drug delivery[78]. Although the large tidal volume may increase aerosol deposition efficiency, caution should be used since it also can cause volutrauma if it is greater than 8-10 mL/kg[79].

1.7.1.3 Volumetric flow rate

Volumetric flow rate dictates flow in the system. High flow rate can increase turbulence and also the inertial impaction of aerosols. Some studies suggested that a lower inspiration flow rate (e.g. 40 versus 80 L/min) improved aerosol delivery in both non-ventilated patients and

ventilated patients[65,80]. Aerosol delivery had a direct correlation with duty cycle (inspiratory time (T_I)/duration of total breathing cycle (T_{TOT}). Investigators found that increasing the duty cycle (T_I)/ (T_{TOT}) improved lower-respiratory-tract aerosol delivery[67]. The longer inspiratory time allows a larger proportion of the nebulizer-generated aerosol to be inhaled with each breath. With the jet nebulizer, more albuterol was delivered with longer inspiratory times[81]. Another study also found greater albuterol delivery to the bronchi with a T_I/T_{tot} of 0.50 than of 0.25 when delivered via MDIs. The study reported a greater effect of volumetric flow rate compared to T_I/T_{tot} on aerosol delivery. The delivery of albuterol was twofold greater when applied at 40 L/min compared to at 80 L/min[65]. Moreover, if the expiratory time is excessively shortened, dynamic hyperinflation may complicate the use of longer inspiratory times. For routine clinical use, a slower inspiratory flow rate was preferred to excessively long inspiratory times to maximize aerosol delivery. The investigators suggested that aerosol delivery in ventilated patients should 'go slow with the flow'[82].

Volumetric flow rate is also important for aerosol delivery when using DPIs. Most all DPIs on the markets are passive inhalers. DPI performance is typically flow dependent. The dispersion of drug powders depends on the inspiration effort of the patient and the resistance within the inhaler. As mentioned previously, a higher flow rate can increase turbulence. Turbulence has an important effect on powder dispersion, potentially resulting in an increased dispersion of powders. Since turbulence and powder impaction may increase when applied at a higher flow rate. The effect of the flow rate on powder performance is, thus, an important parameter when delivering drug aerosol to ventilated patients.

1.7.1.4 Humidity

Ventilator circuits are often humidified and heated. Approximately 40-50% of drugs can be lost when heated/humidified ventilator circuits are used[83,84]. Investigators suggested that drugs were lost in the humidified ventilator circuit because of increasing particle impaction in the ventilator tubing. Fink et al. found greater albuterol deposition in the ventilator circuit and the endotracheal tube caused lower drug delivery to a lung model when the aerosol was delivered via pMDI[65]. Miller et al. also reported an increase of particle size due to droplet growth when albuterol was delivered via a nebulizer in a humidified circuit[85]. The loss of drug aerosol can be reduced by turning off or bypassing a humidifier during aerosol administration. Bypassing the humidifier for a long time, however, can harm the airway mucosa, which could be exacerbated by in the case of some nebulizers that require up to 35 minutes to complete aerosolization[83]. Although the pMDI required a short interval to administer aerosols, disconnecting the ventilator circuit to bypass the humidifier can increase the risk of infection or ventilator-associated pneumonia in both nebulizer and pMDI use. Therefore, humidification may be maintained during pMDI or nebulizer delivery of bronchodilators[84]. For a very expensive drug such as an "antibiotic", a dry circuit could be employed, but drug administration was advised to be achieved within 10 minutes or less[86].

Relative humidity is also known to affect dry powder aerosols. A study showed lower drug delivery efficiency in both dry and humid environments depending on the physicochemical nature (i.e. hygroscopicity) of the drug[87]. For example, drug delivery efficiency can be decreased because of capillary force between the particles in a humid environment[88,89] or due to static charges between the particles in a dry environment[90]. Previous works from our group showed

decreasing drug delivery efficiency when powders were applied through an endotracheal tube at lower relative humidity (40% RH compared to 50% RH)[91]. Conversely, another study showed lower drug delivery efficiency during mechanical ventilation when powder aerosol was applied at 82% RH compared to at 55% RH[92].

1.7.1.5 Endotracheal tube and device placement in the circuit.

Aerosol medication in mechanically ventilated patients is routinely administered through an endotracheal tube. The endotracheal tube is passed through the mouth or nose into the trachea. The narrow diameter of endotracheal tubes, compared to the normal upper airway, create higher air flow resistance in ventilated patients compared to non-ventilated patients. The resistance of the endotracheal tube depends on inspiratory airflow. When a high inspiratory airflow is employed during mechanical ventilation, high shear and turbulence may be generated. Both airway resistance and turbulence influence aerosol deposition in the lung and along the tube. Since the endotracheal tube is the narrowest part of the ventilator circuit, the highest resistance to air flow is created in this area. The endotracheal tube may substantially reduce aerosol delivery in ventilated patients. A study showed that pulmonary deposition decreased 10-14% in these patients compared to ambulatory patients[93].

Aerosol delivery through endotracheal tubes is influenced by many factors such as endotracheal tube size, endotracheal tube material/design, the type of aerosol generator used, and ventilator settings. An *in vitro* study by Crogen and Bishop reported a decrease of aerosol delivery to the filter with reduction of the inner diameter of the endotracheal tube[94]. Some investigators mentioned, however, that these reports might have overestimated the aerosol-delivery impediment created by the endotracheal tube due to the closer placement of the aerosol

generator to the tube[84]. The placement of the aerosol generator also influences the aerosol deposition within the tube. A study showed that the deposition within the tube was decreased and the deposition in the lungs was increased when the aerosol generator was placed farther away from the endotracheal tube[84]. Similar to another study, placing nebulizers closer to the ventilator instead of closer to the patient decreased aerosol impaction in the tube. In addition, using a spacer with a pMDI, a study showed reduction of aerosol loss within the tube when placing the spacer at a distance of at least 15 cm from the endotracheal tube[93]. Although investigators mentioned that the type of aerosol generator and the ventilator settings had a greater effect than the tube size, the appropriate size should be selected to minimize aerosol loss within the tube. "Priming" the tube with a few doses before use may also reduce aerosol deposition within the tube by blocking electrostatic charge on the inner walls of the tube[93].

The endotracheal tube is normally coated with a bacterial biofilm within hours of intubation[95]. A study showed biofilm developed within 6 hours of ventilation. The microorganisms may be transported into the pulmonary tree and cause infection and ventilator-associated pneumonia (VAP)[95,96]. The presence of biofilms in narrow endotracheal tubes can potentially cause tube obstruction[97,98]. This type of obstruction can appear during the first 24 h of mechanical ventilation[98]. The study showed more than 10% obstructions occurred in around 60% of patients and 22% of them showed that the tube inner-diameter reached critical values[98]. Moreover, mucus and secretion within the tube may alter gas flow, create turbulence, increase work of breathing and cause the release of fluid droplets[99,100]. A traditional way to remove mucus lodged within the endotracheal tube is via a suction catheter, which is inserted into the endotracheal tube. Although this method may not remove all secretions, the process of suction can be repeated frequently[100]. A study in sheep showed that the sterile suctioning of the endotracheal tube was performed every

6 hours or as needed[101]. Furthermore, a new technology such as the Muscus Shaver was developed in order to improve the efficiency of removing secretions from the endotracheal tube[96,100].

1.7.2 The differences in direct-to-mouth and ventilator aerosol delivery

Many aerosolized drugs are delivered to conducting airways to minimize systemic exposure and to achieve high local concentration. To treat pulmonary diseases such as asthma or COPD, aerosolized drugs are delivered to patients by direct-to-mouth oral inhalation. The portion that deposits on the tongue or the back of the pharynx may be swallowed and absorbed in the gastrointestinal (GI) tract. Drug could then distribute systemically or be inactivated by first-pass metabolism in the liver. This portion of the aerosol drug can cause adverse side effects or can waste valuable medication.

In contrast to outpatients who receive inhaled drugs direct-to-mouth, patients on mechanical ventilation are delivered inhaled drugs through an endotracheal tube. The endotracheal tube is inserted into the mouth until just above the first bifurcation. Therefore, systemic adverse effects caused following deposition in the mouth and swallowing can be bypassed. Nevertheless, a large amount of drug loss in the endotracheal tube can impede drug delivery in ventilated patients.

1.7.2.1 The effect of airway geometry

In general, deposition patterns in human airways are controlled by three major factors: the aerodynamic particle size distribution, the inhalation flow rate, and airway geometry. The first two factors are related to the aerosol generation system and formulation whereas the airway

geometry is strictly a patient characteristic. The geometric complexity affects flow and particle transport dynamics inside the airways[102]. Low velocity was reported for the oral cavity, whereas a higher velocity was found in the pharynx/larynx region, and the velocity increased rapidly after the glottis[103].

For non-ventilated patient, many studies have tried to understand and predict the behavior of particles and airflows along the airway[104,105] by modeling the geometric surface of the extrathoracic oral airways extending from the mouth through the larynx [103]. Simulated velocity profiles in the pulmonary airway include an oral-tracheobronchial airway model and a bifurcation airway model during inhalation[106]. Numerical predictions have to be developed further to improve geometric and physical realism[107].

In ventilated patients, although using an endotracheal tube can bypass the deposition and variation of airflow in the upper airways, the airway geometry is also important for drug delivery. The airway geometry has an influence on turbulent fluctuations along the airways. Turbulent airflow may enhance the deposition of nanoparticles in the upper airway, especially at a high flow rate. Lower deposition of vapors was observed at a higher flow rate because a longer residence time favors deposition of small particles[102].

1.7.2.2 The effect of device geometry

The device geometry influences aerosol performance in both non-ventilated patients who received inhaled drugs through the mouth and ventilated patients who received the drug through an endotracheal tube. Inhalation devices have been modified to increase drug delivery. For patients who receive inhaled drugs direct-to-mouth, inhaled aerosols can be delivered via different kinds of inhalers. Both geometry of the oral airways and geometry of the device are

important to deliver the drug aerosol to the lung, and avoid deposition in the device or in the extrathoracic cavity.

pMDIs with a spacer and a smaller exit diameter nozzle, for example, reduce droplet size and spray inhalation speed, resulting in higher deposition efficiency. Moreover, drug delivery via the same system with or without a spacer was also simulated[108]. Besides nebulizers and pMDIs, DPIs is an alternative inhaler for widely using with non-ventilated patients. Drug delivery from DPIs depend on a combination of device and formulation properties[109]. Aerosolization performance of different DPIs was studied to identify and understand the key performance attributes of DPI devices at different flow rates[63,110]. Devices have a specific resistance that can affect the fluidization and dispersion of drug powders[111]. In order to compare performance, these behaviors should be investigated in both a test device and a reference device[110]. Generally, the dispersion performance from DPIs depends on both patients' inspiration efforts and the resistance of the device. For instance, patients with different ages such as preschool children and elderly people can have dramatically different abilities to generate inspiratory flows. In preschool children, some studies found that children had to be older than 5 years to maintain dose consistency[112] . At the same time, the ability of children[112,113] and elderly patients[114,115] to provide sufficient inspiration flow depended on the resistance of DPIs. Therefore, the fluid dynamics of DPIs were investigated at different flow rates. The studies suggested that if details of the device flowfield are known, the dispersion performance of DPIs could be predicted[116].

The understanding of device geometry can be applied for ventilated patient also. The important of device that influenced aerosol drug delivery in this kind of patients was discussed in the following section. Although DPIs have not been used with ventilated patient, the

understanding of DPI geometry should benefit the development of DPIs for ventilated patient in advance.

1.7.3 Device-related factors influencing aerosol delivery during mechanical ventilation

Drug medication such as bronchodilators, corticosteroids, and antibiotics are delivered to ventilated patients by using either nebulizers or pMDIs. Nebulizers have been commonly used for drug delivery with these patients for quite some time. Over the past 25 years, however, pMDIs have been accepted for administering inhaled aerosols to ventilated patients because of effective cost, convenience, reliability and the safety of these devices[117]. Since the devices are important determinants of the efficiency of drug delivery, device design and performance factors should be considered.

Nebulizer and pMDI devices have different mechanisms to generate aerosol. The factors that affected aerosol delivery of the nebulizers included nebulizer type, residual volume, nebulizer mode, position of the nebulizer and gas flow[79]. Three different types of nebulizers have been used for aerosol drug delivery during mechanical ventilation but jet nebulizers are the most commonly used in hospitals because of the ease of use and because they are typically cheaper than vibrating mesh and ultrasonic types. Most jet nebulizers, however, are less effective than ultrasonic and vibrating mesh nebulizers. A study showed lower efficiency during mechanical ventilation of jet nebulizers compared to ultrasonic nebulizers although the aerosol deposition in the lungs was poor for both (5.3±1.4 % for ultrasonic nebulizers and 2.3±0.9 % for jet nebulizers)[118]. Different brands and different batches of the same brands can also lead to variability in aerosol deposition[66,119-121]. A study showed broad variability of nebulization time and total amount of drug delivered to the lungs of ventilated patients among different

commercial nebulizers[121]. The high cost, the bulkiness, and the relative inefficiency of nebulizing drug suspensions limits the use of ultrasonic nebulizers in both non-ventilated patients and ventilated patients[86].

Ultrasonic nebulizers and vibrating mesh nebulizers can provide a higher nebulization rate in a shorter period of time compared to jet nebulizers[122]. Since jet nebulizers do not function well with small fill volumes, the medication may have to be diluted to increase the volume, thus increasing the treatment time. Moreover, at the end of nebulization, jet nebulizers retain high residual volumes in the reservoir. The residual volume of jet nebulizers is 0.8-1.4 mL whereas the residual volume ranges from 0.5 - 1.0 mL in ultrasonic nebulizers and 0.1- 0.5 mL in vibrating mesh nebulizers[123]. Because vibrating mesh nebulizers have the lowest residual volume, they usually offer higher efficiency than the other two nebulizers. Moreover, vibrating mesh nebulizers can decrease the risk of contamination entering the mediation reservoir because the medication reservoir is separated from the ventilator circuit by the mesh. On the other hand, jet nebulizers are designed for varied levels of compressed air flow and pressure. The compressor or gas source should be matched with the jet nebulizer design otherwise the particle size of drug aerosols may be increased[124]. The treatment time in jet nebulizers is also affected by gas flow rate, which is not pertinent for electrically powered nebulizers such as ultrasonic and vibrating mesh nebulizers.

Another factor that affects aerosol delivery in nebulizers is the mode of nebulization. Some studies found that intermittent nebulization was more efficient than continuous nebulization since aerosol loss during exhalation was minimized[66]. The position of the nebulizer may also play a role in aerosol delivery efficiency. A study suggested that the nebulizer should

be placed prior to the humidifier to increase drug delivery, especially with the vibrating-mesh nebulizer, although drug delivery with the vibrating-mesh nebulizer was 2-4 fold greater than with the jet nebulizer at all positions[69].

Like nebulizers, the position of a pMDI in ventilator circuit also affected the efficiency of aerosol delivery. Researchers used *in vitro* models of adult mechanical ventilation to compare the efficiency of aerosol generators at different positions in the ventilator circuit under both dry and humidified conditions. They found that the ultrasonic nebulizer, the vibrating mesh nebulizer, and pMDIs with spacer placed in the inspiratory limb 6 inches from the Y adapter yielded the highest deposition[125]. Other factors that affect the efficiency of pMDIs are actuation and priming and shaking the canister. Actuation of the pMDIs should be synchronized with the precise onset of inspiration to maximize aerosol drug delivery. A reduction of inhaled mass by 35% was reported when actuation was not synchronized with inspiration[83]. Also, a study showed at least 40% higher dose when pMDI was actuated at the onset of inspiration compared to actuation during expiration[126]. pMDIs need to be primed and shaken well before the first actuation of each dose administered because the drugs in the pMDI formulation tend to separate from the propellants when standing, resulting in decreasing total and respirable dose by as much as 25 and 35%, respectively[127].

Nevertheless, the variability of drugs delivered to the lungs due to the low efficiency of liquid formulations beckons the development of dry aerosol powder technology for ventilated patients. Although DPIs are not available for ventilated patients, there are some studies of DPIs modified for use during mechanical ventilation. For example, the Turbuhaler® was modified by removing the outer covering of the Turbuhaler® and putting it in a closed chamber that connected

to the ventilator circuit[128]. The researchers suggested that dry powder drug delivery was worthy of further development, especially in the intensive care setting, although some drug was lost in an endotracheal tube. The percent of drug lost should be reduced when a dry endotracheal tube and non-humidified system are applied[79]. Furthermore, an engineered dry powder formulation should increase the drug delivery efficiency during mechanical ventilation.

1.7.4 Dry powders with DPIs in mechanical ventilation

As mentioned previously, only nebulizers and pMDIs are currently used in routine clinical practice for delivering drug aerosols to ventilated patients. Investigators have studied the factors that influence delivery of aerosols and explored factors such as changing the position of the aerosol generator or developing appropriate inhalers. Still, liquid formulations suffer from variability and poor efficiency of aerosol delivery, especially in humid environments. Moreover, aerosols generated from both wet nebulizers and pMDIs can induce bronchoconstriction in ventilated patients with obstructive airway disease[128].

Delivery of inhaled drugs in the form of dry powders to ventilated patients is now an attractive alternative. It can eliminate the problem of 'raining out' or condensation of droplets in the tubing. Advances in particle engineering also compel efforts to explore drug powder formulations in ventilated patients. For example, drug particles have been engineered by applying nanotechnology to pharmaceutical formulation designs. Micron-scale dry powders of nanoparticles have been formulated in the form of agglomeration of nanoparticles known as "NanoClusters." NanoCluster formulations can provide the combination of nano-scale particle and micro-scale particle. In other words, NanoClusters have higher dissolution compared to micronized drug particle[129]. At the same time, the low density of NanoClusters in the size range

of 1-3 um should navigate to the deeper area in the lung and be suitable for deposition in the lower respiratory tract[47,48].

In non-ventilated patients, dry powder is delivered via DPIs. Although DPIs have not been used to deliver inhaled drugs to patients on mechanical ventilation, the development of DPIs in use with ventilation systems is continuously growing[128]. DPIs pose new challenges such as powder dispersion, humidity effects (e.g. hygroscopic powders), device integration, and dose actuation.

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Chapter 2

NanoCluster budesonide formulations enhance drug delivery through endotracheal tubes

2.1 Introduction

Inhalation represents a desirable but largely untapped route for delivering drugs to ventilated patients. In this capacity, both nebulizers and pressurized metered-dose inhalers (pMDIs) have been widely investigated; however, aerosol delivery in this setting is complex. Nebulizers or inhalers must be properly interfaced to efficiently introduce aerosol into the ventilator circuit. Aerosols must then successfully navigate the ventilator tubing and an endotracheal tube. New formulations or devices are desperately needed to enable drug delivery to these patients.

Liquid formulations currently explored for aerosol delivery in ventilated patients often prove ineffective at delivering aerosol to the lungs. Liquid aerosol deposition in the ventilator circuit and the endotracheal tube often results in inefficient and variable dosing. In traditional nebulization, aerosol droplets exhibit relatively large diameters and a broad size distribution. Smaller droplet sizes (~1-3 μm) more efficiently traverse and exit the endotracheal tube [130]. Larger droplets tend to deposit in the ventilation circuit and on artificial airways such as the endotracheal tube by sedimentation or by inertial impaction. Humidification of the ventilator circuit can compound this problem by inducing droplet growth. An overly dry circuit, however, can affect the airway mucosa [79,86,131-133].

The development of new formulations is imperative to enhance drug delivery to the lungs of patients in critical care. Dry powder formulations can provide improved stability compared to liquid formulations. To increase lung coverage, particles should have an aerodynamic diameter of 1-5 μm. Although nanoparticles can enhance the dissolution rate of drugs in the lung, nanoparticles often agglomerate into irregular sizes during drying and can yield poor dry powder

aerosols. Controlling nanoparticle agglomeration provides one attractive formulation option. Well-defined nanoparticle agglomerates (NanoClusters) can also prevent cohesion and adhesion of powders. Such particles have an irregular surface, thus reducing contact area and lowering particle interactions. Nanoparticle agglomerates of poorly water-soluble drugs such as budesonide or paclitaxel can enhance the dissolution and improve the aerodynamic properties of the drugs [134,135].

Endotracheal tubes also play an important role in aerosol delivery to ventilated patients. These tubes have been designed using non-toxic, non-allergic, and non-reactive materials [136]. Many materials such as Teflon, nylon, silicon, polyethylene, and synthetic rubber have been used to make endotracheal tubes. Currently, commercial endotracheal tubes are typically made of polyvinylchloride (PVC). Although tubing surfaces are wet and usually covered with a biofilm [137], researchers have also considered the use of sleeves during drug delivery. Therefore, clean and dry endotracheal tubes were studied here.

Budesonide is a potent glucocorticoid that has been available in inhaled formulations for a long time [138]. Inhaled budesonide has been used for the treatment of asthma [139] and chronic obstructive pulmonary diseases (COPD) [3]. This drug could also be a potent therapy for ventilated patients if delivered effectively. Here, a novel formulation based on agglomerated budesonide nanoparticles was tested for delivery through Teflon tubes or commercial endotracheal tubes. Formulas with or without excipients were compared to micronized stock budesonide and to the Pulmicort Flexhaler powder. Variables such as humidity, diameter of endotracheal tubes, and volumetric flow rates were investigated.

2.2 Materials and methods

2.2.1 Materials

Budesonide was generously provided by Savara Pharmaceuticals. Lactose and L-leucine were purchased from Sigma Chemicals Co. (St. Louis, MO). Double-distilled water was provided by an EASYpure® RODI Barnstead International, Dubuque, Iowa. Hi-Lo® cuffed tracheal tubes (PVC) were provided by clinical collaborates, and Teflon tube were purchased from Fisher Scientific (Fair Lawn, NJ).

2.2.2 Methods

2.2.2.1 Budesonide NanoCluster fabrication

Budesonide NanoClusters were prepared by milling 5 grams of micronized budesonide in 200 mL distilled water for 20 hrs. A Netzsch MiniCer Media Mill was operated using YTZ® grinding media (0.5 mm, Tosoh Corp.) under an agitation speed of 2772 rpm. Particle size of NanoCluster suspensions was determined by dynamic light scattering (Brookhaven Instruments Corp., ZetaPALS) at different time intervals during the milling process. After milling, the collected suspension was frozen at -80ºC and lyophilized for ~36 hours to remove all appreciable water content (Labconco FreeZone 1). Various excipients were added to some of the milled suspensions and homogenized for 30 seconds before freezing and lyophilization. These excipients included lactose and L-leucine in drug:excipient ratios of 1:0.01. Lyophilized NanoCluster powder was stored in glass bottles under desiccant at room temperature for further use.

2.2.2.2 Aerosol characterization

The aerodynamic characteristics of budesonide formulations and commercial budesonide were determined using a Tisch Ambient Cascade Impactor (Tisch Environmental, Inc., Village of Cleves, OH). Experiments were conducted in a clean room and humidity was controlled by using a humidifier with a programmable digital humidistat. Approximately 3-5 mg of each powder was placed on wax paper and vacuumed into the tubing upon actuation of a digital valve. A pre-separator was also added between the tube and the first stage of the cascade impactor. The endotracheal tube was inserted midway through the pre-separator (Figure 2.1). The instrument operated at the reported air flow rate for 4 seconds. Both Teflon tubes and commercial endotracheal tubes had an inner diameter as specified. For the tube size study, endotracheal tubes that had inner diameters of 5.0, 6.5 or 8.0 mm were investigated.

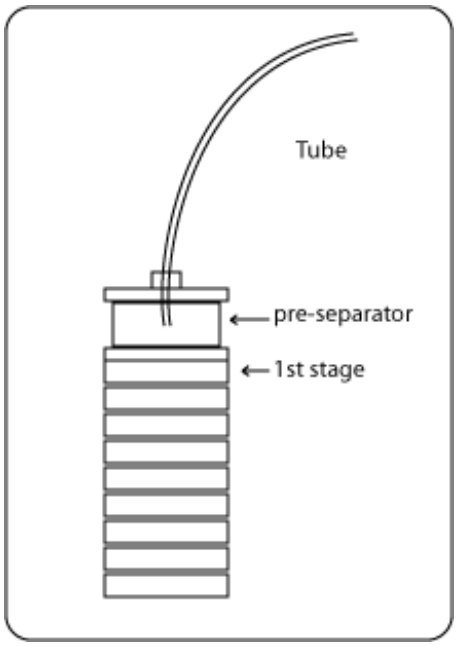

Figure 2.1 A diagram of cascade impactor with a tube.

Dry powders deposited on each stage of the impactor were quantified by the difference in weight of the plate on each stage before and after running the experiment. The percent emitted fraction (%EF), fine particle fractions of the total dose (FPF_{TD}), mass median aerodynamic diameter (MMAD), and geometric standard deviation (GSD) were calculated as follows [140]. The percent emitted fraction (%EF) was calculated as

$$\%\ \text{Emitted fraction (\%EF)} = \frac{\text{Total formulation mass collected in the impactor}}{\text{Total formulation mass introduced into the impactor}} \times 100 \qquad \text{(Eq. 2.1)}$$

The fine particle fraction of the total dose (FPF_{TD}) was calculated as the percentage of aerosolized particles that have aerodynamic diameters below 5.8 μm and the percentage of aerosolized particles that have aerodynamic diameters below 3.3 μm according to the following equation.

$$\%\ \text{Fine particle fraction}\ (FPF_{TD}) = \frac{\text{Formulation mass recovered from terminal stages of the impactor}}{\text{Total formulation mass collected in the impactor}} \times 100 \qquad \text{(Eq. 2.2)}$$

The mass median aerodynamic diameter (MMAD) and the GSD were determined by a linear fit of the cumulative percent less than the particle size range by weight plotted on a probability scale as a function of the logarithm of the effective cut-off diameter. Geometric standard deviation (GSD) was obtained from the following equation:

$$GSD = \left(\frac{d_{84.13\%}}{d_{15.87\%}}\right)^{1/2} \qquad \text{(Eq. 2.3)}$$

When d_n is the diameter at the n^{th} percentile of the cumulative distribution.

2.2.2.3 Particle size and morphology by scanning electron microscopy (SEM)

The size and morphology of the formulated budesonide powders were evaluated using an LEO 1550 field emission scanning electron microscope and compared to that of stock budesonide powders. Prior to imaging, the samples were sputter-coated with gold for 3 min.

2.2.2.4 HPLC analysis

The degradation of budesonide formulations have been performed by using Kromasil C_8 column (4.6 mm x 150 mm, 5 μm). A mixture of 45 percents of water and 55 percents of acetonitrile was used as mobile phase. The system was operated at flow rate 1.1 mL/min and the injection volume was 30 μL. The analysis was monitor at wavelength 244 nm. Budesonide peak was showed at the retention time of 4.55 min and the degradation peak was showed at the retention time of 2.72 min.

2.2.2.5 Thermal analysis

Nanoparticle agglomerates were investigated by differential scanning calorimetry (DSC, Q100 Universal V4.3A TA instruments). A small portion (2-3 mg) of the lyophilized dry mass was sealed and placed in an aluminum pan and heated at a constant rate of 10 °C/min over a temperature range of 25–350 °C for budesonide powder as received, and nanoparticle agglomerate formulations. An inert atmosphere was maintained by purging with nitrogen at 50 mL/min. For TGA, samples weighing 5 ± 2 mg were scanned at a rate of 10 °C/min with a nitrogen flow rate of 40 mL/min.

2.2.2.6 Statistical analysis

The results were presented as the mean and the significant differences were evaluated using Prism 4 GraphPad Software and assessed by one-way ANOVA followed by Tukey's Multiple Comparison Test. One-tailed unpaired t-test was used for assessing the difference between NC-Bud with 1% L-leucine and NC-Bud with 10% L-leucine. A level of confidence of $p<0.05$ was used.

2.3 Results and discussion

2.3.1 NanoCluster budesonide formulations

Budesonide NanoClusters (NC-Bud) were prepared by a wet milling technique. Milling techniques can decrease particles to the nanometer range; however, 1-5 μm particles are desired for effective drug delivery to the lung. In particular, particles in the range of 1-2 μm are preferred to improve lung coverage and to access the alveolar region. If particles are too small (<1 μm), they may be exhaled, whereas large particles (>5 μm) can be trapped in the upper airways, mouth and throat [6,141]. To achieve the desired particle size, nanoparticles were agglomerated to form larger structures.

SEM images showed that budesonide nanoparticles formed micron-sized agglomerates of nanoparticles. NC-Bud contained nanoparticles with a diameter of ~150 nm (Figure 2.2). In addition, the particle size of stock budesonide was much larger than NC-Bud. As budesonide was milled for longer times, particles generally became smaller (Table 2.1). Attrition of the particles produced small particles. The high surface area and increased mobility of the nanoparticles were

expected to induce agglomeration. As shown in the SEM image of NC-Bud, small particles were indeed agglomerated together to form the NanoClusters.

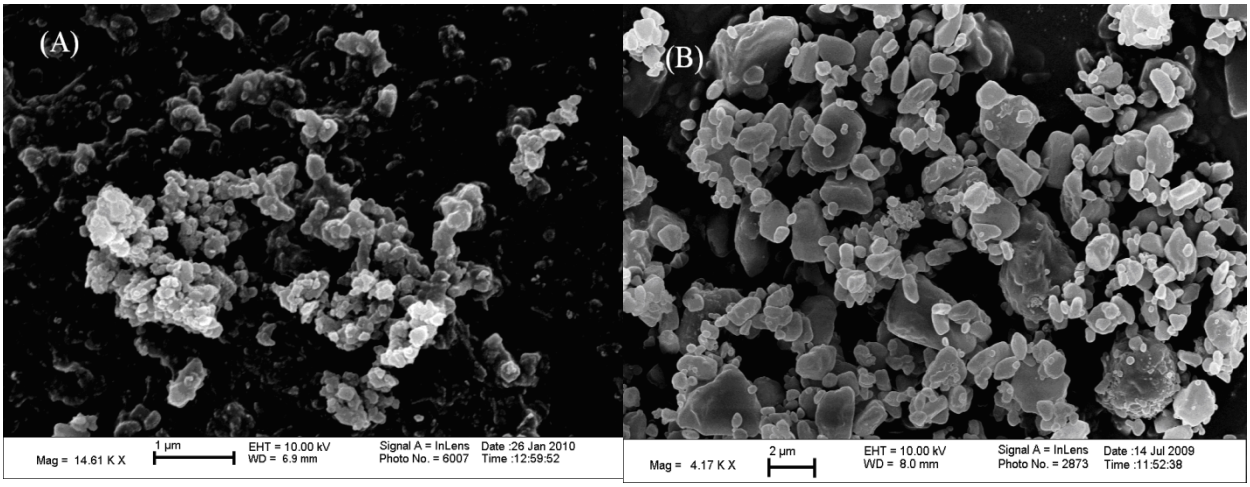

Figure 2.2 SEM images of (A) NanoCluster budesonide formulation and (B) stock budesonide.

Table 2.1 Budesonide particle size over time during media milling.

Time	Particle size (nm)
15 min	1299
30 min	1133
1 hour	1041
2 hours	681-1151*
4 hours	1026-1269*
8 hours	901
12 hours	534
24 hours	670

* bimodal distribution

Cascade impaction was performed to determine the aerosol performance. Powders were entrained into Teflon tubes (ID = 6.5 mm) or commercial endotracheal tubes with an inner diameter of 6.5 mm. NC-Bud had a smaller mass median aerodynamic diameter (MMAD) compared to stock budesonide and Flexhaler powder when applied through a commercial tube (Table 2.2). Generally, the MMAD of NC-Bud was smaller than other budesonide powders. The aerosolization efficiency was quantified as the percent emitted fraction (%EF) and the percent fine particle fraction (%FPF). The %EF of NC-Bud was significantly ($p<0.05$) higher than stock budesonide and Flexhaler powder underscoring the highly efficient aerosolization of NanoCluster formulations. Previous studies using a Turbuhaler, reported that only ~20% of the dose reached the end of an endotracheal tube [132]. NC-Bud showed a higher $\%FPF_{<5.8\mu m}$ than stock budesonide ($p<0.05$) and slightly higher than Flexhaler powder (Table 2.2). The geometric standard deviation (GSD) was also smaller for NC-Bud and was in the range of 2.0-3.0 for all powders.

Table 2.2 Cascade impaction of budesonide when applied through a tube (ID = 6.5 mm) at a flow rate of 28.3 L/min (Values = Average ± SD).

Formulation	**tube**	**% EF**	**% FPF**		**MMAD**	**GSD**
			< 5.8	**< 3.3**	**(µm)**	
Stock budesonide	Teflon	18.8 ± 7.0	51.5 ± 6.2	29.9 ± 5.9	4.4 ± 0.6	2.7 ± 0.4
	commercial	14.9 ± 2.7	50.4 ± 1.2	28.4 ± 5.1	4.7 ± 0.1	2.9 ± 0.3
NC-Bud	Teflon	65.1 ± 1.4	73.9 ± 5.4	27.3 ± 7.0	3.2 ± 0.3	2.0 ± 0.2
	commercial	71.3 ± 1.0	76.5 ± 1.6	31.0 ± 2.9	3.0 ± 0.1	2.2 ± 0.2
Flexhaler powder	Teflon	18.6 ± 4.0	68.7 ± 2.8	34.9 ± 11.8	3.2 ± 0.5	2.7 ± 0.3
	commercial	17.7 ± 2.4	56.4 ± 5.3	30.6 ± 3.6	4.1 ± 0.6	2.6 ± 0.1

2.3.2 Effect of endotracheal tube

Powders were weighed onto slips of wax paper and entrained into different tubing using negative pressure. The Teflon tube and the commercial endotracheal tube showed almost the same size distributions (Figure 2.3). The mass median aerodynamic diameter (MMAD) of budesonide formulations when using Teflon tubing was not different from the MMAD when using a commercial tube (Table 2.2). The percent emitted fraction (%EF), the percent fine particle fraction (%FPF) and the geometric standard deviation (GSD) also did not show a significant difference when using the different tube types (Table 2.2).

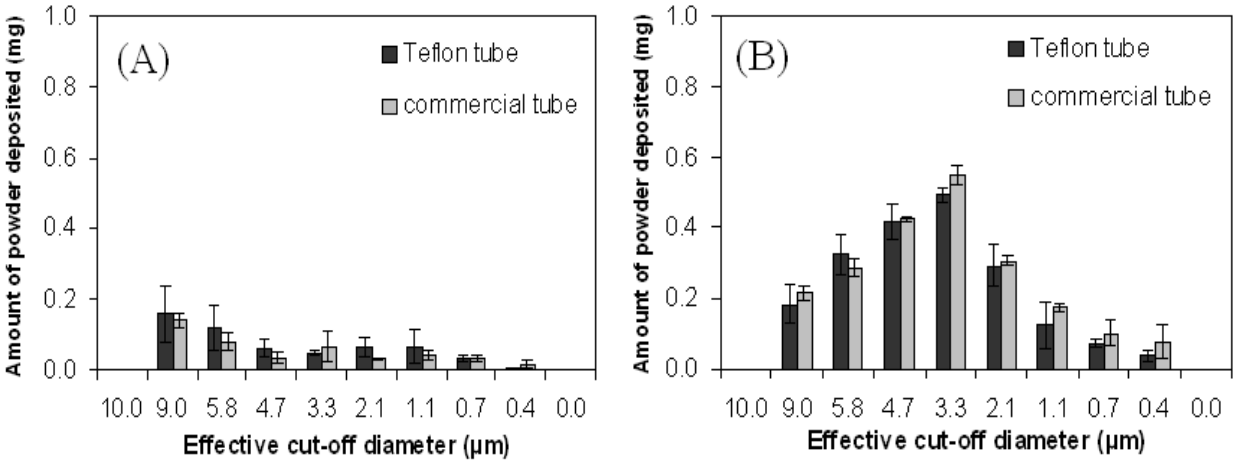

Figure 2.3 The distribution of different budesonide formulations deposited in the cascade impactor when applied through an endotracheal tube (ID = 6.5mm) at a flow rate of 28.3L/min for (A) stock budesonide and (B) NC-Bud.

The flow ability of stock budesonide and NC-Bud was qualitatively assessed by measuring the angle of repose of the powders. The angle of repose was measured after passing powders through the tube and compared to the starting powders. Generally, NC-Bud showed a smaller angle of repose compared to stock budesonide (Table 2.3). The angle of repose increased

for stock budesonide after passing through tubing, suggesting that the tubing may have induced cohesion within the powder, perhaps through inductive charging. There was no statistical difference when applying powders through a Teflon tube or a commercial tube.

Table 2.3 Angle of repose of budesonide formulations (Values = Average ± SD).

Formulation	Angle of repose (degrees)		
	Powder	Teflon tube	PVC tube
Stock budesonide	49.4 ± 0.5	55.3 ± 0.5	53.8 ± 1.5
NC-Bud	39.1 ± 1.1	38.6 ± 0.5	41.3 ± 1.0

Another research has studied the effect of electrostatic charging on aerosol performance. Mannitol powders were studied at a low flow rate of ~30 L/min. Teflon and PVC tubing were used to generate charges on the powder. Researchers found that the initial charges did not influence the aerosolization of powders because the air turbulence may also generate charges on the powder and have more influence on powder aerosolization. Although different materials generated various amounts of charge on the powder depending on the material, the initial charges that were generated by the different tubing materials did not affect the aerosol performance of drug powders [142].

2.3.3 Effect of humidity on powder performance

A humidifier is applied to control the humidity during mechanical ventilation. Some studies showed that during mechanical ventilation, high humidity decreased the aerosol deposition by around 40% and the size of particles increased [79]. Moisture is known to create cohesive forces between particles, thus reducing powder dispersion. The aerosolization of powders at different humidity can also depend on the physicochemical nature of the drug

compounds. Hygroscopic powders commonly absorb moisture in the dispersing air, causing capillary force between particles [88] and these powders often show improved dispersion in a dry environment [89]. Conversely, low humidity can increase static charging of powders [90].

Some researchers studied the effect of relative humidity (RH) on the properties of two inhaled drug products. They found that the relationship between RH and aerosol charging was caused in part by the difference in physicochemical properties of the drugs [143]. For example, terbutaline sulfate, a hygroscopic drug, in Bricanyl® showed decreasing powder charge with increasing RH, whereas budesonide, a non-hygroscopic drug, in Pulmicort® carried the highest charge at low RH (15% RH) and high RH (90% RH) but carried the lowest charge at 40% RH. For budesonide, they inferred that as the moisture adsorption increased, the charges dissipation increased, thus increasing the amount of fine particles. At relative humidity higher than 65%, powder particles were more difficult to disperse in the airstream since the high moisture increased the cohesive force between the particles [88,143]. Here, the budesonide powder performance was indeed affected by relative humidity.

NC-Bud performed better at 55% relative humidity (RH) than at 41% RH. A higher percent emitted fraction (%EF) and percent fine particle fraction (%FPF) were observed at 55% RH in comparison to 41% RH. The mass median aerodynamic diameter (MMAD) of NC-Bud was slightly lower at higher relative humidity (Table 2.4) whereas the size distribution was not dramatically different (Figure 2.4). These results may be due to electrostatic interactions that affected particles at low relative humidity. As a side note, powders that went through an endotracheal tube appeared more cohesive than neat powders, although the angle of repose did not change substantially (Table 2.3). Thus, electrostatic interactions between aerosol particles

and tube surface or between particles may affect aerosolization efficiency by inducing charging of the powder during passage through the tube. The hydrophobic nature of budesonide appeared helpful in maintaining overall performance at both humidities, which may prove beneficial since some moisture is required to prevent drying of the airway mucosa and to minimize bronchospastic responses to breathing cold, dry air. For reference, the percent of relative humidity can vary depending on the area in the respiratory tract such as 40% in the mouth, 60% in the pharynx and around 100% in the deep airways [144].

Table 2.4 Cascade impaction of budesonide NanoClusters when applied through an endotracheal tube (ID = 6.5 mm) at a flow rate of 28.3 L/min at different relative humidities (Values = Average ± SD).

Tube	Relative humidity	% EF	% FPF		MMAD (µm)	GSD
			< 5.8	< 3.3		
Teflon tube	41 %	48.0 ± 3.9	60.6 ± 0.9	25.0 ± 3.3	3.9 ± 0.2	2.0 ± 0.1
	55 %	58.8 ± 8.7	70.8 ± 4.0	28.8 ± 0.2	3.5 ± 0.3	2.1 ± 0.1
PVC tube	41 %	58.8 ± 2.2	56.5 ± 0.1	20.1 ± 1.6	4.3 ± 0.0	1.9 ± 0.0
	55 %	68.5 ± 9.5	62.3 ± 3.3	18.4 ± 2.5	3.9 ± 0.2	1.8 ± 0.1

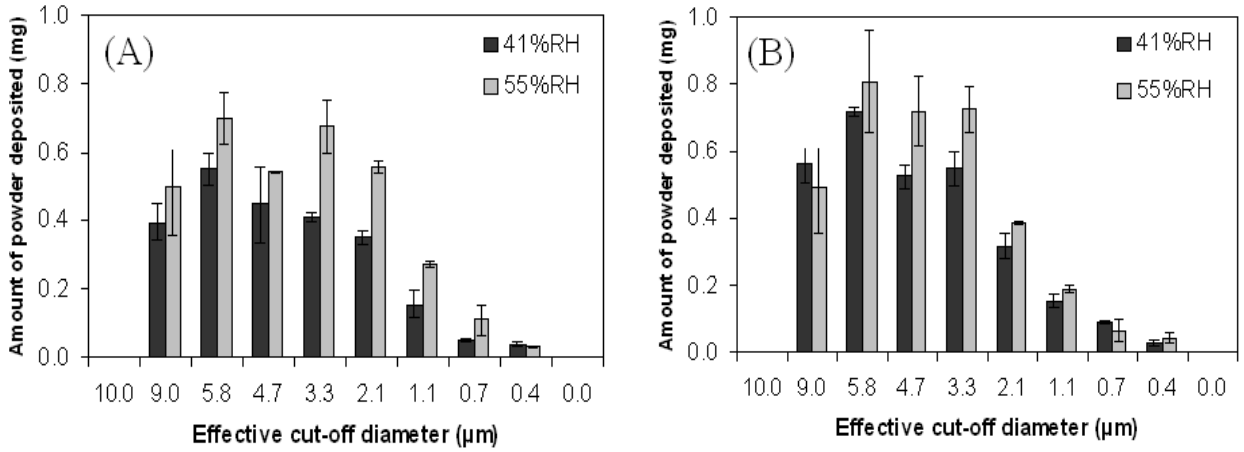

Figure 2.4 The distribution of NanoCluster budesonide formulation (NC-Bud) deposited on the cascade impactor when applied through an endotracheal tube (ID = 6.5mm) at flow rate of 28.3L/min at different relative humidities for (A) Teflon tube and (B) commercial tube.

2.3.4 Effect of excipients on aerosol performance

Lactose and L-leucine were evaluated as excipients for aerosol drug delivery. Lactose is an excipient that is commonly used for aerosol formulation. When used as a carrier particle, lactose is known to help dispersion of aerosol powders [141]. The percent emitted fraction (%EF) of NC-Bud with 1% lactose was higher than the % EF of NC-Bud without excipient ($p<0.05$) (Table 2.5). The MMAD of NC-Bud with 1% lactose was 2.9 ± 0.2 μm when applied through a Teflon tube. This value was slightly lower than other formulations. On the contrary, when applied through a commercial tube, the MMAD of NC-Bud with 1% lactose was 3.4 ± 0.1 μm. This was slightly higher than NC-Bud without excipient. Although those differences were small, results suggested that lactose and tube materials had a measurable effect on this drug formulation.

Table 2.5 Cascade impaction of different budesonide formulations when applied through an endotracheal tube (ID = 6.5 mm) at a flow rate of 28.3 L/min (Values = Average ± SD).

Formulation	% EF	% FPF		MMAD	GSD
		< 5.8	< 3.3	(μm)	
NC-Bud w/o excipients	65.1 ± 1.4	73.9 ± 5.4	27.3 ± 7.0	3.2 ± 0.3	2.0 ± 0.2
NC-Bud w/ 1% lactose	74.5 ± 2.1	71.8 ± 1.4	35.2 ± 2.7	2.9 ± 0.2	2.3 ± 0.2
NC-Bud w/ 1% L-leucine	72.6 ± 5.8	64.7 ± 3.3	27.3 ± 0.6	3.7 ± 0.2	2.1 ± 0.0
Stock budesonide	18.8 ± 7.0	51.5 ± 6.2	29.9 ± 5.9	4.4 ± 0.6	2.7 ± 0.4
Flexhaler powder	18.6 ± 4.0	68.7 ± 2.8	34.9 ± 11.8	3.2 ± 0.5	2.7 ± 0.3

* Teflon tube

Formulation	% EF	% FPF		MMAD	GSD
		< 5.8	< 3.3	(μm)	
NC-Bud w/o excipients	71.3 ± 1.0	76.5 ± 1.6	31.0 ± 2.9	3.0 ± 0.1	2.2 ± 0.2
NC-Bud w 1% lactose	77.8 ± 3.1	67.2 ± 2.9	27.2 ± 1.4	3.4 ± 0.1	2.1 ± 0.0
NC-Bud w 1% L-leucine	66.9 ± 2.0	66.0 ± 1.4	24.3 ± 0.2	3.7 ± 0.1	1.9 ± 0.0
Stock budesonide	14.9 ± 2.7	50.4 ± 1.2	28.4 ± 5.1	4.7 ± 0.1	2.9 ± 0.3
Flexhaler powder	17.7 ± 2.4	56.4 ± 5.3	30.6 ± 3.6	4.1 ± 0.6	2.6 ± 0.1

* Commercial tube

Some researchers have found that L-leucine can also improve powder dispersion. For example, L-leucine was shown to improve the dispersion of aerosol particles even if the applied flow rate was low [145]. The MMAD of NC-Bud with 1% L-leucine was around 3.7 μm when applied through a Teflon tube or a commercial tube (Table 2.5). This was slightly higher than NC-Bud without excipient and NC-Bud with 1% lactose. Different tubes did not affect the performance of these powders. NC-Bud formulations, with or without excipients showed substantially better performance when compared to stock budesonide or Flexhaler powders (Figure 2.5). SEM images of NC-Bud powders with excipients appeared similar to powders without excipient (Figure 2.6).

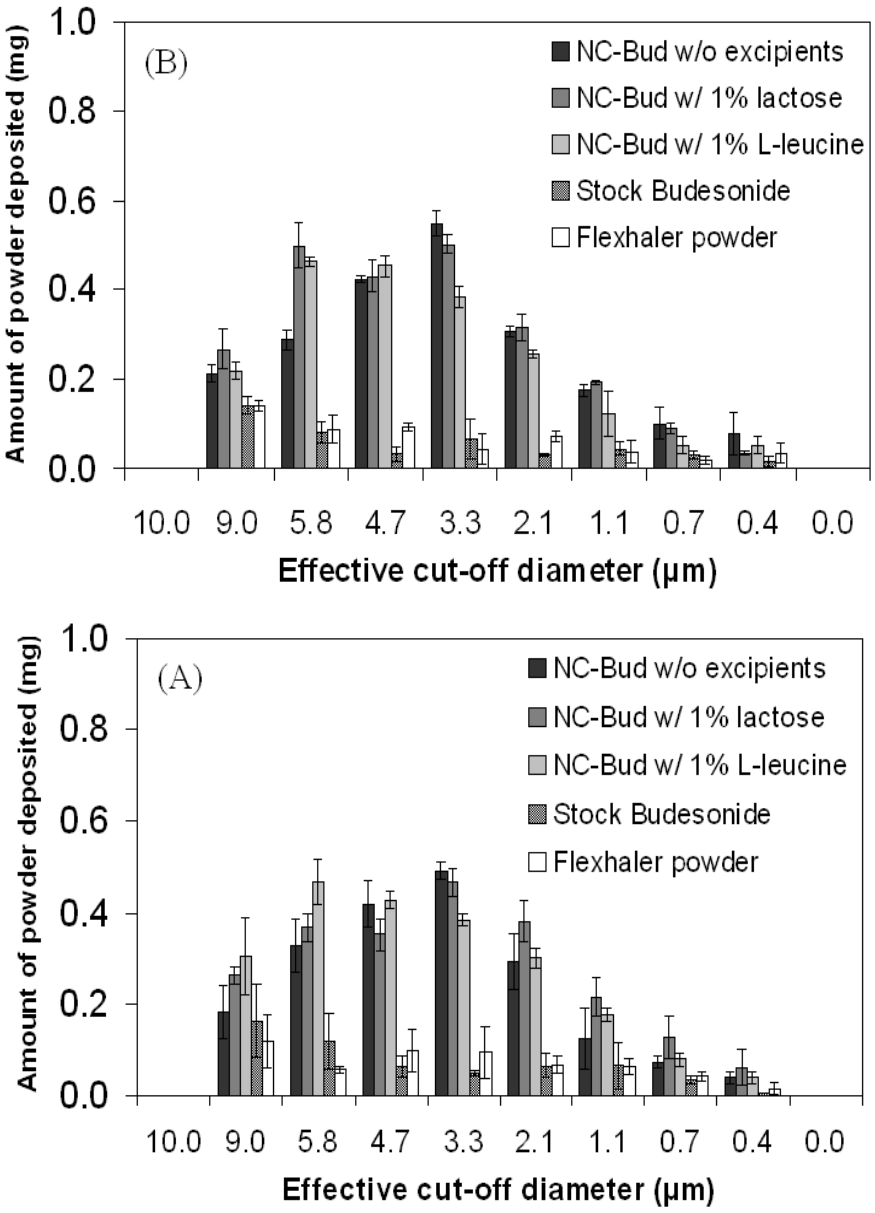

Figure 2.5 The distribution of different budesonide formulations deposited in the cascade impactor when applied through an endotracheal tube (ID = 6.5mm) at a flow rate of 28.3L/min for (A) Teflon tube and (B) commercial tube.

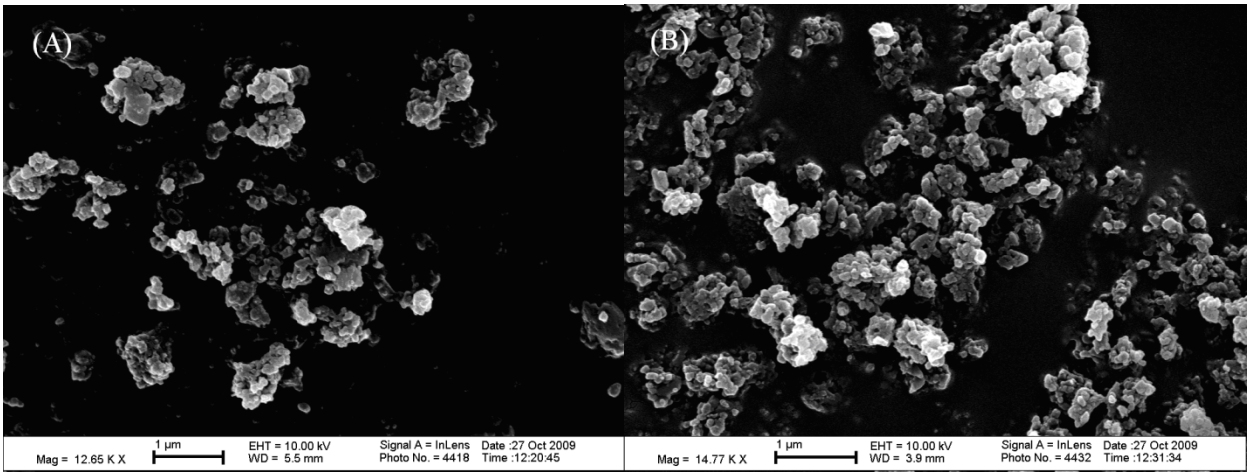

Figure 2.6 SEM images of (A) budesonide with 1% lactose, and (B) budesonide with 1% L-leucine.

2.3.5 Effect of endotracheal tube diameter

The endotracheal tube has been found to affect aerosol delivery during mechanical ventilation. Some studies found that a smaller internal diameter tube reduced the efficiency of aerosol drug delivery [79,146]. The largest diameter endotracheal tube has been suggested to overcome the problem of biofilm and secretions that collect in the endotracheal tube [79]. It should be noted that these studies applied the drug aerosol in the form of liquid aerosol via nebulizers or pMDIs.

Aerosol deposition and distribution of powders changed when applied through endotracheal tubes with different internal diameters (ID). Here, different diameter endotracheal tubes were studied at the same volumetric flow rate of 28.3 L/min, for 4 seconds. When NC-Bud was applied through the 5.0-mm ID tube, the distribution shifted to a smaller size when compared to NC-Bud applied through 6.5-mm ID and 8.0-mm ID tubes (Figure 2.7). The MMAD when applied through a 5.0-mm tube was lower than the MMAD for the 6.5-mm tube

and for the 8.0-mm tube, respectively (Table 2.6). %EF and %FPF also followed this trend. The %EF and %FPF increased when the tube size decreased.

Table 2.6 Cascade impaction of NC-Bud with 1% L-leucine when applied through different sizes endotracheal tubes (Values = Average ± SD) at a flow rate of 28.3 L/min.

Tube Diameter	% EF	% FPF		MMAD (µm)	GSD
		< 5.8	< 3.3		
5.0 mm	77.1 ± 1.3	78.0 ± 3.1	41.3 ± 4.2	2.5 ± 0.2	2.4 ± 0.1
6.5 mm	66.6 ± 1.5	64.8 ± 2.3	24.9 ± 1.0	3.7 ± 0.0	2.0 ± 0.1
8.0 mm	65.5 ± 6.0	57.0 ± 2.4	23.7 ± 6.0	4.2 ± 0.2	2.2 ± 0.4

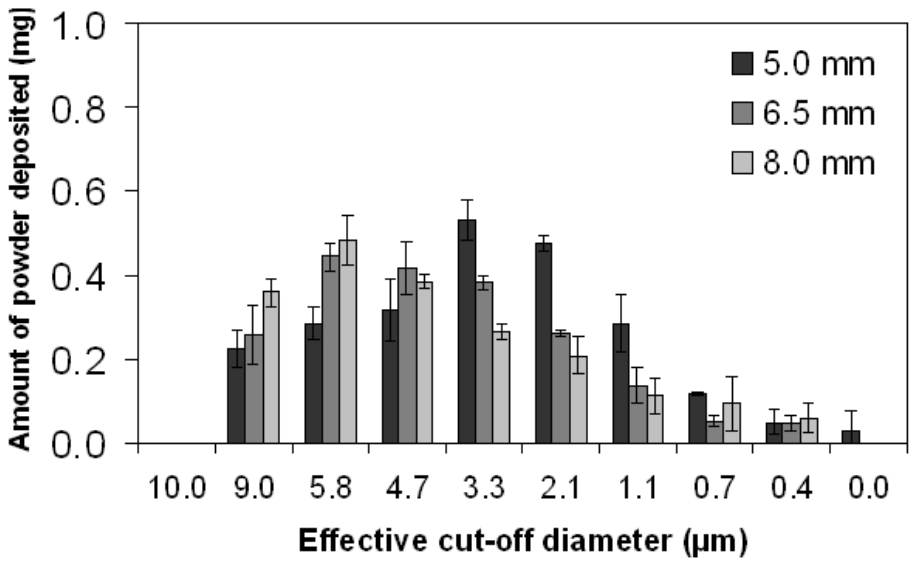

Figure 2.7 The distribution of NC-Bud with 1% L-leucine deposited in the cascade impactor for different tube sizes (commercial tube).

At the same flow rate, a smaller tube would yield more turbulent flow in the tube. Consequently, shear force in the air flow would increase. Turbulent or laminar flow can be described by the Reynolds number (Re). Re is a dimensionless number representing the relationship between inertial forces and viscous forces [147].

$$\text{Reynolds number (Re)} = \frac{\rho v d}{\mu} = \frac{\rho Q d}{\mu A} \qquad \text{(Eq. 2.4)}$$

Here, ρ is the density of air (1.20 kg/m^3 at 20 °C), v is the linear velocity (m/sec), d is the diameter of the tube (m), μ is the dynamic viscosity of air (1.81×10^{-5} kg/(m.sec) at 20 °C), Q is the volumetric flow rate (m^3/sec) and A is the tube cross-sectional area (m^2). Since the same flow rate was applied, differences between endotracheal tubes depended on the ratio of d and A or when simplified d^{-1}. Of course, the 5.0-mm ID tube provided a larger value of d^{-1} in comparison with 6.5-mm ID tubes and 8.0-mm ID tubes, respectively. The Reynolds numbers of 5.0-mm, 6.5-mm and 8.0-mm tube were 8440, 6460, and 5250, respectively, under the conditions studied. All values were in the turbulent flow region (Re>4000).

2.3.6 Effect of flow rate on powder performance

In dry powders, the inspiratory flow rate often plays an important role in powder performance. A minimum inspiratory flow rate can be required to initiate drug delivery through the inhaler and increasing the flow rate can change the performance [148,149]. Some studies found that powders show better dispersibility when the volumetric flow rates from 15 to 90 L/min were applied. They suggested that this flow rate range enhanced the amount of the dispersed fine particles and improved deagglomeration of the particles [145]. Increasing volumetric flow rate will commonly improve the %EF and/or reduce the MMAD. In previous studies, powders showed smaller MMAD when applied at a higher flow rate. On the other hand, particles were suspected to have a higher velocity at the higher volumetric flow rate, which increased the amount of particles that deposited in the induction port (including the USP throat, the glass sampling chamber and the pre-separator) [150].

The influence of flow rate on shear may be considered in a similar manner as tubing diameter, which in this case is ID = 6.5 mm. When applying a high volumetric flow rate, the air flow has a higher velocity, resulting in higher shear force. The Reynolds number equation is directly affected by the volumetric flow rate (Q). The Reynolds number at volumetric flow rates of 30 L/min, 60 L/min and 90 L/min are 6460, 12900, and 19400, respectively. Hence, drug powders deagglomerated when applied at higher flow rates. The MMAD of powders at 30 L/min, 60 L/min and 90 L/min were 3.5 ± 0.3, 1.6 ± 0.1 and 0.7 ± 0.1 μm, respectively (Table 2.7). In addition, the %FPF increased as the flow rate increased, as expected [88,145,150]. The distribution shifted toward a smaller MMAD (Figure 2.8).

Table 2.7 Cascade impaction of NC-Bud with 1% L-leucine when applied through an endotracheal tube (commercial tube, ID = 6.5 mm) at different flow rates (Values = Average ± SD).

Flow rate (L/min)	**% EF**	**% FPF**			**MMAD (μm)**	**GSD**
		< 5.8	**< 6.5**	**< 3.3**		
30	74.1 ± 4.8	66.9 ± 4.5	-	29.3 ± 3.4	3.5 ± 0.3	2.3 ± 0.4
60	70.3 ± 3.9	-	88.8 ± 2.3	61.6 ± 3.4	1.6 ± 0.1	2.4 ± 0.1
90	81.4 ± 9.1	-	89.4 ± 2.2	81.3 ± 2.6	0.7 ± 0.1	6.1 ± 1.6

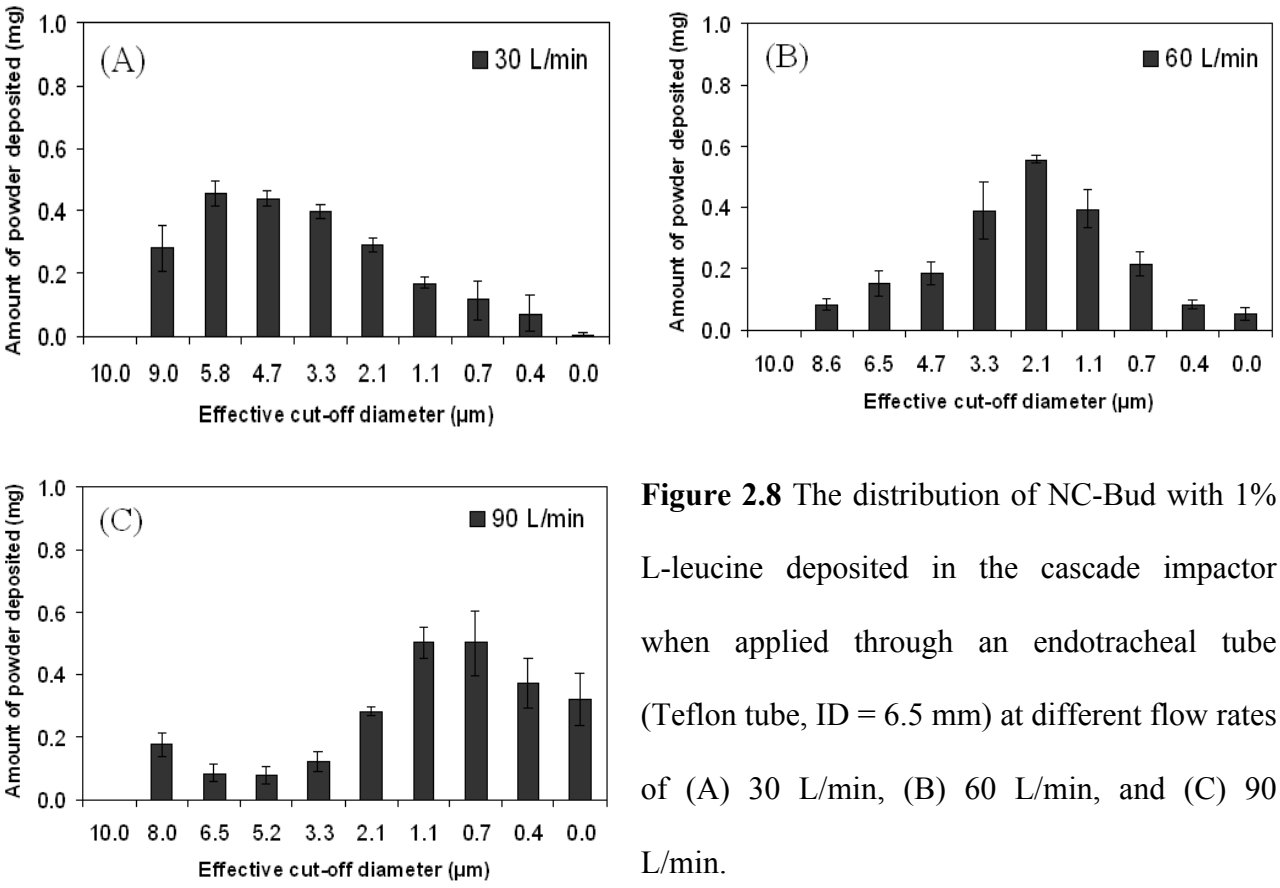

Figure 2.8 The distribution of NC-Bud with 1% L-leucine deposited in the cascade impactor when applied through an endotracheal tube (Teflon tube, ID = 6.5 mm) at different flow rates of (A) 30 L/min, (B) 60 L/min, and (C) 90 L/min.

Generally, the %FPF is low not only for large particles but also due to cohesive small particles. The latter can be partially reduced by applying a higher volumetric flow rate to increase the shear force in the airstreams. Normally, the flow rate influences the %FPF of drug powders, especially in commercial inhalers that have low dispersion efficiencies. For example, 2.7 µm cohesive mannitol powders had a maximal %FPF when delivered from the Rotahaler® at a flow rate of 120 L/min, whereas the maximal %FPF can be achieved at ≥ 60 L/min for the Dinkihaler®, which has a higher dispersion efficiency [88]. Furthermore, the effect of flow rate depended on the physicochemical properties of drug powders. For instance, 5 µm mannitol delivered from the Rotahaler® had a maximal %FPF when applied at 60 L/min but the flow rate had no effect for more cohesive particles of the same particle size [88].

Since the performance of these formulations depends on the flow rate, it will be important to control the inspiration flow rate during mechanical ventilation. Drug powders that are introduced to the patient's lung at different flow rates may yield a difference in distribution or therapeutic effect. At the low flow rate, drug powder may primarily deposit in bronchi and achieve a local effect. If the inspiration flow rate is high, NanoCluster particles may be deagglomerated into small, ~1 μm primary particles. One potential concern is the inertial impaction of these small, high velocity particles, although the velocity would slow as the particles entered the trachea. The performance of NanoCluster formulations will ultimately need to be synergized with ventilator control parameters to optimize delivery to patients.

2.4 Conclusion

NanoCluster formulations of budesonide prepared by a wet-milling technique substantially improved aerosol performance compared to stock budesonide and Pulmicort Flexhaler powders when applied through an endotracheal tube. In general, excipients such as lactose and L-leucine slightly enhanced aerosol properties. Interaction between particles and tube materials was expected to affect delivery efficiency. Budesonide formulations did not differ, however, when applied through a Teflon tube or a commercial endotracheal tube. Volumetric flow rate and tube diameters exhibited a substantial effect on aerosol performance metrics. A higher volumetric flow rate and smaller tube diameter dramatically increased the fine particle fraction, probably due to deagglomeration of the powder as shear force increased. NanoCluster formulations of budesonide represent a potential engineered particle approach for introducing dry powder aerosols to ventilated particles.

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Chapter 3

NanoCluster budesonide formulations enable efficient drug delivery driven by mechanical ventilation

3.1 Introduction

Inhaled drugs are delivered to the lungs via three kinds of aerosol generators; nebulizers, pressurized metered-dose inhalers (pMDIs), or dry powder inhalers (DPIs). Nebulizers and pMDIs are often not efficient in delivering liquid aerosols to patients on mechanical ventilation. A common barrier of drug delivery is the loss of drug aerosols in the humid ventilator circuit and on the endotracheal tube. DPIs have become a popular option for asthma therapy and have been adapted to deliver dry powder formulations to ventilated patients. Optimization of dry powder aerosols and devices, however, is still required to realize the potential of this drug delivery scheme for ventilated patients.

Nebulizers and pMDIs have been historically used during mechanical ventilation. The relative lung deposition efficiencies of different nebulizers and pMDIs have been conducted in many in vitro and in vivo studies [79]. In vitro studies have been highly inconsistent showing highly variable quantities of inhaled drug dose when delivered using either nebulizers or pMDIs. Most liquid formulations have shown poor efficiency because of inertial impaction or gravitational sedimentation of droplets resulting in loss of drug in the ventilator circuit and the endotracheal tube. For example, a study showed a considerable percent of drug was deposited in the spacer chamber, the ventilator circuit and the endotracheal tube when a pMDI was applied [151]. In addition, the humid environment of the ventilator circuit can cause liquid aerosol droplets to increase in size or condense on tubing.

Dry powder inhalers (DPIs) offer an alternative for delivering drugs into ventilated patients. Although many DPIs are available for the treatment of asthma and chronic obstructive pulmonary disease (COPD) [152], few of them have been successfully applied to mechanical

ventilation. Everard et al. used a modified Turbuhaler with the ventilator circuit. The outer covering of the Turbuhaler was removed and the inner cylinder was enclosed to provide spiral disaggregation channels in a chamber. Once the device was loaded, air flowing through the chamber carried the aerosol to the endotracheal tube. They reported that approximately 20% of the nominal dose was delivered to a filter placed at the distal end of the tube [128].

DPIs on the market are passive devices. The fluidization and aerosolization of drug powder in DPIs depends on the inspiratory effort of patients. Some studies indicated that higher airflow dependence might result in higher dose variability due to differences in the patients' inspiration effort [153]. In ventilated patients, however, a patient's inspiration is mainly controlled by the ventilator. Since breathing can be tightly controlled, drug formulations and devices are primary design metrics that would influence DPI performance.

The size and geometry of drug particles play an important role in aerosol performance. To improve deposition in the central airways and peripheral areas in the lungs, drug particles should be in the size range of 1-5 μm. Since cohesive and adhesive forces influence the dispersion of particles in these size ranges, drug formulations should be engineered to reduce interactions between particles and interactions with the surface of the inhaler. NanoCluster budesonide (NC-Bud) was previously shown to enhance drug delivery through endotracheal tubes [154]. The preliminary success of NC-Bud formulations compelled additional studies to assess performance when inspiration is controlled by a ventilator.

Besides drug formulation, device design is another factor that affects drug delivery through a ventilator circuit. A large variability of emitted dose among different DPIs has been reported [155]. The design of DPIs for patients on mechanical ventilation has not been well studied.

Here, a novel device was designed in order to enhance drug delivery and be convenient to connect between the ventilator circuit and the endotracheal tube. The combination of engineered dry powders and a novel device design was expected to improve drug delivery efficiency during mechanical ventilation. The effect of other parameters such as humidity, air flow rate and inspiration pattern were also investigated.

3.2 Materials and methods

3.2.1 Materials

Budesonide (Bud) was obtained from Sicor de Mexico (Lerma,Mexico). Double-distilled water was provided by an EASYpure® RODI Barnstead International, Dubuque, Iowa. Hi-Lo® cuffed tracheal tubes (PVC) and endotracheal catheter tubes (Kimberly-Clark) were provided by clinical collaborators. The ventilator model was 7200® Series Ventilator System (Puritan-Bennett Corporation, Carlsbad, CA). The details of Monodose® inhaler (Plastiape Monodose Inhaler RS01 Model 7) and the novel device are reported in Table 3.1 and in Figure 3.1.

Table 3.1 Dimensions of the Monodose® inhaler and the novel device.

Device	Device geometry	Mesh size (mm)	Inlet opening (mm)	outlet opening (mm)
Monodose® inhaler	cylinder	1.28 ± 0.03	4.00x5.66	10.72
Novel device	cylinder	0.15 ± 0.05	2.5	15

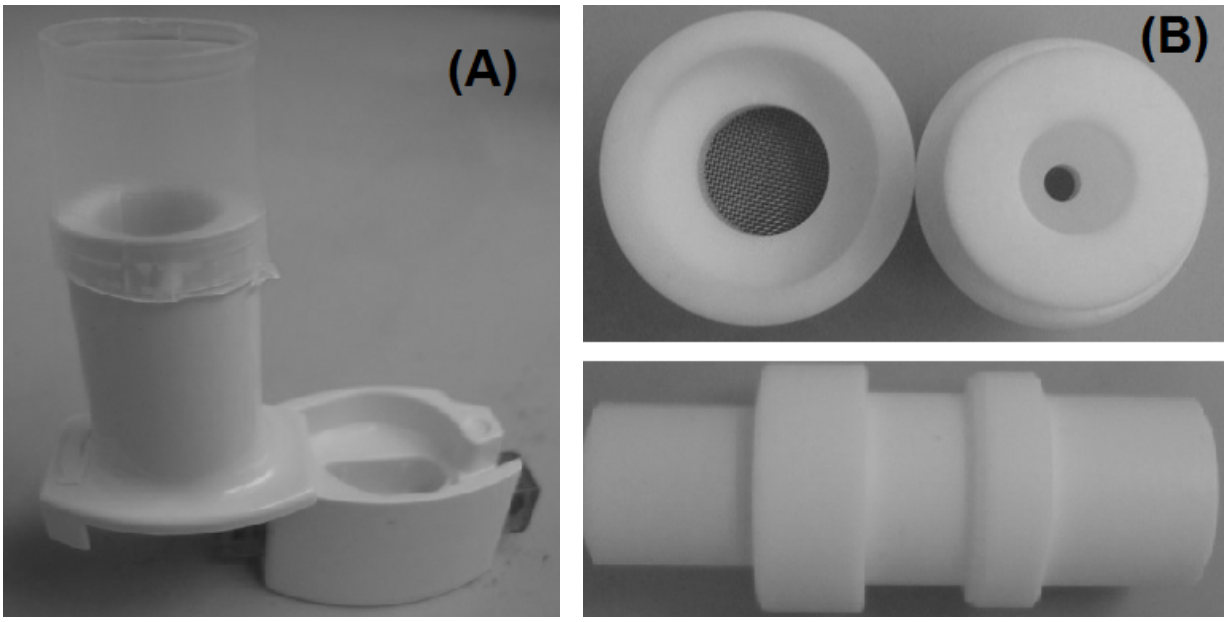

Figure 3.1 Photographs of (A) the modified Monodose® inhaler and (B) the novel device.

3.2.2 Methods

3.2.2.1 Budesonide NanoCluster fabrication

Budesonide NanoClusters were prepared by milling 5 grams of micronized budesonide in 200 ml distilled water for 20 hrs. A Netzsch MiniCer Media Mill was operated using YTZ® grinding media (0.5 mm, Tosoh Corp., Tokyo, Japan) under an agitation speed of 2772 rpm. Particle size of the suspensions was determined by dynamic light scattering (Brookhaven Instruments Corp., ZetaPALS) at different time intervals during the milling process. After milling, the collected suspension was frozen at -80ºC and lyophilized for ~36 hours to remove all appreciable water content (VirTis Feezemobile-12XL, The Virtis Company,Gardiner, NewYork). Lyophilized powder was stored in glass bottles under desiccant at room temperature for further use.

3.2.2.2 Aerosol characterization

The aerodynamic characteristics of budesonide formulations and commercial budesonide were determined using a Tisch Ambient Cascade Impactor (Tisch Environmental, Inc., Village of Cleves, OH). Approximately 5 mg of each powder was filled in a capsule (HPMC type, size 3, generously provided from Capsugel®, NJ, USA). Powder was introduced to the cascade impactor via a modified Monodose® inhaler or a novel dry powder inhaler. Conditions and parameters such as volumetric flow rate were controlled by a ventilator. The endotracheal tube was placed between the ventilator and the cascade impactor (Figure 3.2).

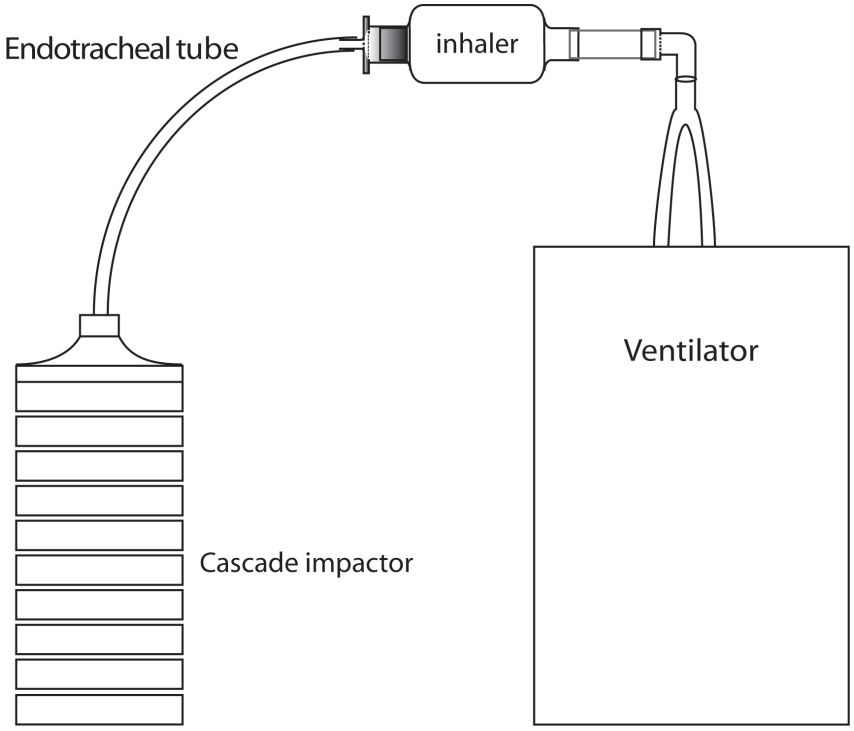

Figure 3.2 A schematic diagram of the DPI connected between the ventilator and the cascade impactor.

Dry powders deposited on each stage of the impactor were quantified by the difference in weight of the plate on each stage before and after running the experiment. For the humidity study, the amounts of drug in each stage were determined by ultraviolet-visible (UV-Vis) spectroscopy. The percent emitted fraction (%EF), fine particle fractions of the emitted dose (FPF_{ED}), mass median aerodynamic diameter (MMAD), and geometric standard deviation (GSD) were calculated as previously reported [154]. The fine particle fraction of the emitted dose (FPF_{ED}) was calculated as the percentage of aerosolized particles that have aerodynamic diameters below 5.8 μm and the percentage of aerosolized particles that have aerodynamic diameters below 3.3 μm (Eq. 3.1). The $\%FPF_{ED}$ was calculated as

$$\%\ \text{Fine particle fraction}\ (FPF_{ED}) = \frac{\text{Formulation mass recovered from terminal stages of the impactor}}{\text{Total formulation mass collected in the impactor}} \times 100 \quad \text{(Eq. 3.1)}$$

The mass median aerodynamic diameter (MMAD) was determined at the 50th percentile of the cumulative mass distribution curve. Geometric standard deviation (GSD) was calculated as the square root of the ratio of diameters at the percentile of 84.13% and 15.87% of the cumulative distribution [135].

3.2.2.3 Evaluation of particle size and morphology by scanning electron microscopy (SEM)

The size and morphology of the formulated budesonide powders were evaluated using an LEO 1550 field emission scanning electron microscope and compared to that of stock budesonide powders. Prior to imaging, the samples were sputter-coated with gold for 3 min.

3.2.2.4 Ultraviolet-visible (UV-Vis) spectroscopy analysis

The amount of drug in each stage was determined by an Agilent 8453 UV-Vis Spectroscopy System (Agilent Technologies 95-03, Waldbronn, Germany). The instrument was equipped with a quartz cell with a path length of 0.1 cm. The measurement was made at a wavelength of 244 nm.

3.2.2.5 Measurement of pressure drop across the devices

The pressure drop of the devices was measured using critical flow controller (CFC) (type TPK 2000, Copley Scientific, Nottingham, UK). The CFC was connected to a vacuum pump and a Dosage Unit Sampling Apparatus (DUSA) (Figure 3.3). The pressure drops were measured at flow rates of 30, 60, and 90 L/min. The square root of the pressure drop at different flow rates was plotted against the flow rate. The slope of the fitted line was the resistance of the device [156].

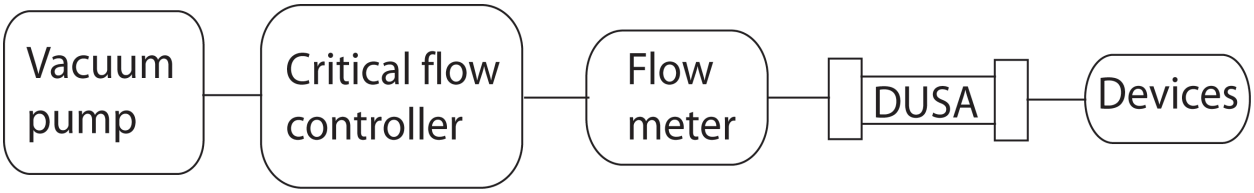

Figure 3.3 Experimental set-up for pressure drop measurement.

3.2.2.6 Statistical analysis

The results were presented as the mean and the significant differences were evaluated using Prism 4 GraphPad Software and assessed by one-way ANOVA followed by Tukey's Multiple Comparison Test. One-tailed unpaired t-test was used for assessing the differences between NanoCluster budesonide and budesonide as received, modified Monodose® inhaler and

new dry powder inhaler, ventilator and ventilator bag, and one-time respiration and three-time respiration. A level of confidence of $p<0.05$ was used for all statistic tests.

3.3 Results and discussion

In patients who have problems of breathing, mechanical ventilation is often necessary to assist or control respiration. Here, a ventilator was used to provide and control airflow in the circuit. Cascade impaction was performed to determine aerosol and DPI performance. A cascade impactor was connected to a ventilator and the Monodose®. DPI was integrated as shown (Figure 3.2). Parameters such as flow rate, inspiratory volume, inspiration pattern, and humidity were controlled by the ventilator.

3.3.1 NanoCluster budesonide (NC-Bud) formulation

Scanning electron microscopy images showed the increased surface area in nanoparticle agglomerates (NanoClusters) of budesonide compared to stock micronized budesonide (Figure 3.4). NanoCluster budesonide (NC-Bud) formulations previously showed effective performance on the cascade impactor when pulled through an endotracheal tube using negative pressure. The studies demonstrated that NC-Bud did not require excipients to achieve excellent aerosol performance [154,157]. NC-Bud without excipients was studied here and these studies were extended to ventilator control (positive pressure).

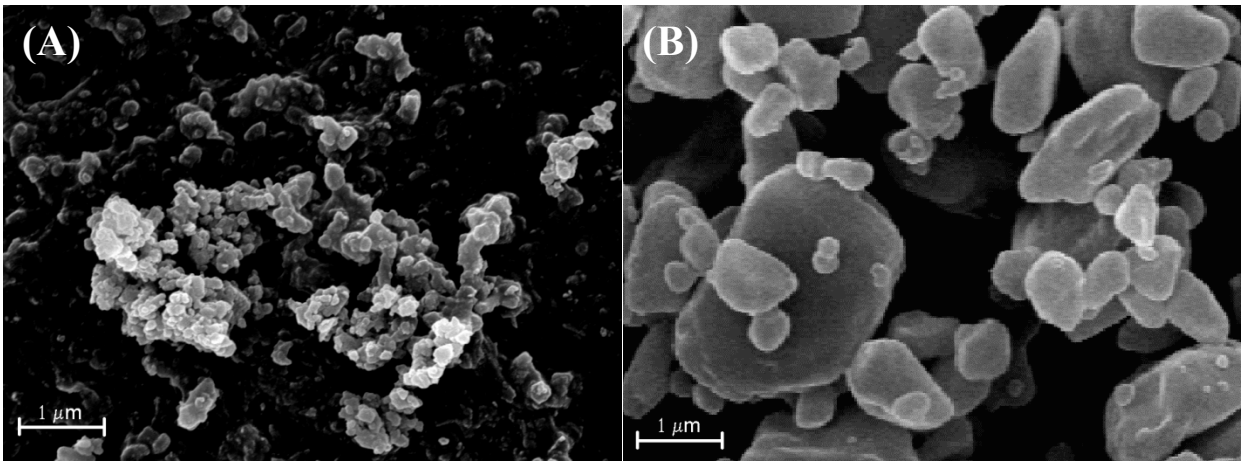

Figure 3.4 SEM images of (A) NanoCluster budesonide (NC-Bud) and (B) Stock budesonide (Scale bar equals to 1 μm).

NC-Bud without excipients and stock budesonide were delivered via a Monodose® inhaler through an endotracheal tube (5.0 mm ID). The drug aerosol was delivered by fitting the Monodose® inside an aerosolization chamber attached to the end of an endotracheal tube (Figure 3.2). The ventilator was operated at 30 L/min for one inspiration cycle controlled by the ventilator. An inspiratory volume of 2.5 L and sine-wave-form inspiration pattern were applied. The NC-Bud showed a percent emitted fraction (%EF) much higher than the %EF of stock budesonide (Table 3.2) although the mass median aerodynamic diameter (MMAD) was not different between NC-Bud and stock budesonide ($p<0.05$). The percent fine particle fraction (%FPF) of NC-Bud and stock budesonide were 85.2 ± 3.3 and 72.8 ± 12.3, respectively. It should be noted that %FPF is calculated as a percentage of the emitted dose, which was quite small for stock budesonide. The geometric standard deviation (GSD) of NC-Bud was 2.4, smaller than the GSD of stock budesonide (3.6) (Figure 3.5).

Table 3.2 Cascade impaction results of budesonide when applying through an endotracheal tube (ID = 5.0 mm) at a flow rate of 30 L/min (Values = Average ± SD).

Formulation	% EF	% FPF		MMAD	GSD
		< 5.8	< 3.3	(μm)	
NC-Bud	64.6 ± 7.3	85.2 ± 3.3	48.8 ± 6.6	2.2 ± 0.3	2.4
Stock budesonide	15.9 ± 3.3	72.8 ± 12.3	52.1 ± 12.7	2.1 ± 0.8	3.6

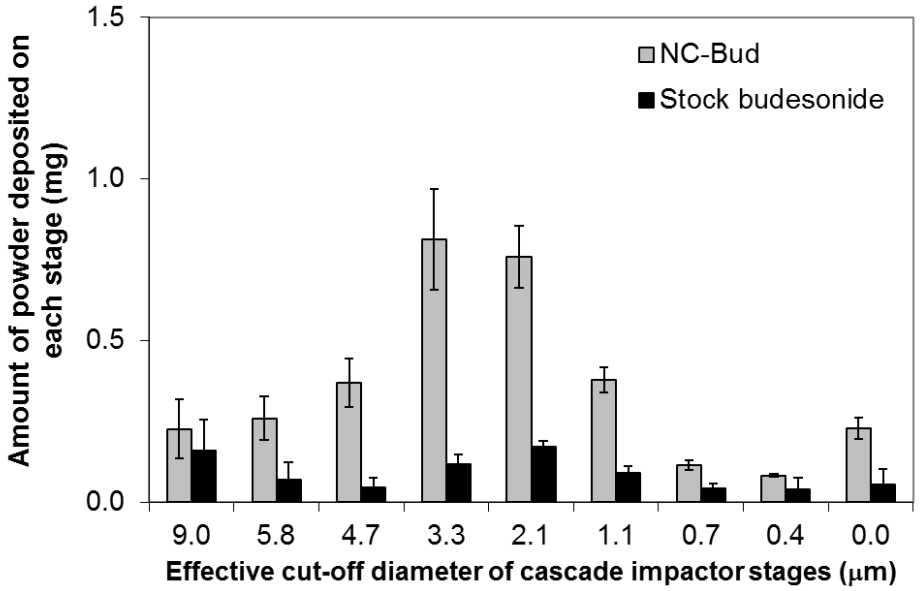

Figure 3.5 The distribution of different budesonide formulations deposited on the cascade impactor when applied through an endotracheal tube (ID = 5.0 mm) at a flow rate of 30.0 L/min for NC-Bud and stock budesonide.

3.3.2 Effect of inspiration pattern, flow rate, and inspiratory volume on powder performance

In patients who cannot aid in respiration, the ventilator will fully control breathing. When using assisted modes on the ventilator, mismatching ventilator air delivery and patient inspiration causes patient-ventilator asynchrony and can cause injury [159,160]. Furthermore, the relationship between the ventilator operation and patient inspiration affects ventilation efficiency. Since the value of peak inspiratory flow depends on waveforms, a specific waveform may influence the

success of mechanical ventilation [161]. The basic waveforms can be categorized into three patterns; square, ramp and sine wave [74]. Some studies showed that the sinusoidal or ramp waveforms delivered drug aerosols more effectively than the square waveform [79]. They suggested that the different inspiration pattern affected the sudden onset and duration of peak flows, and the turbulence. Other studies showed the influence of inspiration flow patterns on aerosol drug delivery when the drug was delivered via nebulizer compared to pMDI [81]. Flow patterns influenced albuterol delivered via nebulizer but there was no difference when delivered via pMDI. Other studies consistently showed no effect of the flow patterns during bronchodilator delivery by pMDI [78,162]. Nevertheless, the influence of inspiration flow pattern on aerosol drug delivery via dry powder inhalers (DPIs) should be investigated.

As mentioned previously, DPIs are preferred to pMDIs due to ease of use and the absence of propellant[163-165]. In general, the efficiency of DPIs has depended on the inspiration effort of the patient and the resistance in the inhalers. DPI performance is typically flow dependent [166]. For example, three different dry powder inhalers (Rotahaler®, Monodose® and Handihaler®) were studied at various flow rates (30-180 L/min). The emitted mass of salbutamol sulphate increased with flow rates when using the Monodose® inhaler. For Rotahaler® and Handihaler®, the emitted mass increased with flow rates until it reached a plateau at 60 L/min. The variability of emitted mass also decreased with increasing flow rate. The Monodose® showed less variability in emitted mass compared to other inhalers due to a different mechanism of capsule emptying [167]. A computational study of fluid dynamics showed that increasing flow rate generated higher turbulence in the device. The turbulence has an effect on fine particle dispersion. Although the high flow rate can increase de-agglomeration of powders, it can also increase powder impaction [168]. Beside inspiration pattern, therefore, in order to optimize drug

delivery efficiency in ventilated patients, the effect of inspiratory flow rate must also be considered especially considering that ventilators apply a positive pressure to control breathing [169].

The aerosol powder was applied through the endotracheal tube (ID = 5.0 mm) at different volumetric flow rates for one cycle of inspiration. Three inspiration flow patterns (square, ramp, and sine waveforms) were applied. Moreover, since patients with COPD have low inspiratory capacity [77], the different lung volumes may lead to variable efficiency of drug delivery to these patients. The maximum inspiratory volume on the ventilator used here was 2.5 L. Different inspiratory volumes of 1.5, 2.0 and 2.5 L were studied.

The results showed that the %EF of NC-Bud at flow rate of 20-40 L/min was consistently around 70-80% and it did not vary with inspiration patterns. The powder performance was not significantly different ($p<0.05$) for volumetric flow rates in the range of 20 – 40 L/min although the %FPF of NC-Bud at a flow rate of 40 L/min was slightly higher than the %FPF at lower flow rates ($p<0.05$). The %FPF was around 80-90% at the cut off diameter of 5.8 μm and around 50% at the cut off diameter of 3.3 μm. The MMAD of NC-Bud at a flow rate of 40 L/min was 1.9 μm compared to 2.1 μm at flow rates of 20 or 30 L/min (Table 3.3, Figure 3.6).

Table 3.3 Cascade impaction results of NC-Bud when applying with different inspiration patterns, different flow rates, and different inspiration volumes (Values = Average ± SD).

Inspiration pattern	**% EF**	**% FPF**		**MMAD**	**GSD**
		< 5.8	**< 3.3**	**(µm)**	
Square waveform	78.9 ± 3.8	82.6 ± 2.3	51.5 ± 3.6	2.0 ± 0.2	2.6 ± 0.0
Ramp waveform	76.1 ± 8.2	83.5 ± 4.0	49.6 ± 1.4	2.1 ± 0.1	2.5 ± 0.2
Sine waveform	77.8 ± 10.0	83.5 ± 3.7	49.7 ± 2.6	2.1 ± 0.1	2.4 ± 0.1
Flow rate	**% EF**	**% FPF**		**MMAD**	**GSD**
(L/min)		**< 5.8**	**< 3.3**	**(µm)**	
20	72.4 ± 5.4	83.8 ± 1.0	51.2 ± 0.8	2.1 ± 0.1	2.4 ± 0.1
30	77.8 ± 10.0	83.5 ± 3.7	49.6 ± 2.6	2.1 ± 0.1	2.4 ± 0.1
40	75.6 ± 5.8	90.2 ± 0.7	55.8 ± 2.1	1.9 ± 0.1	3.0 ± 0.1
Volume	**% EF**	**% FPF**		**MMAD**	**GSD**
(L)		**< 5.8**	**< 3.3**	**(µm)**	
1.5	74.6 ± 7.7	84.8 ± 2.9	48.8 ± 2.1	2.2 ± 0.1	2.5 ± 0.1
2.0	79.8 ± 3.2	84.3 ± 2.6	48.0 ± 4.0	2.2 ± 0.2	2.5 ± 0.3
2.5	77.8 ± 10.0	83.5 ± 3.7	49.6 ± 2.6	2.1 ± 0.1	2.4 ± 0.1

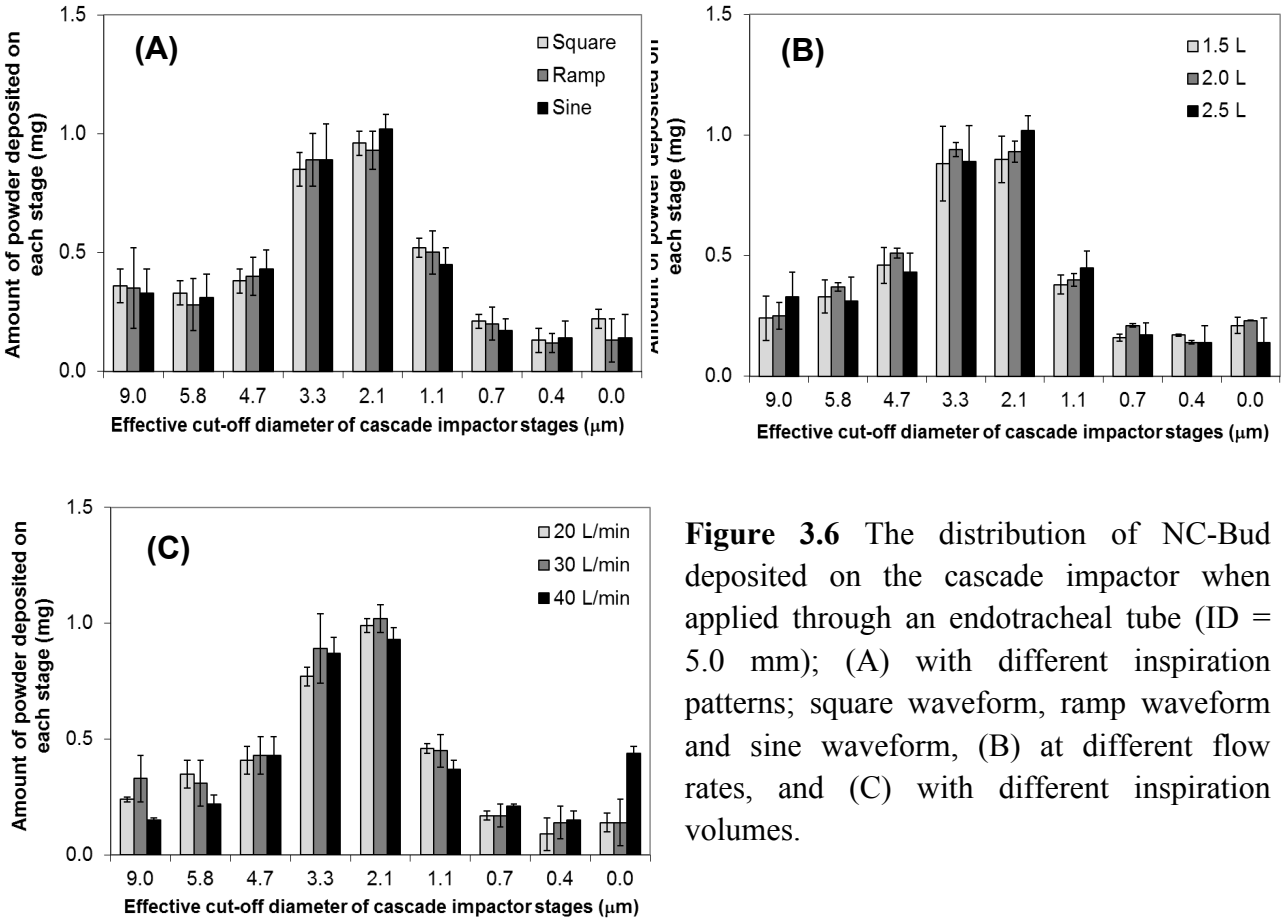

Figure 3.6 The distribution of NC-Bud deposited on the cascade impactor when applied through an endotracheal tube (ID = 5.0 mm); (A) with different inspiration patterns; square waveform, ramp waveform and sine waveform, (B) at different flow rates, and (C) with different inspiration volumes.

Also, the same %EF, %FPF, MMAD and GSD were achieved for the study of the different volumes. Studies investigating the role of capsules on the inhaler performance suggested that, although the size of the capsule has an insignificant effect on inhaler performance, the capsule-particle impaction could play a role in the de-agglomeration mechanism depending on the size of the capsule hole [170,171]. Here, the same inhaler and the same size of capsules were applied to all experiments. A study that evaluated the effect of inhaled volume on dry powder inhalers showed that the inhaled volumes can have no significant effect on fine particle fraction (FPF) at flow rates of 30 and 60 L/min [172]. It is likely, therefore, that the

time for emptying the capsule was shorter than the time required for delivering 1.5 L of air or more through the cascade impactor.

3.3.3 Effect of humidity of inspiratory airflow on powder performance

Traditionally, liquid aerosols are used in mechanically ventilated patients. The efficiency of aerosol delivery is affected by humidity of inspired air and by humidity in the circuit[65,78,173]. In one study [173], albuterol was delivered via nebulizers through an endotracheal tube. The size of aerosol droplets increased when the humidifier was applied due to hygroscopic growth. A greater impaction in the ventilator tubing decreased the total aerosol delivery as humidity increased. An *in vivo* study delivering antibiotics to patients showed higher sputum levels (sputum levels provide a direct index of drug delivery) in a non-humidified ventilator compared to humidified [173]. Similarly, albuterol delivered via pMDIs showed lower percent drug delivery in a humidified circuit due to impaction in the ventilator circuit [78]. Therefore, humidity is an important parameter that affects the efficiency of drug delivery during mechanical ventilation.

Besides liquid aerosol delivery, relative humidity can have an effect on dry powder aerosol delivery. The efficiency of drug delivery can be decreased in both dry and humid environments [87]. At high humidity, capillary force between the particles can decrease drug delivery efficiency. Moisture can create cohesive forces between particles, decreasing powder dispersion [88,89]. Conversely, static charges between the particles play a role in powder dispersion at low humidity [90]. Furthermore, each drug has a different physicochemical nature (i.e. hygroscopicity).

Budesonide is a relatively non-hygroscopic drug, yet the powder performance depends on the humidity. At low relative humidity (RH), budesonide particles were reported to carry static

charge. That charge can be dissipated when the RH increases; however, if the %RH was higher than 65, the dispersion of budesonide was decreased due to cohesive forces between particles [88,90]. NanoCluster budesonide (NC-Bud) was previously investigated under 2 conditions of relative humidity (41% and 55%). The results showed that the formulations performed better at the higher %RH [154]. Here, the ventilator was used to control the percent relative humidity at around 51 and 82% (under operating of the humidifier, the equilibrated humidity in the circuit was 82%).

The NC-Bud aerosol was delivered as before (Monodose® inhaler, 5.0 mm endotracheal tube, 30 L/min). The sine wave form and the inspiratory volume of 2.5 L were applied for all experiments. The %EF of NC-Bud when operated at 82% was lower than the %EF of NC-Bud when operated at 51% RH ($p<0.05$) although the distribution of aerosol powder at 51 and 82% RH were the same (Figure 3.7). The MMAD at 51% and 82% RH were 2.2 ± 0.3 and 2.1 ± 0.1, respectively (Table 3.4).

Table 3.4 Cascade impaction results of NC-Bud when applying through an endotracheal tube (ID = 5.0 mm) at different relative humidity of airflows (Values = Average ± SD).

Relative humidity	**% EF**	**% FPF**		**MMAD**	**GSD**
(%RH)		**< 5.8**	**< 3.3**	**(μm)**	
51	64.6 ± 7.3	85.2 ± 3.3	48.8 ± 6.6	2.2 ± 0.3	2.4
82	36.6 ± 2.1	86.4 ± 3.6	50.8 ± 2.8	2.1 ± 0.1	2.0

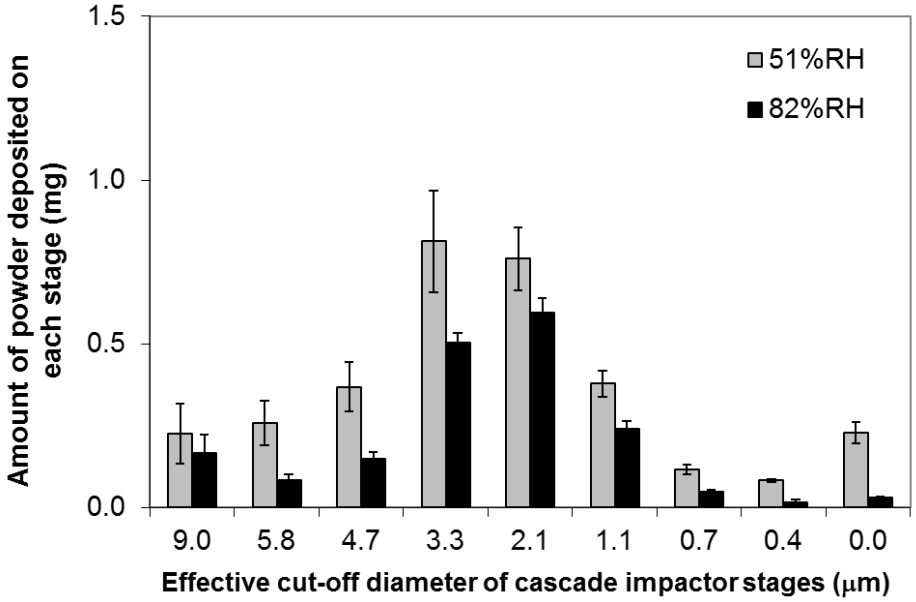

Figure 3.7 The distribution of NC-Bud deposited on the cascade impactor when applied through an endotracheal tube (ID = 5.0 mm) at a flow rate of 30.0 L/min at different humidity of airflows.

3.3.4 Powder performance using a novel inhaler

Most dry powder inhalers (DPIs) were not designed for use with patients on mechanical ventilation. In order to apply dry powder technology to ventilated patients efficiently, DPIs should fit with the connections of the ventilator circuit. Here, a novel device was designed for this purpose. The novel inhaler was compared to the Monodose® inhaler (Figure 3.8). The resistance of the device is an important parameter that should be considered when designing a new inhaler. The resistance and the design of the inhaler can determine the efficiency of drug delivery [113,174]. The specific flow resistance of the device is related to the pressure drop across the device and the volumetric flow rate as follows:

$$R = \frac{\sqrt{\Delta P}}{Q}$$

where R is the specific flow resistance, Q is the volumetric flow rate and ΔP is the pressure drop across the device [153]. Based on the resistance, dry powder inhalers can be categorized into three groups; low, medium, and high resistance devices [90,156]. The resistance of the device determines the turbulence of the airflow within the device. A high resistance inhaler such as the Inhalator Ingelheim® should generate higher turbulence, often resulting in higher %FPF compared to a medium resistance inhaler (Cyclohaler®, Diskhaler®) or a low resistance inhaler (Rotahaler®).

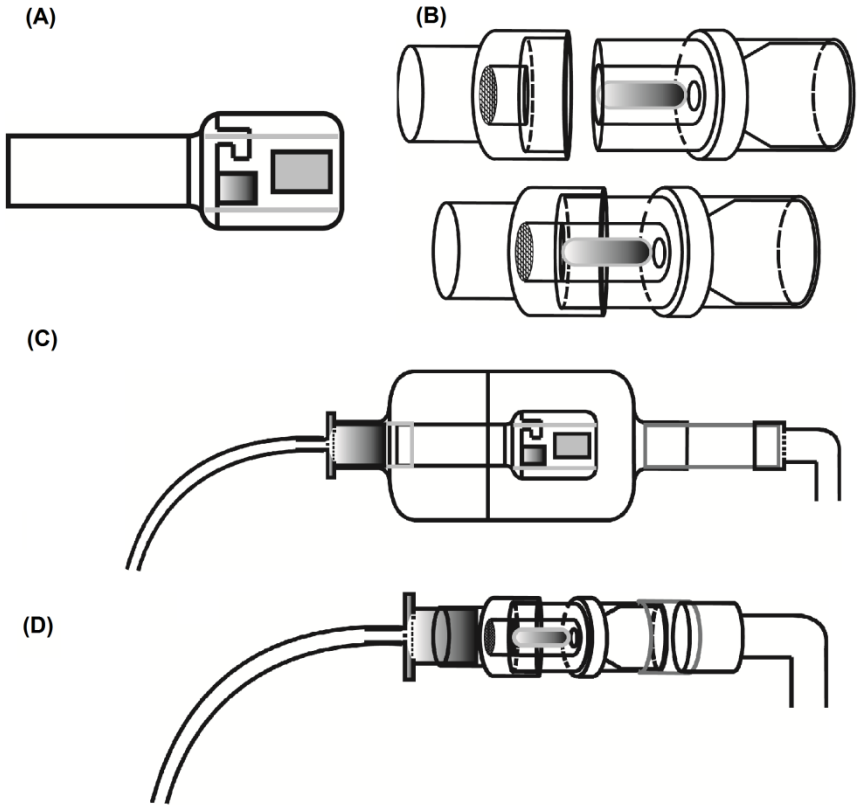

Figure 3.8 Schematic diagrams of (A) the Monodose® inhaler, (B) the novel device, (C) the Monodose® inhaler connected to the mechanical ventilator circuit, and (D) the novel device connected to the mechanical ventilator circuit.

Besides the resistance of the device, the flow rate has an influence on the turbulence across the device. The inhalers should, therefore, be tested at the proper flow rate. Some recommended that inhalers should be tested at flow rates of 30 L/min, 60 L/min and 90-100 L/min for high, medium and low resistance devices, respectively [90]. Other studies reported that devices with different specific resistances can generate the same FPF if they are operated at a specific flow rate and using the same formulation [156]. Thus, both device resistance and flow rate are critical.

Moreover, the different patterns of turbulent airflow through the device depend on the internal geometry of the device. The effect of device design on the FPF of drug delivered was reported [156]. For example, a study of aerosol performance delivered using medium resistance devices (Diskhaler® and Cyclohaler®) at different flow rates showed the same FPF at 60 L/min and different FPF at 30 L/min. Cyclohaler® was less sensitive to flow rate in the terms of FPF compared to Diskhaler®. The FPF of drug substantially decreased from the Diskhaler® at 30 L/min [156]. The authors suggested that the preferred device should generate a higher turbulence at low flow rate so that a high FPF could be obtained.

The pressure drop across a device should be investigated when designing a new device. A pressure drop ranging from 2.9 – 16.0 kPa was reported for patients with varied lung disease states [175]. From the United States Pharmacopeia (USP) standard, DPIs have to test at a constant inspiratory volume of 4 L and a constant pressure drop of 4 kPa [176]. A study of relationships between the percent of relative de-agglomeration (%RD) of drug powder and the pressure drop in different inhalers showed that the Monodose® had the best %RD at the 4 kPa pressure drop. The report suggested that the Monodose® was the most efficient device compared to Rotahaler®

and Handihaler®, however, the relationship was different with different drug powders[175]. Here, the novel device had resistance of 0.0514 $kPa^{0.5}L^{-1}min$ whereas the Monodose® was 0.018 $kPa^{0.5}L^{-1}min$. The resistance of the novel device was significantly higher than the Monodose®.

The NC-Bud aerosol was applied via the novel device through a 5.0 mm endotracheal tube at a flow rate of 30 L/min. The sine wave form and an inspiration volume of 2.5 L were used as before. The MMAD of NC-Bud when applied via the new inhaler was 1.7 ± 0.1 compared to 2.2 ± 0.3 when applied via the Monodose®. The %EF of NC-Bud when applied via the novel device or Monodose® inhaler were not significantly different ($p<0.05$). The GSD of both experiments was around 2.3 to 2.4. (Table 3.5, Figure 3.9). Although the powder performance was not dramatically improved when applying the novel device, the novel device provided the convenience of a direct fit with the ventilator and the endotracheal tube connections.

Table 3.5 Cascade impaction results of NC-Bud when applying through an endotracheal tube (ID = 5.0 mm) via different devices (Values = Average ± SD).

Device	**% EF**	**% FPF**		**MMAD**	**GSD**
		< 5.8	**< 3.3**	**(µm)**	
Monodose® inhaler	64.6 ± 7.3	85.2 ± 3.3	48.8 ± 6.6	2.2 ± 0.3	2.4
Novel device	68.0 ± 6.2	89.6 ± 0.7	60.0 ± 1.5	1.7 ± 0.1	2.3

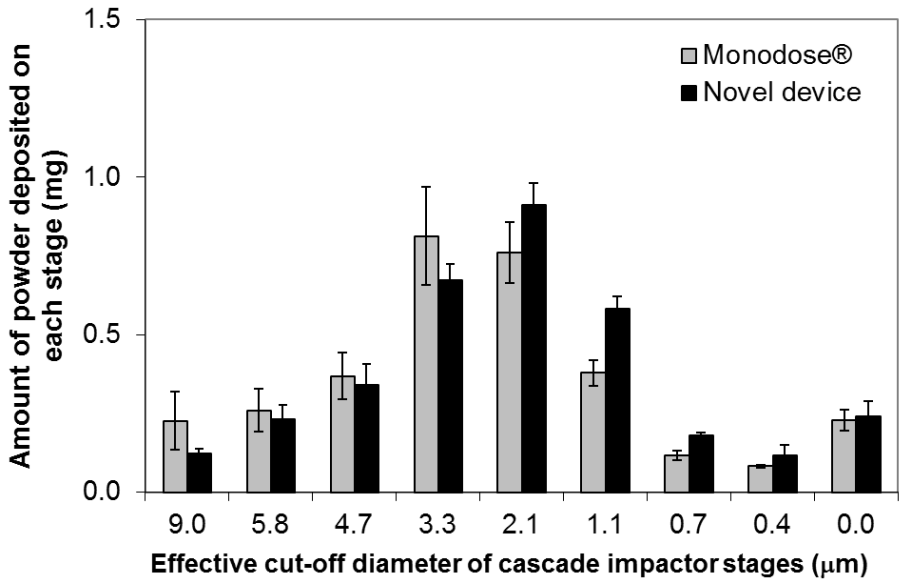

Figure 3.9 The distribution of NC-Bud deposited on the cascade impactor when applied through an endotracheal tube (ID = 5.0 mm) at a flow rate of 30.0 L/min via different inhalers.

3.3.5 Effect of inhalation cycles on powder performance

To ensure that NC-Bud formulations emptied from the capsule, three inspiration cycles were applied. The data were then compared to the data when one inspiration cycle was applied. The aerosol was applied via the new inhaler through a 5.0 mm endotracheal tube. A flow rate of 30 L/min, an inspiration volume of 2.5 L and the sine wave form inspiration pattern were controlled by the ventilator. The result showed the same performance of NC-Bud formulation when either one inspiration or three inspiration cycles was applied. The MMAD was the same (1.7 ± 0.1) and the %EF was not significantly different. (Table 3.6, Figure 3.10).

Table 3.6 Cascade impaction results of NC-Bud when applying through an endotracheal tube (ID = 5.0 mm) via the novel device. (Values = Average ± SD).

Number of inspiration cycles	% EF	% FPF		MMAD (μm)	GSD
		< 5.8	< 3.3		
1-time	68.0 ± 6.2	89.6 ± 0.7	59.9 ± 1.5	1.7 ± 0.1	2.3
3-times	72.9 ± 4.7	86.8 ± 1.9	58.9 ± 2.8	1.7 ± 0.1	2.5

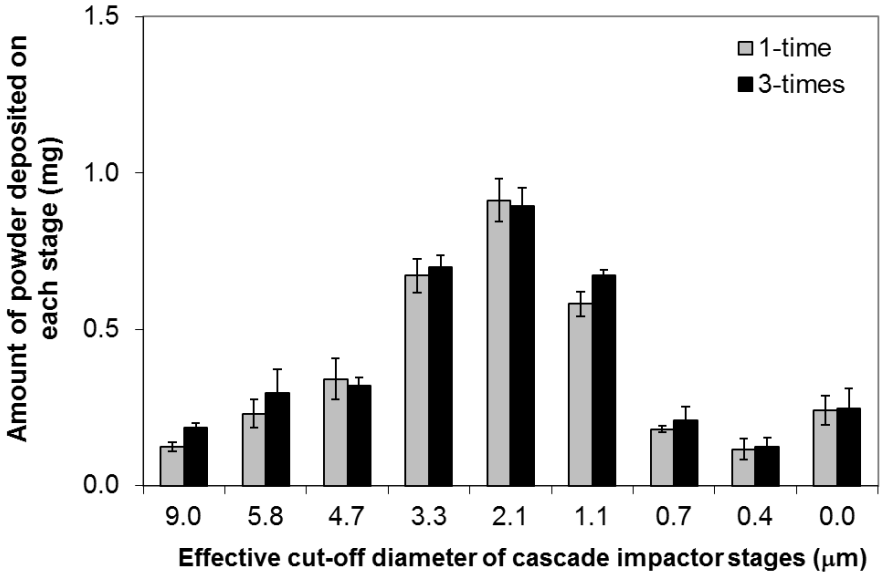

Figure 3.10 The distribution of NC-Bud deposited on the cascade impactor when applied through an endotracheal tube (ID = 5.0 mm) at a flow rate of 30.0 L/min with different inspiration cycles.

As discuss previously, the capsule emptying time depended on the size of the capsule hole more than the size of the capsule [170]. An optimal range of the capsule hole of 1.00 to 2.38 mm was suggested for delivering high FPF with minimal impaction loss [171]. A study found that the capsule with one hole of 1.5 mm can be emptied in 0.8 ± 0.05 seconds at a flow rate of 60 L/min [170]. Here, capsules with two holes of 1.5 mm were used to deliver NC-Bud formulations at a flow rate of 30 L/min. A 2.5 L inspiratory volume was applied for one cycle, so the inspiration time for one cycle equaled 5 seconds. Although, the ventilator flow rate was only 30 L/min, one

may suspect that, the capsule should be emptied in the first half of the first inspiration cycle. The remaining time of the inspiration cycle and the delayed time before the next cycle represent the time that powders can travel through and deposit in the cascade impactor. Since the data showed the same powder performance of NC-Bud for either one or three cycles, it can be deduced that NC-Bud also completely deposited within the cascade impactor before the next inspiration cycle started.

3.3.6 Effect of tube diameter on powder performance

The tube diameter can play a role in drug delivery efficiency in ventilated patients. For liquid formulations, larger diameter tubes are often preferred to small ones. Some studies reported that a reduction of the inner diameter of endotracheal tubes (5 to 7.5 mm) decreased aerosol delivery [79,146]. NC-Bud formulations were previously delivered through different endotracheal tubes. The MMAD of NC-Bud powders actually decreased with a decrease in tubing diameter [154].

Airflow in a tube can be categorized into three types; laminar, transient or turbulent. These types of airflows are described by the Reynolds number (Re). Re represents the relationship between the inertial forces and viscous force as follows[154]:

$$\mathrm{Re} = \frac{\rho v d}{\mu} = \frac{\rho Q d}{\mu \mathrm{A}} = \frac{4\rho Q}{\pi d \mu} \qquad \text{(Eq. 3.2)}$$

where ρ is the density of air (1.20 kg/m^3 at 20°C), ν is the linear velocity (m/s), d is the diameter of the tube (m), μ is the dynamic viscosity of air [1.81× 10^{-5} kg/(m.s) at 20°C], Q is the volumetric flow rate (m^3/s), and A is the tube cross-sectional area (m^2). Laminar flow and

turbulent flow are typically observed at Re less than 2100 and Re more than 4000, respectively. Flow in the transient region (2100 < Re < 4000), can have laminar or turbulent character[147].

The NC-Bud aerosol was again delivered using a ventilator combined with the novel device. The flow rate of 30 L/min was applied. NC-Bud was applied through four different diameter tubes with inner diameters of 3.0, 5.0, 6.5 or 8.0 mm. NC-Bud showed smaller MMAD when applied through the catheter tube (3.0 mm ID) compared to endotracheal tubes (5.0, 6.5 and 8.0 mm ID). The $\%FPF_{<5.8}$ was not significantly different ($p<0.05$), however, the $\%FPF_{<3.3}$ of NC-Bud applied through the catheter tube was higher than the other tubes. The %EF of NC-Bud was lower in the catheter tube compared to 6.5 and 8.0 mm endotracheal tubes but was not different when compared to the 5.0 mm tube. (Table 3.7, Figure 3.11).

Table 3.7 Cascade impaction results of NC-Bud when applying through an endotracheal tube (ID = 5.0, 6.5, 8.0 mm) and a catheter tube (ID = 3.0 mm) via the novel device (Values = Average ± SD).

Tube size (mm)	**% EF**	**% FPF**		**MMAD (µm)**	**GSD**
		< 5.8	**< 3.3**		
3.0	68.0 ± 3.2	90.8 ± 1.8	82.3 ± 2.8	0.9 ± 0.0	2.1
5.0	68.0 ± 6.2	89.6 ± 0.7	59.9 ± 1.5	1.7 ± 0.1	2.3
6.5	77.9 ± 2.3	89.2 ± 2.8	65.9 ± 6.6	1.4 ± 0.3	2.6
8.0	78.2 ± 5.2	89.1 ± 2.5	63.9 ± 3.0	1.5 ± 0.1	2.6

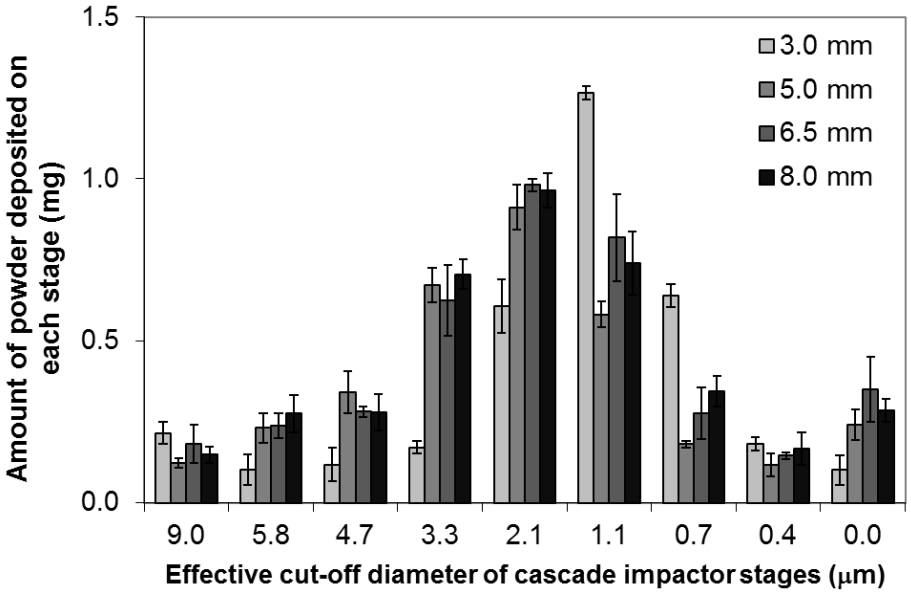

Figure 3.11 The distribution of NC-Bud deposited on the cascade impactor when applied through catheters and endotracheal tubes with different diameters at a flow rate of 30.0 L/min.

The Re has an inverse relationship with diameter (d). The calculated Re values of 3.0, 5.0, 6.0, and 8.0 mm tubes were 14067, 8440, 6492, and 5275, respectively. Although all of these are in the turbulent regime (Re>4000), the smallest ID tube had a very high Re compared to the other tubes. The higher turbulent flow corresponded to higher shear force in the airflow, probably improving de-agglomeration of the drug powders.

3.3.7 Powder performance using a ventilation bag

A ventilation bag was also used to provide the inspiration airflow and compared to ventilator using the same experimental conditions as described above. The %EF of NC-Bud was not significantly different when either the ventilator or the ventilation bag was used. The ventilator can provide a higher flow rate compared to the ventilator bag, resulting in a slightly higher %FPF of NC-Bud. A peak flow rate of approximately 23.0 L/min was measured for the ventilator bag. Consistently, the data showed a smaller MMAD ($p<0.05$) on the ventilator as well

(Table 3.8); however, the overall performance of NC-Bud was almost the same when using the ventilator or the ventilation bag (Figure 3.12).

Table 3.8 Cascade impaction results of NC-Bud when applying through endotracheal tube (ID = 5.0 mm) comparing between the ventilator and the ventilator bag. (Values = Average ± SD).

Airflow supply	% EF	% FPF		MMAD (μm)	GSD
		< 5.8	< 3.3		
Ventilator	68.0 ± 6.2	89.6 ± 0.7	60.0 ± 1.5	1.7 ± 0.1	2.3
Ventilator bag	63.9 ± 0.7	84.4 ± 1.4	49.2 ± 2.5	2.2 ± 0.1	2.4

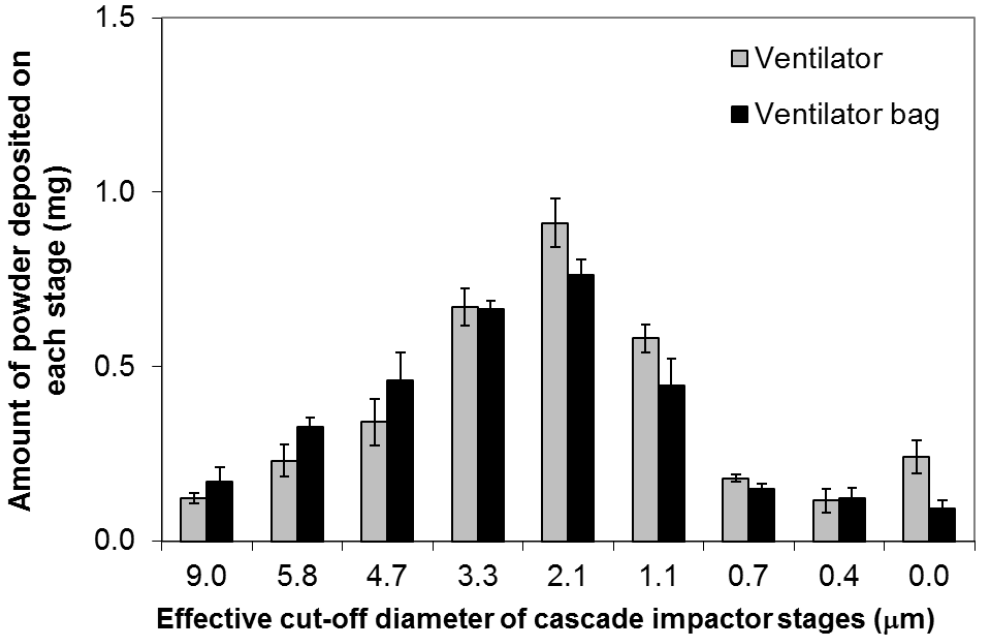

Figure 3.12 The distribution of NC-Bud deposited on the cascade impactor when applied through an endotracheal tube (ID = 5.0 mm) comparing between the ventilator and the ventilator bag.

Other studies conducted using the ventilation bag confirmed the trends found when using the ventilator as the air source. Applying three cycles of inhalation using the ventilation bag showed a %EF was not different from a single inhalation ($p<0.05$) (Table 3.9). However,

multiple inhalation cycles resulted in a shift of the distribution towards a smaller MMAD ($p<0.05$) (Figure 3.13).

Table 3.9 Cascade impaction results of NC-Bud when applying through an endotracheal tube (ID = 5.0 mm) via the novel device. The ventilator bag was used as the air source (Values = Average ± SD).

Number of inspiration cycles	% EF	% FPF		MMAD (μm)	GSD
		< 5.8	< 3.3		
1-time	63.9 ± 0.7	84.4 ± 1.4	49.2 ± 2.5	2.2 ± 0.1	2.4
3-times	68.6 ± 9.0	89.3 ± 1.8	63.6 ± 1.8	1.4 ± 0.1	2.6

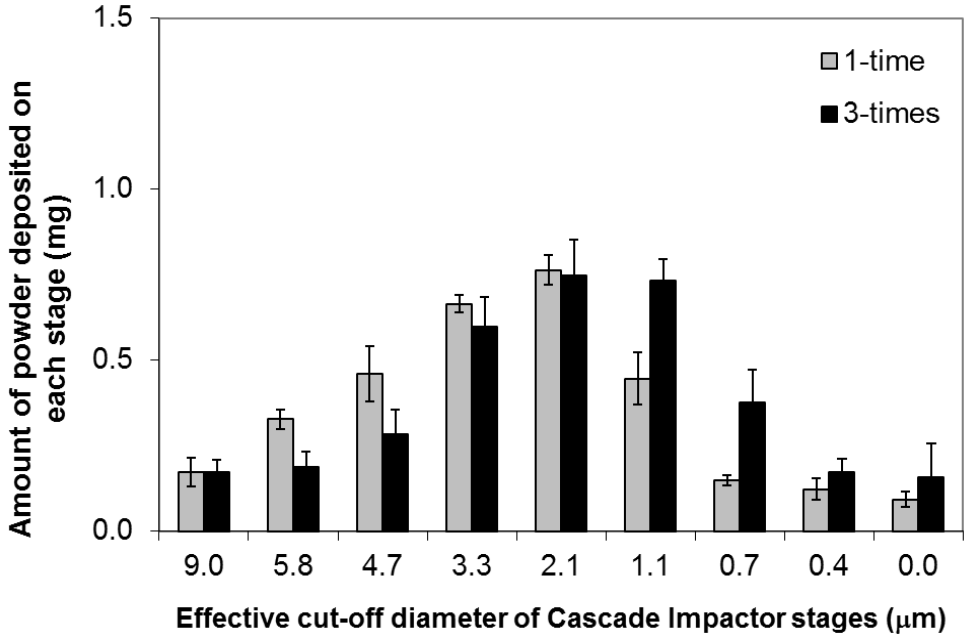

Figure 3.13 The distribution of NC-Bud deposited on the cascade impactor when applied through an endotracheal tube (ID = 5.0 mm) at a flow rate of 23 L/min. The ventilator bag was used as the air source.

NC-Bud was also applied through different diameter endotracheal or catheter tubes (3.0, 5.0 and 6.5 mm). As observed in ventilator experiments, larger diameter tubes provided a higher %EF of NC-Bud. The distribution of the NC-Bud shifted towards a smaller MMAD when applied through the smaller diameter tubes, especially the catheter tube (Table 3.10, Figure 3.14).

Table 3.10 Cascade impaction results of NC-Bud when applying via the novel device through an endotracheal tube (ID = 5.0, 6.5 mm) and a catheter tube. The ventilator bag was used as the air source (Values = Average ± SD).

Tube size (mm)	% EF	% FPF		MMAD (μm)	GSD
		< 5.8	< 3.3		
3.0	54.6 ± 2.6	92.0 ± 2.1	77.6 ± 2.2	1.1 ± 0.0	2.1
5.0	63.9 ± 0.7	84.4 ± 1.4	49.2 ± 2.5	2.2 ± 0.1	2.4
6.5	74.3 ± 4.5	86.0 ± 3.1	52.9 ± 1.4	1.9 ± 0.1	2.5

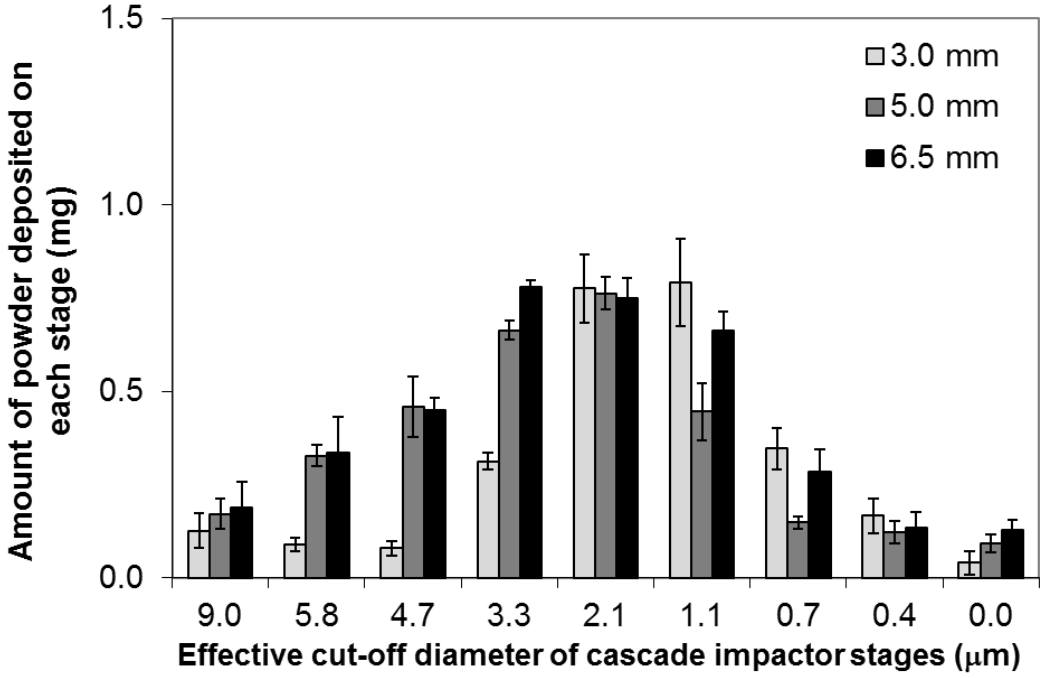

Figure 3.14 The distribution of NC-Bud deposited on the cascade impactor when applied through catheters and endotracheal tubes with different diameters. The ventilator bag was used as the air source.

3.4 Conclusion

NanoCluster budesonide (NC-Bud) dramatically improved aerosol performance compared to stock budesonide when delivered using a ventilator. Parameters controlled by the ventilator such as inspiration pattern and inspiratory volume, did not affect the performance of NC-Bud formulations. Volumetric flow rates in the range of 20 to 40 L/min also did not change the powder performance. Higher humidity in the ventilator circuit, however, decreased the percent emitted fraction although the fine particle fraction was not significantly changed. A novel device was preferred to the Monodose® due to the convenience of connecting with the ventilator and endotracheal tubing while maintaining efficient aerosol delivery. Studies using a ventilation bag were consistent but slightly different from the ventilator, probably due to the better inspiratory controls of the ventilator. NanoCluster technology combined with a new device may offer effective drug delivery to ventilated patients.

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Chapter 4

NanoCluster itraconazole formulations provide a potential engineered drug particle approach to generate effective dry powder aerosols

4.1 Introduction

Drug nanoparticle formulations can be created by two approaches: bottom-up processes and top-down processes. Bottom-up processes such as precipitation builds up particles from the molecular state whereas the top-down processes such as milling breaks down large micron-sized particles into smaller particles[1]. In both processes, the large surface area of particles increases free energy of the particles. To reduce the free energy, smaller particles generated during processing tend to agglomerate together, and, potentially, crystalline nuclei will dissolve and precipitate onto other particles via Ostwald ripening[2]. Excipients such as surfactants and viscosity modifiers have been used to minimize agglomeration, but few have attempted to actually control the agglomeration process.

In previous studies, budesonide was used to explore the phenomenon of nanoparticle agglomeration to create particles known as "NanoClusters". NanoCluster dry powder aerosols demonstrate a desirable microstructure for efficient lung deposition and nanostructure for rapid dissolution of poorly water-soluble drugs. Studies have shown that budesonide NanoClusters provided efficient aerosolization with a high fine particle fraction and faster dissolution when compared to the stock micronized powder[3]. The success of NanoCluster technology encouraged the development of other inhaled drug formulations.

Itraconazole (ITZ), a triazole antifungal agent, is a poorly water-soluble drug that has been explored as an inhaled therapeutic. The solubility of this drug in water is less than 1 μg/ml, across pH values from 1 – 12.7[4]. ITZ has a broad spectrum of activity including *Aspergillus* species[5]. ITZ interferes in sterol biosynthesis in fungal cell membrane by inhibiting cytochrome P450 of the fungi, leading to cell death[4]. ITZ is orally administered for treatment of fungal

infections such as allergic bronchopulmonary aspergillosis (ABPA)[6]. Oral formulations must be given in high doses to achieve effective concentrations in the lungs. One study showed a partial but significant improvement of pulmonary function when patients were treated with oral ITZ for 1 year[7]. Oral ITZ significantly improved clinical symptoms and reduced the mean dose of oral glucocorticoids required in patients with ABPA. Poor oral bioavailability and variable absorption of oral solutions limit ITZ to a second or third line treatment option. Absolute oral bioavailability of the oral capsule is 55% in the fed state and 40% lower in the fasted state[8]. Moreover, hydroxypropyl-β-cyclodextrin often used in the oral solution can cause gastrointestinal toxicity in patients[9].

Since invasive *aspergillosis* primarily occurs in immunocompromised patients via inhalation of conidia into the lungs, the treatment of this fungal infection has been a focus for pulmonary drug delivery. Delivery of aerosolized ITZ to the lung tissue offers local treatment and prophylaxis against invasive aspergillosis at the primary site of infection in the lungs. Aerosolized ITZ can minimize systemic side effects, and eliminate the need for formulation with cyclodextrin. *In vivo* studies showed prolonged survival, high lung tissue concentrations, and low systemic exposure of aerosolized nanostructured ITZ formulations compared to commercially available ITZ oral solution[10]. A study showed the lung tissue concentration increased nearly 10 times when ITZ was administered by inhalation compared to the oral route in a mouse model[11]. The ITZ administered as an aerosol significantly enhanced the survival of mice infected with *Aspergillus fumigatus* and achieved greater ratios of lung:serum drug concentration compared to the orally dosed ITZ compositions. High and sustained lung tissue concentrations were achieved while serum levels were maintained above the minimum lethal concentration (MLC) of *A. fumigatus*. Researchers mentioned that the high and sustained lung concentrations of inhaled ITZ

demonstrated effective local delivery to prevent fungal spore germination, growth, and dissemination of the fungus from the lung to the body[12].

Although an inhaled formulation of ITZ has not been available to enable local treatment of aspergillosis, inhalation of aerosolized ITZ in the form of a nanosuspension has been studied *in vivo*[13]. More, recently, an ITZ dry powder was prepared and studied *in vitro*. Researchers reported the fine particle fraction (FPF) of amorphous ITZ was 16-47% when investigated using a multi-stage liquid impinger[14]. The variation of the percent FPF and percent of the emitted dose depended on excipients that were used in the formulation. Here, ITZ NanoCluster formulations were created via wet milling without using any excipients. The formulations obtained by wet milling (top-down process) were compared to formulations that were prepared by precipitation methods (bottom-up processes) and to stock itraconazole. The physicochemical properties and aerosol performance of different ITZ NanoClusters suggested an optimized top-down process might be the preferred process.

4.2 Materials and methods

4.2.1 Materials

Itraconazole was purchased from Sigma Chemicals Co. (St. Louis, MO). Methanol, ethanol and methylene chloride were purchased through Fisher Scientific. Double-distilled water was provided by an EASYpure® RODI (Barnstead International, Dubuque, Iowa).

4.2.2 Methods

4.2.2.1 Itraconazole formulations (ITZ) prepared by precipitation methods

NanoCluster suspensions of itraconazole were prepared using anti-solvent precipitation. Solutions of the drug in 1,3-dioxolane were prepared at concentrations of 0.4% (w/v). ITZ solution (5 ml) was directly injected into water at a rate of 5 mL/min using ultrasonication (probe-type sonicator, Fisher Scientific, Sonic Dismembrator) at amplitude of 30% in an ice bath or alternatively using homogenization (probe-type homogenizer, Tissue tearor, Biospec Products, Inc.) at 25,000 rpm. Suspensions were analyzed by dynamic light scattering. All measurements were performed in triplicate. After precipitation, the collected suspension was frozen at -80ºC and lyophilized for ~36 hours to remove all appreciable water content.

4.2.2.2 Itraconazole formulations (ITZ) prepared by wet milling

Itraconazole formulations (ITZ) were prepared by milling 1 gram of micronized itraconazole in 300 mL of 10%EtOH. The suspension was collected at 0.5, 1, and 2 hours milling time. A Netzsch MiniCer Media Mill was operated using YTZ® grinding media (0.2 mm, Tosoh Corp., Tokyo, Japan) under an agitation speed of 2004 rpm. Particle size of the suspensions was determined by dynamic light scattering (Brookhaven Instruments Corp., ZetaPALS, Holtsville, NY) at different time intervals during the milling process. After milling, the collected suspension was frozen at -80ºC and lyophilized for ~36 hours to remove all appreciable water content (VirTis Feezemobile-12XL, The Virtis Company,Gardiner, New York).

4.2.2.3 Aerosol characterization

The aerodynamic characteristics of itraconazole (ITZ) formulations were determined using an Andersen Cascade Impactor (ACI) (Tisch Environmental, Inc., Village of Cleves, OH). Approximately 5 mg of each powder was filled in a capsule (gelatin type, size 3, generously provided from Capsugel, NJ, USA). Powder was introduced to the cascade impactor via a Monodose® inhaler (a Plastiape Monodose Inhaler RS01 Model 7) at ~90 L/min for 2.6 seconds or at ~30 L/min for 8.0 seconds. Cut-off particle aerodynamic diameters at 90 L/min for each stage of the impactor were: stage 0 (8.0 μm), stage 1 (6.5 μm), stage 2 (5.2 μm), stage 3 (3.3 μm), stage 4 (2.1 μm), stage 5 (1.1 μm), stage 6 (0.7 μm), stage 7 (0.4 μm), and filter (0.0 μm). Cut-off particle aerodynamic diameters at 30 L/min for each stage of the impactor were: stage 0 (9.0 μm), stage 1 (5.8 μm), stage 2 (4.7 μm), stage 3 (3.3 μm), stage 4 (2.1 μm), stage 5 (1.1 μm), stage 6 (0.7 μm), stage 7 (0.4 μm), and filter (0.0 μm).

Dry powders deposited on each stage of the impactor were quantified by the difference in weight of the plate on each stage before and after running the experiment. All experiments were performed under controlled conditions (21 ± 2 °C, 50 - 55% RH) in triplicate. The emitted dose (ED) was defined as the mass of drug collected from all stages of ACI. The emitted fraction (EF) was determined as the percent of the emitted dose divided by the initial mass delivered into the impactor. The fine particle fraction of the emitted dose (FPF_{ED}) was calculated as the percentage of aerosolized particles that have aerodynamic diameters below 6.5 μm (or 5.8 μm for 30 L/min) and the percentage of aerosolized particles that have aerodynamic diameters below 3.3 μm. The mass median aerodynamic diameter (MMAD) was determined at the 50th percentile of the cumulative mass distribution curve. Geometric standard deviation (GSD) was calculated as the

square root of the ratio of diameters at the percentile of 84.13% and 15.87% of the cumulative distribution. The percent emitted fraction (%EF), fine particle fractions of the emitted dose (FPF_{ED}), mass median aerodynamic diameter (MMAD), and geometric standard deviation (GSD) were calculated as previously reported[15].

4.2.2.4 Evaluation of particle size and morphology by scanning electron microscopy (SEM)

The size and morphology of the formulated itraconazole powders were evaluated using an LEO 1550 field emission scanning electron microscope and compared to that of stock itraconazole powders. Prior to imaging, the samples were sputter-coated with gold for thickness of 30 nm.

4.2.2.5 Thermal analysis

ITZ formulations were investigated by differential scanning calorimetry (DSC) (Q100 Universal V4.3A; TA Instruments, New Castle, Delaware). Accurately weighed portions (2–3 mg) of the lyophilized dry mass was sealed and placed in hermetic aluminum pans and heated at a constant rate of 10 °C/min over a temperature range of 40–300 °C for ITZ formulations. An inert atmosphere was maintained by purging with nitrogen at 50 mL/min. For thermal gravimetric analysis (TGA), samples weighing 5±2 mg were scanned at a rate of 10 °C/min with a nitrogen flow rate of 40 mL/min.

4.2.2.6 Powder x-ray diffraction (PXRD)

The crystallinity of the dried powders was determined by using powder x-ray diffraction (PXRD). PXRD was performed by using a monochromated CuKa radiation (l= 1.54178 Å) on a Bruker Proteum Diffraction System equipped with Helios multilayer optics, an APEX II CCD detector or a Platinum 135

CCD detector and a Bruker MicroStar microfocus rotating anode x-ray source operating at 45 kV and 60 mA. The powder sample was suspended in Paratone N oil then loaded on a nylon loop. The loop was then loaded on the goniometer where either 3 or 2, 180° 10 minute scans (based on the detector) were taken using the Bruker Apex2 V2010.3-0 software package. Scans were taken at 30°, 60° and 90° with the detector 50.0 mm away. The patterns were analyzed using the Bruker EVA powder diffraction software package version 13.0

4.2.2.7 High-performance liquid chromatography analysis

The chemical stability of ITZ during processing was determined by using a Kromasil C8 column (4.6 × 150 mm^2, 5 μm). A mixture of methanol (MeOH) and 0.5% ammonium acetate (in water) in the ratio of 80/20 was used as mobile phase. The system was operated at a flow rate of 0.8 mL/min and the injection volume was 30 μL. Samples were monitored at a wavelength of 261 nm. Itraconazole peak appeared at a retention time of around 6.6 min.

4.2.2.8 Statistical analysis

The results were presented as the mean and the significant differences were evaluated using Prism 4 GraphPad Software and assessed by one-way ANOVA followed by Tukey's Multiple Comparison Test. One-tailed unpaired t-test was used for assessing the differences. All tests were evaluated at a level of confidence of $p<0.05$.

4.3 Result and discussion

4.3.1 Formulation of itraconazole (ITZ)

In dry powder formulations, specific excipients are often required to achieve the desired aerosol properties. Inhaled excipients should be chemically and physically stable and harmless

on the respiratory tract. The number of authorized inactive ingredients, however, is quite limited and documentation of the safety profile of potential excipients for pulmonary administration is usually incomplete[16]. The lack of acceptable excipients can hinder formulation approaches, especially when attempting to develop aerosols of poorly water-soluble drugs. Therefore, engineering the drug itself has become attractive for developing inhaled drugs. Here, itraconazole (ITZ) was formulated without using any excipients. The ITZ formulations were prepared by two approaches: solvent precipitation (bottom-up method) and wet milling (top-down method).

4.3.1.1 Anti-solvent precipitation

Many anti-solvent precipitation techniques have the same principles. The main steps consist of local supersaturation, nucleation, solute diffusion and particle growth[17]. In other words, nanoparticles are generated during mixing of solution and anti-solvent. When the drug concentration in the mixture is supersaturated, nucleation occurs and is followed by particle growth and agglomeration[18]. The nucleation and particle growth depend on the level of supersaturation. Thorat et.al. suggested that rapid and high supersaturation was the main driving force for precipitation[18]. If supersaturation is attained at a slow rate, the metastable zone is crossed very slowly, resulting in large crystals due to the dominance of particle growth. Conversely, if supersaturation is attained rapidly, the metastable zone is also crossed rapidly, resulting in small particles due to the dominance of nucleation in the precipitation process[19,20]. Therefore, particle formation depends on the speed of supersaturation that is partially controlled by the mixing process.

In order to enhance nucleation and suppress particle growth, rapid micro-mixing is normally used to generate particles, leading to 'ultrafine' nanoparticles[21]. Sufficient micro-mixing can provide a uniform growth and a narrow particle size distribution. Rigorous micro-mixing can be achieved in various ways such as anti-solvent precipitation, high-gravity controlled precipitation, supercritical fluid (SCF) technology, flash nanoprecipitation, and sonoprecipitation[21].

Sonoprecipitation is a method of precipitation that uses ultrasound to facilitate micro-mixing. Many studies reported a decrease of mixing time when precipitation occurred under ultrasound[22,23]. One study showed that ultrasound provided more than 10 times higher mixing rate compared to mechanical stirring[24]. Ultrasound creates bubbles by generating alternate cycles of compression and rarefaction within a liquid. The critical size of bubbles then collapse (cavitation process)[25]. Ultrasonic waves can cause faster and fairly uniform nucleation throughout the sonicated volume, leading to smaller and more uniform-sized particles. Ultrasound effects supersaturated system by inducing high supersaturation and decreasing metastable zone width due to the higher mixing rate, resulting in faster nucleation and precipitation of smaller particles[21,25,26]. Ultrasound can also reduce agglomeration of particles by breaking up large agglomerates[27].

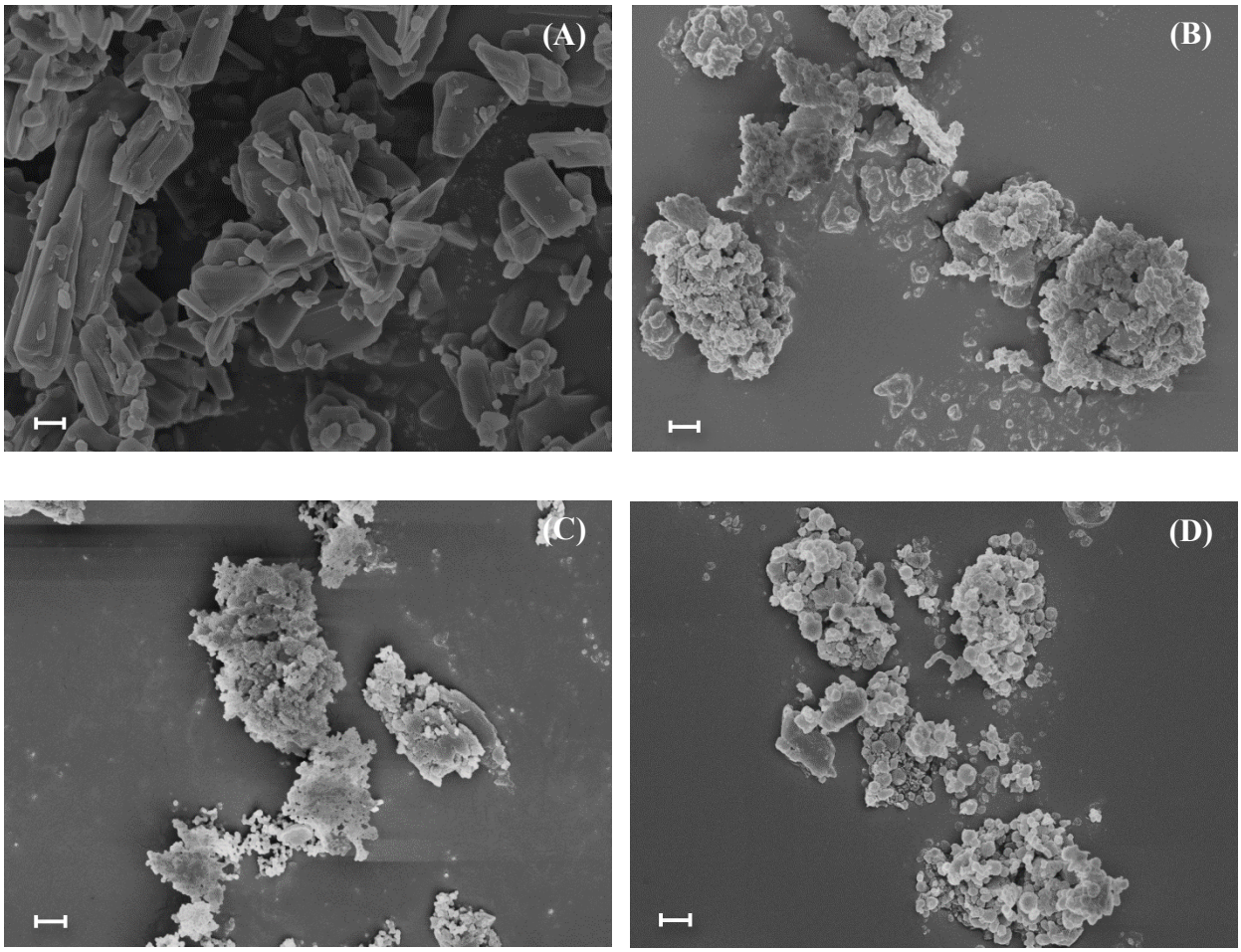


Figure 4.1 SEM images of (A) ITZ as received, (B) milled ITZ, (C) ITZ (sonication) and (D) ITZ (homogenization) (Scale bar equals 1 μm).

Itraconazole NanoClusters were prepared by anti-solvent precipitation using sonication or homogenization. Factors such as drug concentration, solvent type, rate of injection, homogenization speed, and amplitude of sonication were varied to optimize the precipitation conditions. Scanning electron microscopy (SEM) images showed different morphology of drug particles depending on the precipitation process (Figure 4.1). The size of particles decreased compared to ITZ as received. Consistently, increasing ultrasound power led to smaller particles[21]. Nevertheless, DSC thermograms of precipitated ITZ formulations showed an

amorphous state in formulations prepared using sonication or using homogenization (Figure 4.2). The amorphous character of ITZ formulations was confirmed by PXRD (Figure 4.3).

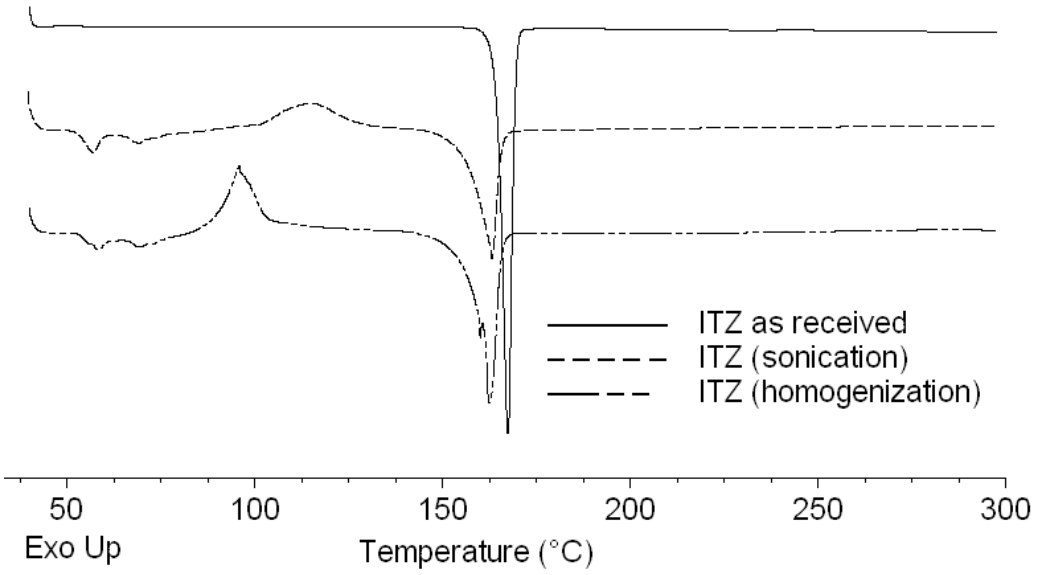


Figure 4.2 DSC profile of ITZ prepared by precipitation compared to ITZ as received.

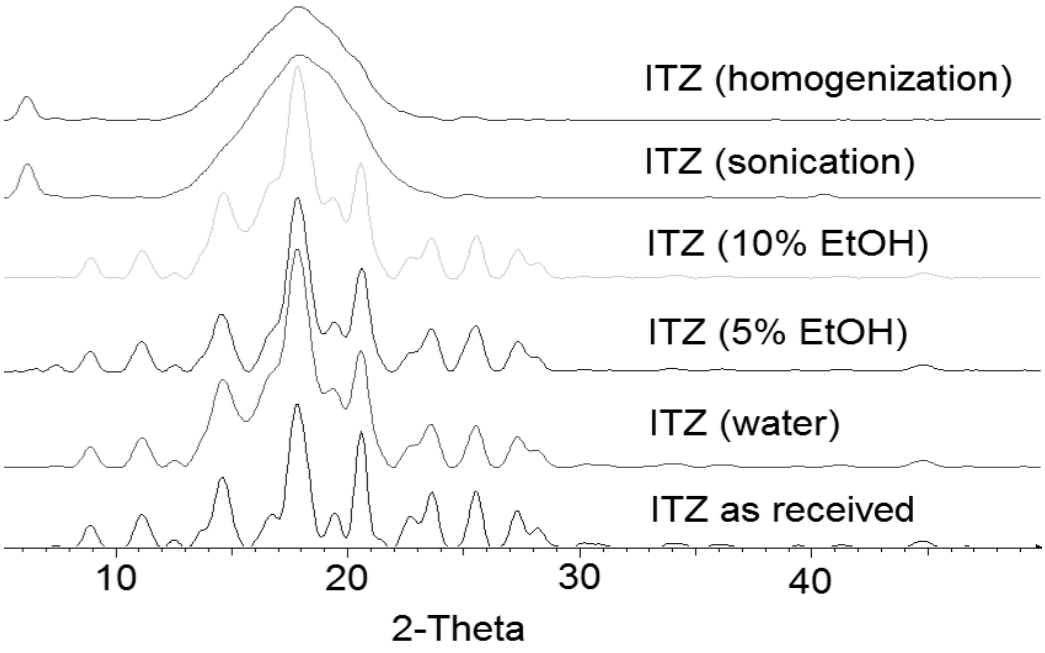


Figure 4.3 PXRD pattern of ITZ formulation.

A study by Dalvi and Dave[28] also found that precipitated itraconazole showed amorphous character due to high nucleation rates and rapid precipitation of itraconazole particles. They mentioned that hydrophobic molecules such as ITZ had a higher supersaturation compared to moderately water-soluble molecules due to a very low equilibrium solubility in anti-solvent-solvent mixture. The nucleation rate is, therefore, always higher. Other researchers also indicated limitations of sonoprecipitation for drug nanoparticles[21]. Sonoprecipitation was reported to work better for production of amorphous nanoparticles although it has also been successfully used for preparing micron-size particles of drugs[29,30]. Although the amorphous state of drug particles may increase the dissolution rate[14,31], amorphous forms can crystallize with time and the physical properties of the drug particles may change[32].

4.3.1.2 Wet milling

Although there are many different mills, ball mills and jet mills are normally used for milling drug to a particle size range of 1–5 μm for inclusion in dry powder aerosol formulation[16]. Wet milling, another top-down method, was chosen to prepare ITZ NanoClusters. Wet milling is a process that utilizes milling media to grind a suspension of insoluble drug. The milling media can be ceramic[33], metallic, glass[34] or highly crosslinked polystyrene resin-coated beads[35,36]. Drugs are dispersed into medium, usually water with surfactant and possibly a viscosity modifier. The movement of the milling media generates shear force and pulverizes the drug particles, leading to size reduction down to 100nm or less[37,38]. The wet milling technique can be scaled up without batch-to-batch variation[37]. Wet milling processes have been used to produce a few FDA approved drugs[38]. Several inhaled drugs such as beclomethasone dipropionate,

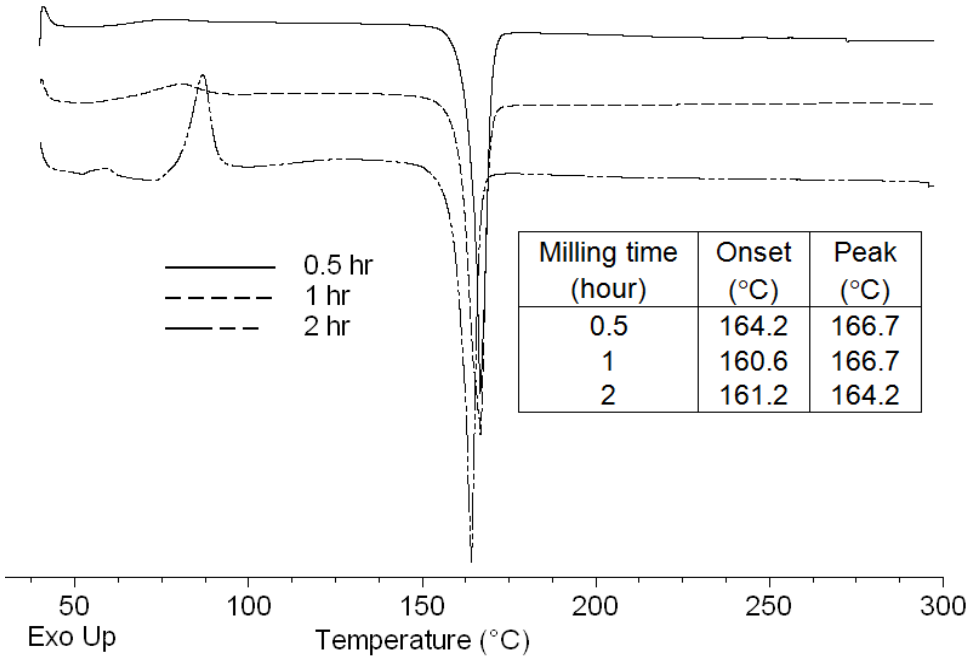
budesonide, fluticasone propionate, and itraconazole[39] have been investigated. Itraconazole, for example, was studied in the form of nanosuspension in order to use with pMDI[40].

In this study, itraconazole (ITZ) NanoClusters were formulated in the form of dry powder for DPI applications. Micronized itraconazole as received was milled using different conditions and durations. Milling time depends on many factors such as the surfactant content (none in this case), hardness of the drug, viscosity, temperature, energy input, and size of the milling media[35]. The milling time can be less than 30 minutes to hours or several days[41]. Several studies showed that processing time could be very lengthy for itraconazole. For example, Yang et. al. itraconazole was milled for 10 days under nitrogen gas to get amorphous ITZ[33] while Tam et. al. spent 2 weeks to mill ITZ in water to get spherical nanoparticles[40]. Here, ITZ suspensions were collected at milling times of 0.5, 1, and 2 hours.

SEM images showed changes in drug morphology and particle sizes of ITZ after milling process (Figure 4.1). The smaller particles and higher porosity of ITZ NanoClusters compared to micronized itraconazole as received increased the surface area. The SEM images showed structures consistent with agglomeration of nanoparticles to form NanoClusters, which would be expected to reduce the free energy of nanoparticles.

ITZ powders milled for larger times showed increasing amorphous state on the DSC thermograms (Figure 4.4). The peak of recrystallization appeared at around 80-90 °C. The degree of amorphousness of ITZ was confirmed by x-ray diffraction patterns (Figure 4.5). It is noteworthy that x-ray diffraction patterns of ITZ prepared by precipitation were different from x-ray diffraction patterns of ITZ formulation prepared by milling technique. This difference of x-ray diffraction patterns indicated different polymorphs of ITZ powders. A study mentioned that

polymorphism might be controlled by solvent selection although metastable polymorphs are sometimes produced in preference to the thermodynamic form[25].



Milling time (hour)	Onset (°C)	Peak (°C)
0.5	164.2	166.7
1	160.6	166.7
2	161.2	164.2

Figure 4.4 DSC thermogram of ITZ milled in water for different durations.

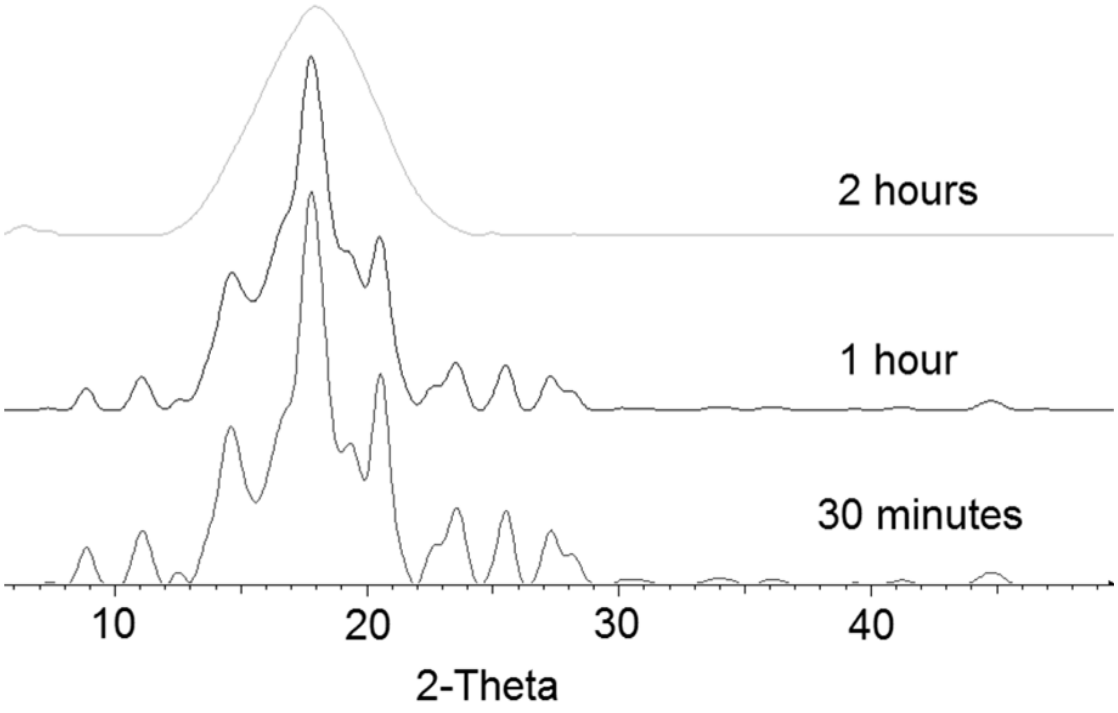


Figure 4.5 PXRD pattern of ITZ milled in water for different durations.

4.3.1.3 Characterization of aerosolized ITZ formulations

The aerosolization of NanoCluster itraconazole formulations was assessed by cascade impaction. ITZ powders were delivered via Monodose® inhaler at flow rate of 90 L/min for 2.6 seconds. The aerosolization efficiency was quantified as the percent emitted fraction (%EF), the percent fine particle fraction (%FPF), and mass median aerodynamic diameter (MMAD). The aerosol performance of ITZ NanoClusters prepared by milling in water was assessed. The particle size distributions of ITZ formulations were not significantly different ($p<0.05$) when ITZ suspension was collected at milling times of 0.5, 1, or 2 hours (Figure 4.6). Although the %EF was slightly lower when the powder was milled longer, the %FPF and MMAD of these powders were not different ($p<0.05$) (Table 4.1).

Table 4.1 Cascade impaction of milled itraconazole at a flow rate of 90 L/min (Values = Average ± SD).

Milling time (hour)	% EF	% FPF		MMAD (μm)	GSD
		< 6.5	< 3.3		
0.5	86.6 ± 6.5	93.8 ± 1.1	79.7 ± 1.7	0.9 ± 0.0	2.2 ± 0.1
1	74.8 ± 11.4	91.1 ± 0.9	66.8 ± 3.3	1.3 ± 0.1	2.2 ± 0.2
2	66.6 ± 3.2	97.5 ± 0.6	78.2 ± 3.8	1.0 ± 0.1	2.0 ± 0.1

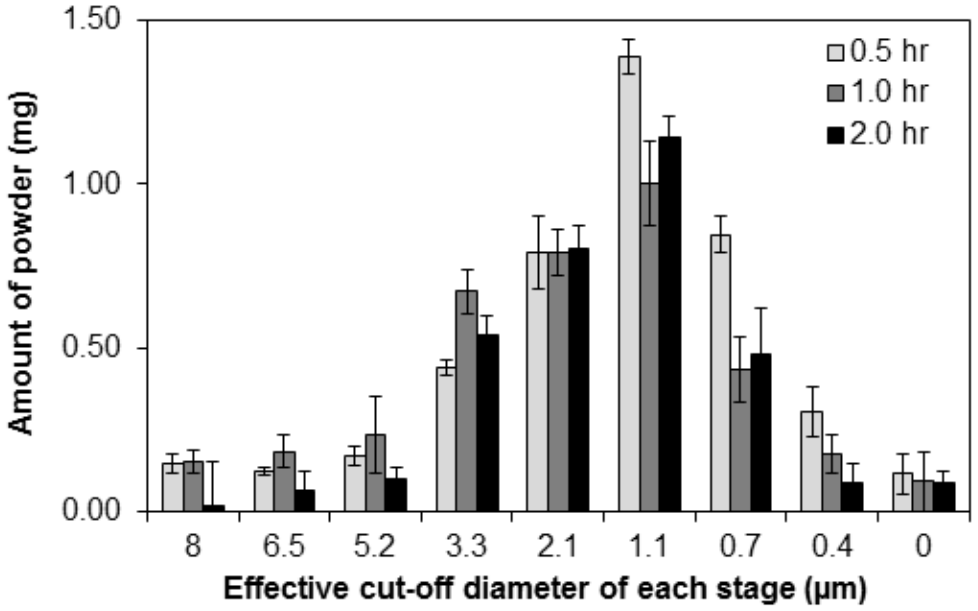


Figure 4.6 The distribution of ITZ milled in water for different durations (flow rate of 90 L/min).

Milled ITZ showed better aerosolization performance compared to ITZ as received and precipitated ITZ (Figure 4.7). The %EF of milled ITZ was 86.6 ± 6.5%, much higher than the %EF of precipitated ITZ (around 50-60%) and ITZ as received (44.4 ± 1.3%). Milled ITZ also showed the smallest MMAD of around 1 μm. The GSD of milled ITZ (around 2) was also smaller than the GSD of precipitated ITZ and ITZ as received, which were around 3 (Table 4.2).

Table 4.2 Cascade impaction of different itraconazole formulation at a flow rate of 90 L/min (Values = Average ± SD).

Formulation	% EF	% FPF		MMAD	GSD
		< 6.5	< 3.3	(µm)	
ITZ as received	44.4 ± 1.3	73.2 ± 1.2	47.5 ± 0.8	2.3 ± 0.0	3.1 ± 0.0
Milled ITZ*	86.6 ± 6.5	93.8 ± 1.1	79.7 ± 1.7	0.9 ± 0.0	2.2 ± 0.1
ITZ(sonication)	50.2 ± 7.3	75.6 ± 3.7	57.7 ± 5.9	1.5 ± 0.4	3.5 ± 0.2
ITZ(homogenization)	58.1 ± 5.3	75.0 ± 1.4	48.2 ± 8.9	2.2 ± 0.5	3.1 ± 0.1

* 30 minutes milled ITZ.

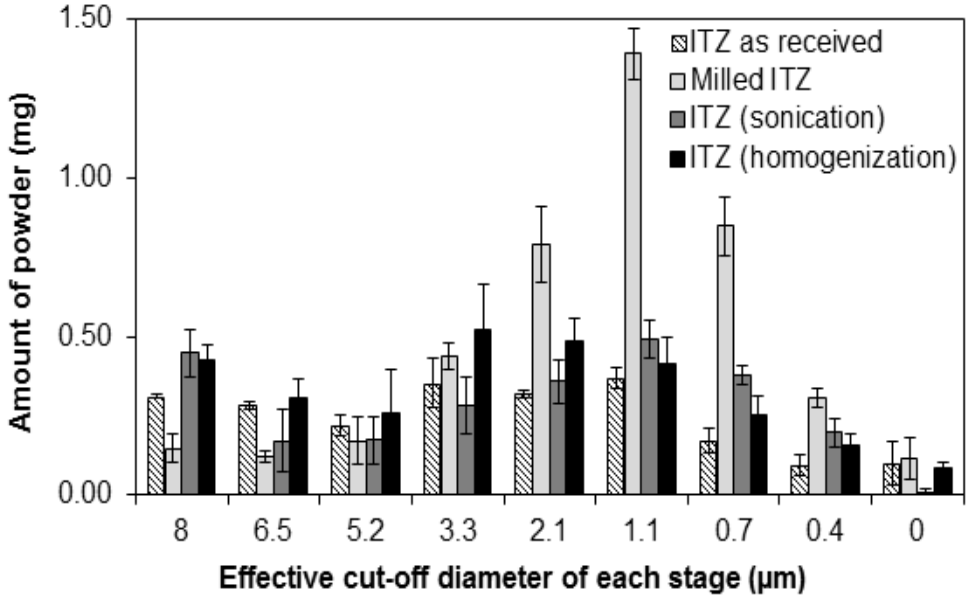


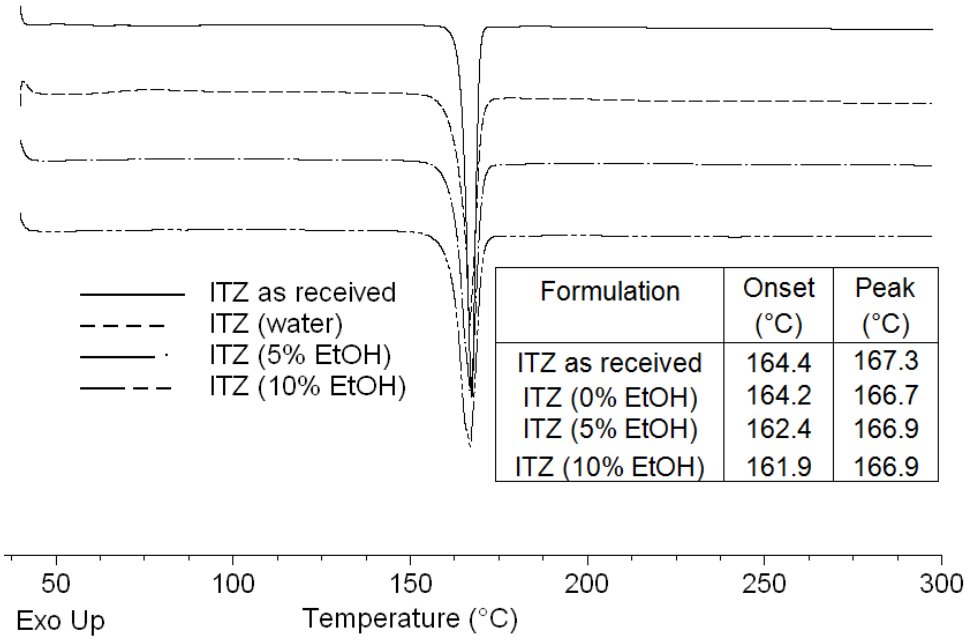
Figure 4.7 The distribution of different ITZ formulations (flow rate of 90 L/min).

4.3.2 Effect of dispersion solvent

4.3.2.1 Characteristic of powders

Although milled ITZ showed better aerosolization performance compared to precipitated or stock ITZ, DSC thermograms showed that milled ITZ contained some of amorphous characteristics. According to our studies and some studies in literature[25], a dispersion solvent may play a significant role in the physical characteristics of formulated powders. If

polymorphism or amorphousness can be controlled by solvent selection[25], a suitable dispersion solvent should affect a preference towards amorphous or crystalline ITZ powders.



Formulation	Onset (°C)	Peak (°C)
ITZ as received	164.4	167.3
ITZ (0% EtOH)	164.2	166.7
ITZ (5% EtOH)	162.4	166.9
ITZ (10% EtOH)	161.9	166.9

Figure 4.8 DSC thermogram of ITZ milled in different dispersion solvent.

Itraconazole is insoluble in water and very slightly soluble in alcohols. Alcohols such as ethanol (EtOH) are known to affect on wettability and dispersibility of a poorly water-soluble drug during milling. Since the solvent has an influence on the polymorphism or amorphousness[25], adding ethanol to the dispersion expected to affect the physical character of ITZ NonaClusters. Micronized ITZ as received was, therefore, milled in water, 5% EtOH and 10% EtOH. The DSC thermogram showed crystalline character of powders when ITZ was milled in media that contained ethanol (Figure 4.8). The crystalline state was confirmed by PXRD patterns. Moreover, the DSC profiles and PXRD patterns of ITZ milled in 10% EtOH did not show amorphous character across mill times of 2 hours (Figure 4.9, 4.10).

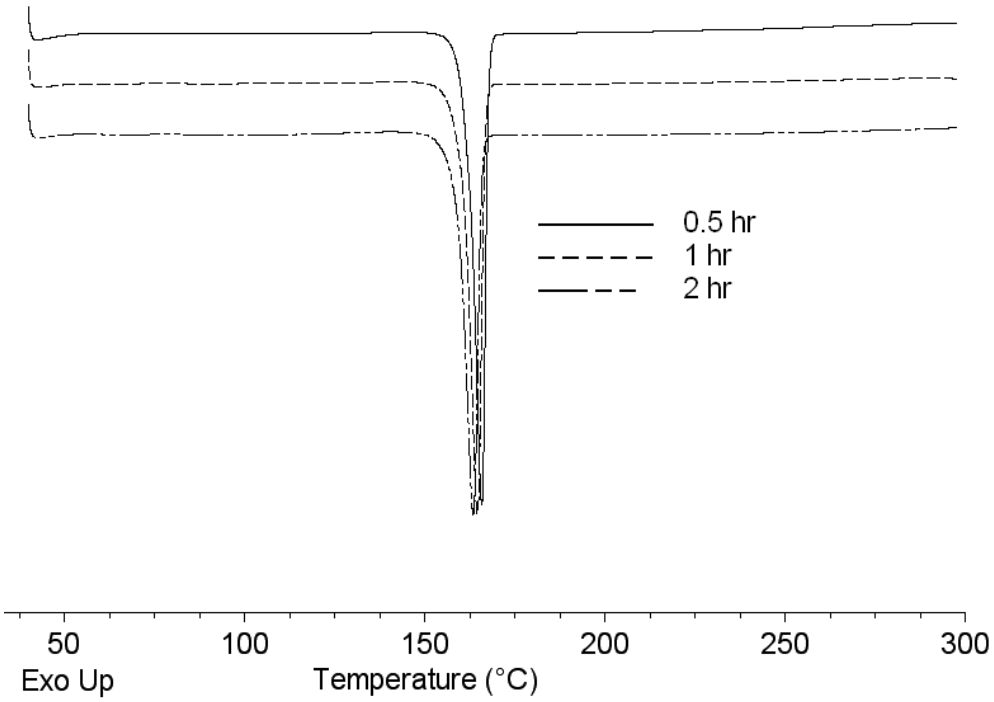


Figure 4.9 DSC thermogram of ITZ milled in 10% EtOH for different durations.

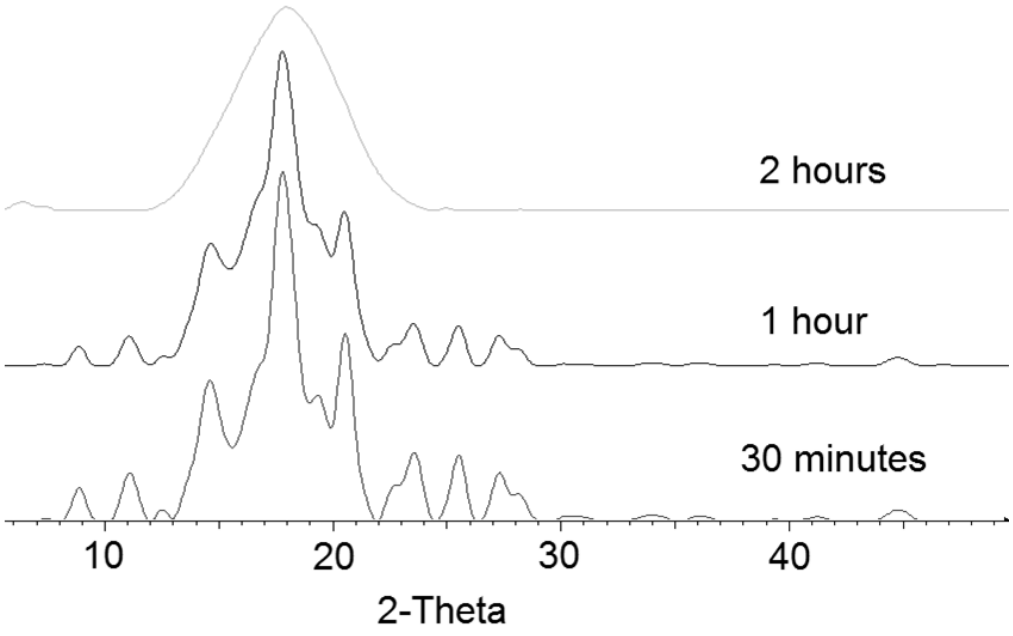


Figure 4.10 PXRD pattern of ITZ milled in 10% EtOH for different durations.

The amorphous state of a drug formulation is important and should be considered when formulating drug powders. Although amorphous phases can improve the dissolution and bioavailability of poorly soluble compounds[33], they can be thermodynamically unstable and change to a more stable state over time[42]. The presence of amorphous phases, even in small quantities, may affect the physical and chemical stability of pharmaceutical products[43]. Recrystallization depends on both molecular mobility and configurational entropy of the drug[44]. Molecules with lower configurational entropies require less mobility for spontaneous crystallization. Therefore, drugs with high configurational entropy barriers and low molecular mobility (i.e. crystals) should exhibit greater physical stability compared to amorphous forms[45].

4.3.2.2 Aerosolization performance of ITZ milled in water/ethanol mixtures

ITZ milled for half an hour in water, 5% EtOH, or 10% EtOH were compared to ITZ as received (Figure 4.11). The distribution of milled ITZ shifted toward a smaller MMAD compared to stock ITZ. The MMAD was decreased from 2.3 to 1.0 after milling (Table 4.3). At the same time, the %EF of milled ITZ formulations was two times higher than the %EF of ITZ as received. The %FPF of milled ITZ formulations was around 90-95% at a cut-off diameter of 6.5 μm and around 75-80% at a cut-off diameter of 3.3 μm. The GSD of milled ITZ formulations was also smaller than the GSD of ITZ as received. Although ITZ milled in different percentages of ethanol did not show a difference in aerosolization performance, as discuss previously, the ethanol helped to control the crystallinity of drug during the milling process.

Table 4.3 Cascade impaction of itraconazole prepared by milling for 30 minutes in different media at a flow rate of 90 L/min (Values = Average ± SD).

Formulation	% EF	% FPF		MMAD	GSD
		< 6.5	< 3.3	(µm)	
ITZ (water)	86.6 ± 6.5	93.8 ± 1.1	79.7 ± 1.7	0.9 ± 0.0	2.2 ± 0.1
ITZ (5% EtOH)	82.5 ± 3.9	94.5 ± 2.5	78.0 ± 3.4	1.0 ± 0.1	2.2 ± 0.2
ITZ (10% EtOH)	80.3 ± 10.7	91.8 ± 1.2	75.8 ± 0.8	1.2 ± 0.1	2.0 ± 0.1

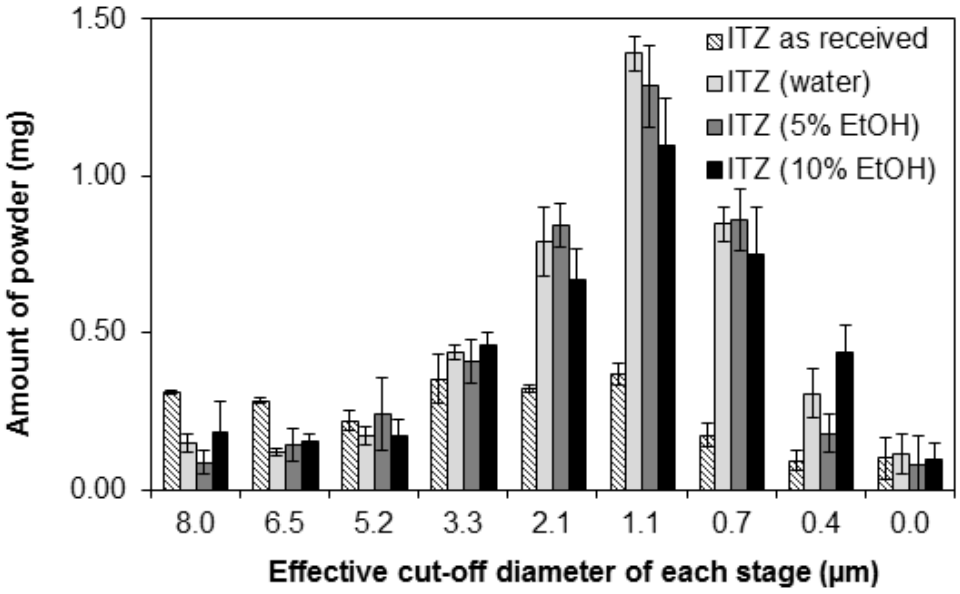


Figure 4.11 The distribution of ITZ milled for 30 minutes in different dispersion solvent (flow rate of 90 L/min).

4.3.3 Effect of flow rate on aerosolization performance

Dry powder formulations are delivered to patients via dry powder inhalers (DPIs). Each DPI has a specific resistance across the device, leading to different required flow rates to disperse the powder. DPIs on the market are normally breath-actuated devices. The efficiency of dry powder drug delivery using these devices depends on the patient's inspiration efforts. During inhalation, flowing air will generate shear force across the device, resulting in dispersion of drug

powders[46]. Besides drug formulations, therefore, flow rate across the DPIs should be investigated.

In general, powder dispersion increases when the flow rate is increased from 15 to 90 L/min[47]. High flow rate increases agglomerate break-up into smaller particles, resulting in a shift of the distribution towards smaller sizes. In our previous study, budesonide NanoCluster size distribution shifted toward a smaller MMAD when a higher flow rate was applied[15].

ITZ formulations were introduced to a cascade impactor via a Monodose® inhaler. Changing the flow rate affected both ITZ as received and the milled ITZ formulation. The MMAD of ITZ as received decreased from 5.5 ± 0.6 μm to 2.3 ± 0.0 μm while the MMAD of milled ITZ decreased from 3.7 ± 0.4 μm to 1.2 ± 0.1 μm when the volumetric flow rate was increased from 30 L/min to 90 L/min (Table 4.4). The particle size distribution of ITZ powders was also shifted toward smaller MMAD when flow was applied at 90 L/min compared to 30 L/min (Figure 4.12). Increasing flow rate had a slight effect on the %EF of ITZ as received (from 33.7 ± 8.6 μm to 44.4 ± 1.3 μm); however, it did not show any effect on the %EF of milled ITZ (from 79.2 ± 0.6 μm to 80.3 ± 10.7 μm). Furthermore, it is worth noting that the %FPF of milled ITZ increased substantially when the flow rate was 90 L/min. The $\%FPF_{<3.3}$ of milled ITZ increased from 21.4 ± 2.0 % to 75.8 ± 0.8 % compared to $\%FPF_{<3.3}$ of ITZ as received (from 20.9 ± 3.1 % to 47.5 ± 0.8 %). Milled ITZ in the form of NanoClusters was easy to deagglomerate into small particles, leading to a large fine particle fraction. On the contrary, a high flow rate can increase the dispersion of ITZ as received but it cannot dramatically change the size of the particles due to the solid form of particles.

Table 4.4 Cascade impaction of itraconazole at different flow rates (Values = Average ± SD).

Flow rate L/min	Formulation	% EF	% FPF			MMAD (μm)	GSD
			< 5.8	< 6.5	< 3.3		
30	ITZ as received	33.7 ± 8.6	46.4 ± 6.2	-	20.9 ± 3.1	5.5 ± 0.6	2.2 ± 0.2
	Milled ITZ*	79.2 ± 0.6	64.4 ± 4.0	-	21.4 ± 2.0	3.7 ± 0.4	2.0 ± 0.0
90	ITZ as received	44.4 ± 1.3	-	73.2 ± 1.2	47.5 ± 0.8	2.3 ± 0.0	3.1 ± 0.0
	Milled ITZ*	80.3 ± 10.7	-	91.8 ± 1.2	75.8 ± 0.8	1.2 ± 0.1	2.0 ± 0.1

* 30 minutes milled ITZ.

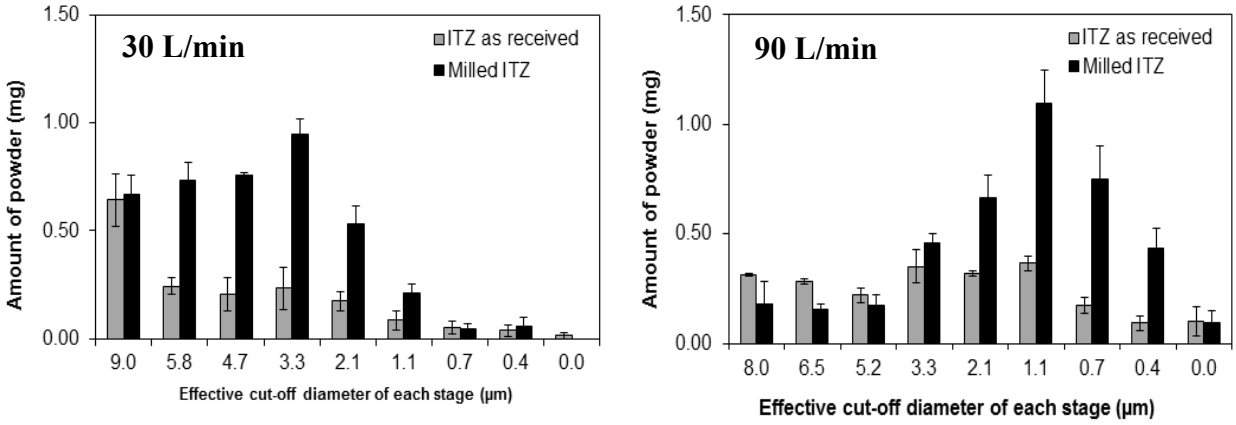


Figure 4.12 The distribution of ITZ when applied at different flow rates.

The effect of flow rate normally plays a greater role for DPIs that have low dispersion efficiencies. A study demonstrated the different effect on powder performance at any flow rate when tested via a low dispersion efficiency inhaler and a high dispersion efficiency inhaler[48]. Using the same particles, the low dispersion efficiency inhaler required higher shear force to maximize powder delivery. They also found flow dependence for the smaller powder (2.7 μm) but less dependence on larger particles (5 μm) when using the low dispersion efficiency inhaler.

Conversely, the FPF of 5 μm particles was decreased when applied via the higher dispersion efficiency inhaler at the same flow rate used for the low dispersion efficiency inhaler. The Monodose® inhaler is a low resistance DPI device that was designed for use at flow rate of 90 L/min.

4.4 Conclusion

Itraconazole (ITZ) NanoCluster formulations prepared by a wet-milling technique showed better aerosol performance compared to micronized ITZ as received and ITZ NanoCluster formulations prepared by precipitation methods. An amorphous state appeared, however, when ITZ was milled in water for half an hour or more. Adding a small amount of ethanol during milling played an important role in dictating the crystallinity of drug particles. ITZ milled in 10% EtOH maintained the crystalline character of ITZ even during a 2 hour milling time. Milled ITZ NanoClusters also showed substantially improved of aerosol performance when tested at a high inspiration flow rate. In conclusion, ITZ NanoCluster formulations represent a potential engineered particle approach for inhalation drug development, providing effective aerosol properties and stability due to crystalline state of the drug powders.

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Chapter 5

Conclusion

5.1 Conclusion

NanoCluster technology provides an effective approach for engineering dry powder aerosols especially for inhalation. Particles in the size range of 1-5 μm are desired for efficient deposition in the lungs. NanoClusters are agglomerated nanoparticles that combine the advantages of nanoparticles and microparticles. Nanoparticles increase dissolution rate of drug powders as a result of high surface area while microparticle structure provides a suitable size for aerosolization and deposition along the respiratory tract.

For patients on mechanical ventilation, liquid formulations have been used for routine treatment. Liquid formulations perform poorly with variable delivery due to in efficient aerosol conduction or condensation of wet aerosols in the endotracheal tube. Dry powder technology, was investigated as an alternative approach for inhalation drug delivery in these patients. Dry powder formulations have been primarily used in non-ventilated patients. Dry powder inhalers that are available on the market are also not designed for use with ventilated patients, leading to inefficient drug delivery.

In this thesis, we formulated NanoClusters of inhaled drugs and developed a dry powder inhaler for use with mechanical ventilation. In chapter 2, budesonide NanoClusters were prepared by a wet-milling technique and delivery through an endotracheal tube was investigated. We found that budesonide NanoClusters showed better aerosolization performance compared to stock micronized budesonide and Pulmicort Flexhaler powder. Budesonide NanoCluster powders showed a high emitted fraction and a high percentage of fine particles were achieved when NanoClusters were applied through the endotracheal tube. Comparing, a Teflon tube and a commercial (PVC) endotracheal tube did not show any difference. Other parameters such as

volumetric flow rate, tube diameter and humidity were also studied. A higher volumetric flow rate and smaller tube diameter substantial increased the fine particle fraction and particle size distribution shifted toward smaller MMAD.

NanoCluster budesonide formulations efficiently navigated through the endotracheal tube, which is a major barrier for liquid formulations. The success of introducing NanoClusters though the endotracheal tube led to a study of aerosol performance of this formulation driven by mechanical ventilation in chapter 3. The study showed that the NanoCluster budesonide formulation dramatically improved aerosol performance delivery compared to stock budesonide. Parameters such as inspiration pattern, inspiration volume and volumetric flow rate did not affect the aerosolization performance of budesonide NanoClusters when delivered through endotracheal tubes using the ventilator. It is noteworthy that only the volumetric flow rate in the range of 20 – 40 L/min was studied because of backpressure limitations on the ventilator. The ventilator shuts down automatically when applied at very high flow rate to protect patients from injury.

As mentioned previously, DPIs on the market have not been designed for use with the ventilation system. In this study, a novel inhaler was developed and compared with a modified Monodose® inhaler. The study also demonstrated that a novel device could be connected directly to the ventilator and endotracheal tubing while maintaining efficient aerosol delivery.

Using a ventilation bag instead of the ventilator remarked consistency of particle size distribution for either air source. The ventilator should provide better inspiratory control compared to the ventilation bag, yet our results suggested inconsistency to the air source.

NanoCluster technology combined with a new device, therefore, offers an effective drug delivery option for patients on mechanical ventilation.

The better aerosolization performance of budesonide NanoClusters compared to stock budesonide and Pulmicort Flexhaler powders (chapter 1) represented an effective engineered particle approach, therefore, this technology was applied to formulate other inhaled drugs such as itraconazole (chapter 4). For treatment of local disease in the lung, inhalation of itraconazole is preferred to oral administration because of low bioavailability in the gastrointestinal tract. In this chapter, itraconazole NanoClusters were prepared by wet-milling and precipitation methods. Itraconazole NanoClusters formulated by wet milling showed better aerosolization performance than precipitated itraconazole formulations and stock itraconazole. Moreover, wet-milling maintained the crystalline character of itraconazole when stock itraconazole was milled in 10% ethanol whereas the precipitation process generated amorphous itraconazole. Milled itraconazole NanoClusters, therefore, represent an effective drug formulation for inhalation compared to precipitated itraconazole and stock itraconazole due to the better aerosol performance and crystallinity of the drug formulation.

5.2 Future directions

The next steps of dry powder drug development can be divided into three main areas. The first area is formulation development. NanoCluster technology is worthy of exploration for other inhaled drugs. Although NanoCluster technology was useful to develop budesonide and itraconazole powder, the processing conditions should be optimized for each drug. Each drug has different properties such as hardness and brittleness. For example, low grinding speed and short milling time were preferred in order to diminish the formation of amorphous material during

milling of itraconazole and to optimize the aerosol properties of dry powder formulation. Conversely, optimal budesonide milling used higher grinding speed and longer times.

The second area is device design for use with the ventilator. Since dry powder inhalers have not been routine used for patients on mechanical ventilation, we designed a novel inhaler to fit the connection and endotracheal tube. Although our device performed effectively, modification of the design should be continued in order to optimize efficiency. Moreover, the new device has to be modified for use with rat, rabbit or other animal model.

Last but not least, NanoCluster formulations should be studied both *in vitro* on human ventilators and *in vivo* in animal ventilators. Budesonide NanoClusters were successfully delivered on a ventilator system that connected to a cascade impactor. The next step for budesonide NanoClusters is animal studies. The device should be modified to deliver budesonide NanoClusters to the animals such as rats or rabbits. The ventilation system for any animal has to be studied and optimized. Parameters such as the dose of the drug powder, delivery time, and volumetric flow rates have to be adjusted to suit the animal. For furthering itraconazole NanoCluster formulations, the next step is an *in vitro* mechanical ventilation study. An exploration of ventilator parameters should be performed. Then, an animal study should be considered.