The Use of Excipients to Stabilise Pressurised Metered Dose Inhalers

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by

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Abbreviations

A: Alveolar

ACI: Andersen Cascade Impactor

AFM: Atomic Force Microscopy

ANOVA: Analysis of Variance

API: Active Pharmaceutical Ingredient

APSD: Aerodynamic Particle Size Distribution

BAC: Benzyltriethyl Ammonium Chloride

BACIS: Benzyltriethyl Ammonium Chloride Internal Standard Solution

BDP: Beclomethasone Dipropionate

BP: British Pharmacopoeia

c-AMP: Cyclic Adenosine Monophosphate

CFC: Chlorofluorocarbon

COA: Certificate of Analysis

COPD: Chronic Obstructive Pulmonary Disease

DPI: Dry Powder Inhaler

DUSA: Dosage Unit Sampling Apparatus

ECD: Effective Cut-off Diameters

ELPI: Electrical Low-Pressure Impactor

EPDM: Ethylene-Propylene Diene Monomer

FCP: Fluorocarbon Polymerisation

FDA: Food and Drug Administration

FEV1: Forced Expiratory Volume in One Second

FFFE: “Fast Fill, Fast Empty” Valve

FP: Fluticasone Propionate

FPF: Fine Particle Fraction

FPD: Fine Particle Dose

GOLD: Global Initiative for Chronic Obstructive Lung Diseases

GSD: Geometric Standard Deviation

HFA: Hydrofluoroalkane

HPLC: High Performance Liquid Chromatography

ICH: International Conference on Harmonisation

ICS: Inhaled Corticosteroids

IP: Induction Port

IgA/E/G: Immunoglobulin A/E/G

LABA: Long Acting Beta Agonist

Lac: Lactose

Leu: Leucine

LOD: Limit of Detection

LOQ: Limit of Quantitation

MF: Mometasone Furoate

MHRA: Medicines and Healthcare Products Regulatory Agency

MMD: Mass Median Diameter

MMAD: Mass Median Aerodynamic Diameter

MOC: Micro Orifice Collector

NEB (s): Nebuliser (s)

NICE: National Institute for Health and Care Excellence

NGI: Next Generation Impactor

NP: Nasopharyngeal

PDA: Phase Doppler Anemometry

PEG: Polyethylene Glycol

PET: Polyethylene Terephthalate

Ph. Eur.: European Pharmacopoeia

PIL: Patient Information Leaflet

ppm: Parts per Million

RI: Refractive Index

RH: Relative Humidity

pMDI (s): Pressurised Metered Dose Inhaler (s)

RSD: Relative Standard Deviation (Coefficient of Variation)

RP-HPLC: Reverse Phase - High Performance Liquid Chromatography

SABAs: Short Acting Beta Agonist

SEM: Scanning Electron Microscope

SIGN: Scottish Intercollegiate Guidelines Network

SOP: Standard Operating Procedure

SP: Secondary Particulate

SS: Salbutamol Sulphate

SVLI: Small Volume Liquid Inhaler

TB: Tracheobronchial

Tio: Tiotropium Bromide Monohydrate

TIOSPIR: Tiotropium Safety and Performance in Respimat

TLP: Through Life Performance

TOF: Time of Flight

THC: Tetrahydrocannabinol

UPLIFT: Understanding Potential Long-Term Impacts on Function with Tiotropium

USP: United States Pharmacopeia

WHO: World Health Organisation

Summary

This thesis concerns investigations of novel pressurised metered dose inhaler (pMDI) formulations containing tiotropium (Tio) in association with a secondary particulate (SP). A number of formulation and hardware variables were studied using in vitro methods to determine their influence on the performance of these novel formulations.

Initial studies indicated that Tio was practically insoluble in HFA propellants and its solubility was not increased under raised moisture levels during long-term stability tests. Formulations with L-leucine (Leu) or lactose (Lac) as SP's were investigated in Tio:SP ratios ranging from 1:2.5 to 1:25 and with different SP sieve fractions from <20 µm to <63 µm. Many formulations demonstrated improved aerosol characteristics compared with Tio alone, particularly in through life performance (TLP). The inclusion of fine SP's (<20 µm) was found to significantly improve dose uniformity, fine particle fraction (FPF) and fine particle dose (FPD). Tio:Lac formulated in HFA 227 resulted in slightly greater FPF and FPD but also a higher mass median aerodynamic diameter (MMAD) than when formulated in HFA 134a. With respect to the hardware parameters investigated, smaller actuator orifices (in the range 0.25-0.46 mm) and lower valve volume (25 µl instead of 50 µl) were generally associated with significantly increased FPF and reduced MMAD, whereas a smaller canister volume and fluorocarbon polymerization canisters tended to improve TLP. In comparison with marketed Tio products, comparable FPF's to Spiriva Handihaler® (41%) and Spiriva Respimat® (53%) were demonstrated with bespoke Tio:Lac and Tio:Leu formulations respectively. The Tio:Leu formulation also had a much lower submicron fraction of Tio than Spiriva Respimat®.

This research concerning Tio:SP pMDI formulations has demonstrated the advantages of including a SP to promote drug-SP association in the HFA suspension and promoting particle de-aggregation during propellant atomisation. Further research regarding direct measurement of particle interactions and aerodynamic behavior is warranted.

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1 Introduction

1.1 Background

This chapter aims to provide understanding of the requirements for an effective and efficient inhaled drug delivery system for the treatment of a range of pulmonary disorders. It is therefore, vital that appropriate background on the physiology and function of the respiratory tract is provided to give understanding of the barriers that devices need to overcome to provide a therapeutic effect. A short overview will provide information related to the current available devices used as treatment for the pulmonary disorders with particular attention given to pressurised metered dose inhalers (pMDIs), as these devices will be the main focus of this project. The most significant change to the pMDIs in the last 50 years has been the shift from ozone depleting propellants to more environmentally friendly ones and this change has raised issues with the performance of formulations using the new propellants. The difference in propellant polarity causes solubility difficulties of both drugs and excipients, and in many cases, it has taken more than two decades, since the late 1980's, to introduce acceptable reformulations of certain drugs (Myrdal et al, 2014). Based on research carried out by Dickinson and Warren (2004) and Jones (2004) it has been shown that the inclusion of secondary particles in pMDIs assisted in overcoming some of the issues caused during by the transition. The main purpose in this study is to further the understanding of the mechanisms and potentials of secondary particulate systems within pMDIs.

1.2 Anatomy and Physiology of the Respiratory Tract

The primary functions of the respiratory system are to enable gas exchange between the blood and air, maintain homeostatic systemic pH, facilitate the production of sounds, and protect respiratory airways from external environment. The respiratory system is made up of organs that help with breathing. Based on its

anatomical structure, the three main components are the nasopharyngeal (NP) region consisting of the nose, nasal passages, pharynx and the larynx, the tracheobronchial (TB) region consisting of the trachea, bronchi and conducting bronchioles, and the alveolar (A) region, which contains the respiratory bronchioles, alveolar ducts, and alveoli. A morphological model of the respiratory tract, first reported by Weibel (1963), illustrates the diameter, length, number and cross-sectional area of the 23 "Generations" in the model (Figure 1.1). Based on its function, the respiratory tract can also be divided into the conducting zone (Generations 0-16) and the respiratory zone (Generations 17-23). The conducting zone consists of the trachea, bronchi, bronchioles and terminal bronchioles. In this area, there is no gas exchanging, and in normal conditions, as the temperature and humidity of the inspired air is varied, the air is heated and humidified before being transported into the deeper respiratory zone, where the gas is fully saturated with water vapour at body temperature (Altiere and Thompson, 2007; Shelly et al, 1988). The respiratory zone includes the respiratory bronchioles, alveolar ducts and alveolar sacs. From the conducting airways to these distal respiratory airways, there is a progressive decrease in thickness of the cell and fluid layers and an increase of the total surface area. There is a thin and extensive diffusion pathway between alveolar spaces and red blood cells in capillaries. Coupled with the pressure gradients of oxygen and carbon dioxide, the main function of this area is dominated by gas exchange under the mechanism of ventilation (Patton and Byron, 2007; Smyth and Hickey, 2011).

	Generation		Diameter, cm	Length, cm	Number	Total cross-sectional area, cm^2
Conducting zone	Trachea	0	1.80	12.0	1	2.54
	Bronchi	1	1.22	4.8	2	2.33
		2	0.83	1.9	4	2.13
		3	0.56	0.8	8	2.00
	Bronchioles	4	0.45	1.3	16	2.48
	Terminal bronchioles	5 ↓ 16	0.35 ↓ 0.06	1.07 ↓ 0.17	32 ↓ 6×10^4	3.11 ↓ 180.0
Transitional and respiratory zones	Respiratory bronchioles	17	↓	↓	↓	↓
		18				
		19	0.05	0.10	5×10^5	10^3
	Alveolar ducts	T_3 20	↓	↓	↓	↓
		T_2 21				
		T_1 22				
	Alveolar sacs	T 23	0.04	0.05	8×10^6	10^4

Figure 1. 1 Weibel's Model of the Human Lung Airways
(Adapted from Levitzky 2013, Weibel, 1963)

The therapeutic effect of inhaled drugs may depend on the physiology and histology of the respiratory tract, which determine the deposition and absorption of the inhaled particles. The lining of the conducting airways, except the glottis and the larynx, is a pseudo-stratified columnar epithelium consisting of ciliated cells, mucus-secreting goblet cells and short basal stem cells. Beneath the epithelium, serous glands, lymphatic follicles and C-shaped hyaline cartilages, mainly in trachea and the primary bronchi, form the elastic connective tissue that provides support to breathing movement. The mucus-secreting goblet cells and serous glands are involved in mucus secretion to form a thin and adhesive mucosal layer covering the surface of the epithelium (Ballard and Inglis, 2004). In secondary and tertiary bronchi, C-shaped cartilages are replaced by overlapping plates of cartilages, while in bronchioles, there is a lack of supportive cartilage when the diameter is 1 mm or

less. The presence of an additional smooth muscle layer makes bronchioles more contractible and flexible. Smooth muscle cells are present in the epithelium, and gradually increase from trachea to bronchioles to regulate the airflow in the airway to prevent hypoventilation and hyperventilation. While down to the final branches of the conducting airway, the terminal bronchioles, with diameters of 0.5 mm or less, have serous cells rather than goblet cells and mucous glands. In these regions, the epithelial cells are connected via tight junctions, which form a physicochemical barrier to the absorption of drugs. The ciliated cells, goblet cells, mucous and serous glands coordinate to produce mucociliary clearance. In the respiratory airway, the walls of respiratory bronchioles are comprised of non-ciliated cells and smooth muscle, while alveolar ducts and sacs only have non-ciliated simple squamous epithelium, comprised of Type 1 pneumocyte cells and Type 2 pneumocyte cells (Saladin, 2007). Type 1 pneumocyte cells cover 95% of alveolar surface area where gas exchange occurs, and Type 2 pneumocyte cells account for the remaining and their primary roles are to produce Type 1 pneumocyte cells and pulmonary surfactants (Smyth and Hickey, 2011).

Exposed to the environment, the respiratory tract works as a defence system to protect the body from numerous insults of bacteria, virus, fumes and particles (Gerritsen, 2000). The anatomic structure and physiological properties of the respiratory tract contribute to the clearance of these exogenous substances and also bring limitations for the pulmonary delivery of therapeutic agents. The pulmonary defence mechanism consists of a series of physical defences e.g. filtration mechanisms to remove these insults. In the upper airways, large particles (>10 μm) e.g. dust are trapped and eliminated by the filtering mechanisms in the nasal cavity and pharynx, which are then cleared by mucociliary clearance, sneezing and coughing. In the central and some peripheral airways, mucociliary transport mechanisms are involved in the removal of smaller particles through the support of

expectoration or swallowing into the gastrointestinal tract. The viscous mucous layer above the epithelium binds the deposited particles by adhesion and transports them in the proximal direction through the movement of cilia. In addition, immunoglobulins e.g. IgA and IgG secreted by plasma cells into both lower and upper airways assist with clearance of foreign matter. In the alveolar region where there is a lack of ciliated cells, the main defence system is provided by alveolar macrophages, surfactants and complement proteins for clearance of microorganisms or foreign matters (Labiris and Dolovich, 2003b; Gerritsen, 2000). Deposited particles, especially in the range of 1.5 - 3 μm, on the alveolar surface tend to be phagocytosed by alveolar macrophages, and then are transported to upper airways or directly degraded by internal enzymes (Oberdorster, 1998). Due to these biological barriers, the drug particles for pulmonary delivery need to be manipulated to overcome these clearance mechanisms to reach and remain in the target area.

1.3 Pulmonary Drug Delivery

The lungs provide a huge mucosal surface area of around 120-140 m^2 of some extremely thin membranes and good blood supply, and this facilitates drug absorption. This structure and facilitation has made the pulmonary route the preferred choice for treatments of asthma and other local diseases due to the quick onset of drug action, fewer systemic adverse reactions and potentially long residence times associated with peripheral lung deposition. A range of drugs such as anti-allergics, beta-agonists and mast cell stabilisers have been used via this route to achieve direct effects on local tissues (Pilcer and Amighi, 2010). Recently, with an increased understanding of pulmonary biology, more attention has been focused on the systemic delivery of drugs. Compared with oral administration, pulmonary delivery avoids hepatic first-pass elimination and degradation in the digestive tract. Small lipophilic molecules e.g. nicotine, tetrahydrocannabinol (THC)

and rizatriptan show high bioavailability with rapid absorption kinetics from lung epithelium (Patton, 1996). As novel therapeutics for treating major disorders e.g. cancer, macromolecules only permeate with difficulty through multiple biological barriers between the administration sites and targets by traditional delivery routes. Injection is effective but hindered by a lack of patient convenience and compliance and may be challenged by slow onset (e.g. subcutaneous injection). In the deep lung, the large surface area, extremely thin membrane and good vascularisation present the advantage of high permeability for large molecules (Andrade et al, 2011). In addition, with a small fraction of the drug metabolizing and efflux transporter activity, pulmonary administration provides a potential pathway to deliver macromolecular drugs such as proteins or DNAs for the treatment of both lung conditions and systemic diseases (Jorgenson and Nielson, 2009). Currently, pulmonary delivery of antibiotics and antivirus or antifungal agents e.g. tobramycin (Amighi et al, 2009), azithromycin (Zhang et al, 2010) and rifampicin (Son and McConville, 2011) have been studied for infectious disease therapy.

1.4 Aerosol Particle Deposition Mechanisms

Aerosol deposition in the lungs is clearly an essential part of successful pulmonary drug delivery, and the deposition characteristics of particles are dependent upon the physicochemical properties of the drug, formulation, delivery devices and the patient's breathing patterns. For airborne particles, there are various factors e.g. particle size, shape and density, which are recognised to affect their deposition, and the equivalent aerodynamic diameter ($d\alpha$) is considered the most fundamental physical factor that predicts the aerodynamic behaviour of particles. Aerodynamic diameter is defined as the physical diameter of a unit density sphere with the same settling velocity as the irregular particle of interest. This is defined in Equation 1, as follows:

$$d_{\alpha} = d_g \sqrt{\frac{\rho}{\rho_{\alpha}}} \qquad (1)$$

(d_{α}: aerodynamic diameter; d_g: geometric diameter; ρ: mass density of the particle; ρ_{α}: unit density (1 g/cm^3) (Hinds, 1999)

There are three essential mechanisms responsible for deposition of an aerosol particle onto the airway walls of the respiratory system consisting of inertial impaction, gravitational sedimentation and Brownian diffusion (Groneberg et al, 2003). For inertial impaction, particles with dα >5 µm tend to deposit in the mouth and upper airways (Hinds, 1999; Sung et al, 2007). As the incoming airflow goes through the whole airway branching structure and each time flow direction is changed, the larger particles will keep moving in their original direction by their inertia. The particles will deposit and the probability of deposition depends on the particle inertia (i.e. mass x velocity) (Equation (2)). This is the primary deposition mechanism in large airways especially at the bifurcations where the streamlines bend most sharply (Hinds, 1999).

$$DE_{Im} \sim d_{\alpha}^{\,2} \cdot v \qquad (2)$$

(DE_{Im}: probability of aerosol particle deposition on the airway wall by inertial impaction; d_{α}: aerodynamic diameter of particle; v: linear velocity of the particle) (Scheuch et al, 2006)

Small particles which evade impaction tend to be deposited by gravitational forces, and sedimentation rate is increased by particle size and density (Equation (3)). In smaller airways and alveolar regions, due to low air velocities and small airway dimensions, gravitational sedimentation dominates as the deposition mechanism. The deposition can be increased by longer breath holding (Newman et al, 1982). The settling distance can be calculated by residence time and terminal settling

velocity, and particles with a size range of 3 µm to 5 µm tend to be deposited in the horizontally oriented distal airway (Hinds, 1999).

$$DE_{sed} \sim d_{\alpha}{}^{2} \cdot t \qquad (3)$$

(DE_{sed}: probability of aerosol particle deposition on the airway wall by sedimentation; d_{α}: aerodynamic diameter of particle; t: residence time of particle) (Scheuch et al, 2006)

Very small aerosol particles are removed irregularly by collisions with gas atoms or molecules, which is called diffusion or Brownian motion. This diffusion mechanism is predominant for deposition of particles with diameters below 0.5 µm (Labiris and Dolovich, 2003b). The possibility of particle deposition by diffusion is determined by the particle geometric size and residence time as illustrated by Equation (4). Generally, the small airways show low flow rates and long passage times for particles. Ultra-fine particles may evade all of the deposition mechanisms and be exhaled out without attaching to the mucous membrane (Heinemann et al, 1997). Consequently, it is considered that in the range of 1 to 3 µm, more particles penetrate deeper into the lung or alveoli, while very small aerosols may be exhaled without deposition.

$$DE_{dif} \sim \frac{t}{d_g} \qquad (4)$$

($DE_{dif:}$ probability of aerosol particle deposition on the airway wall by diffusion; t; residence time of particle; d_g: geometric diameter of particle) (Scheuch et al, 2006)

1.5 Airway Diseases

Pulmonary drug delivery is mainly used for treating lung local diseases e.g. asthma (e.g. using Ventolin®, salbutamol sulphate), chronic obstructive pulmonary disease (COPD) (e.g. using Spiriva Handihaler®, tiotropium bromide), lung infections (e.g. using Tobi Podhaler®, tobramycin) and cystic fibrosis (e.g. using Cayston®, aztreonam). As a targeted delivery route, inhalation therapy is recommended by Scottish Intercollegiate Guidelines Network (SIGN) and National Institute for Health and Care Excellence (NICE) as the optimal route of first-line therapy for treating asthma and COPD, and also alternatively used for treating other local diseases e.g. cystic fibrosis (Labiris and Dolovich, 2003b). In this section, the definition and causes of asthma and COPD, as the most common diseases treated by inhalation therapy, are described.

1.5.1 Asthma

Asthma is a life-threatening chronic disease of the lung characterized by variable obstruction of the airways, causing breathing difficulties such as coughing, wheezing, chest tightness and shortness of breath that affects patients of all ages. Inflammatory conditions e.g. airway hyper-responsiveness are always associated with obstructive symptoms (Kaplan et al, 2009). The reasons leading to asthma are complex and varied, and genetic, environmental, and pathogenic factors can each increase the risk. Pathophysiology studies have shown that macrophages could be activated by allergen attached to immunoglobulin E (IgE), eosinophils were identified in bronchoalveolar lavage fluid at the late reaction and neutrophils had been proved relevant to acute exacerbation of asthma. Other inflammatory mediators e.g. leukotrienes (LTC_4, LTD_4 and LTE_4) or histamine were all involved (Barnes, 2000a). Based on the British guideline on the management of asthma (SIGN, 2016), short-acting beta agonists (SABAs) by inhalation are recommended to

treat mild and acute exacerbation of asthma. Inhaled SABAs are efficacious and preferable for quick relief of bronchospasm than other administration routes i.e. intravenous (Travers et al, 2001). Corticosteroids and long-acting beta agonists (LABAs) administered by inhalation are either used alone or as a combination for treating stable asthma.

1.5.2 Chronic obstructive pulmonary disease (COPD)

Chronic obstructive pulmonary disease (COPD) is an under-diagnosed and life-threatening lung disease ranked as the fourth leading cause of death in 2004, and in the trends between 2004 and 2030, COPD-induced mortality is projected to rise up to the third leading cause of death by 2030 (WHO, 2008). According to the Global Initiative for Chronic Obstructive Lung Diseases (GOLD) guidelines, COPD has been referred to as a preventable and treatable disease accompanied by severe extrapulmonary effects in some individuals. The pulmonary component is usually characterised by progressive airflow limitation, not fully reversible obstruction and associated abnormal inflammatory response of the lung to noxious particles or gases (GOLD, 2017). COPD is the name of a collection of different airway diseases, which consist of small airway diseases e.g. obstructive bronchiolitis, chronic bronchitis and parenchymal destruction e.g. emphysema. The basic clinical symptoms include airflow limitation or breathlessness, ciliary dysfunction, mucus hypersecretion, gas exchange abnormalities and pulmonary hypertension (Koumis and Samuel, 2005). COPD causes a structural change of the airways, and is a progressive disease. The primary risk factor for COPD is tobacco smoke including tobacco use or secondary smoke inhalation (WHO, 2008). Exposure to particles such as occupational dust and chemicals, or outdoor pollution could also increase the burden to the lung. Both viral and bacterial infection could cause airway inflammation and contribute to the pathogenesis and progression of COPD (Eisner et al, 2005). It has been found that increased respiratory symptoms in adulthood

are relevant to reduced lung function due to a history of severe childhood respiratory infection (Burge et al, 2003). According to GOLD (2017), the pharmacologic therapies for acute symptoms includes SABAs i.e. salbutamol, and short-acting anticholinergics i.e. ipratropium, and for stable COPD includes LABAs and long-acting anticholinergics or a combination therapy.

1.6 Treatment of Airway Diseases

According to the guidelines for diagnosis and management of asthma and COPD (SIGN, 2016; GOLD, 2017), bronchodilators e.g. beta-agonists are recommended to treat asthma, along with inhaled corticosteroids for regular prevention treatment, while beta-agonists and anticholinergics are recommended to treat COPD.

1.6.1 Beta2-agonists

Beta2-agonists are a type of bronchodilator prescribed to treat asthma and COPD, which target beta-adrenergic receptors that distribute throughout the lung (Barnes, 2004). Beta2-agonist molecules are analogs of adrenaline, which is a very short acting bronchodilator (Barnes, 1995). The mechanism of beta2-agonists is to increase the release of cyclic adenosine monophosphate (c-AMP) and produce functional antagonism to bronchoconstriction to relax airway smooth muscle by stimulating beta-adrenergic receptors (GOLD, 2017). Short-acting beta agonists (SABAs) include salbutamol, terbutaline and fenoterol, and the effects can last 4 to 6 hours. SABAs are normally used as first-line medication to treat patient with acute asthma either through a pressurised metered dose inhaler (pMDI) i.e. Ventolin® (salbutamol sulphate, SS) or a nebuliser driven by oxygen. Long-acting beta agonists (LABAs) were developed based on the understanding of the chemistry of short-acting drugs to increase the residence time at receptors. Licensed LABAs included salmeterol, formoterol, olodaterol and Indacaterol, and

the duration of effects extended to 12 hours or more, which reduced the daily dosing frequency and relieved the exercise limitation (Tashkin and Fabbri, 2010). A regular use of LABAs could improve the symptoms of breathlessness and rate of exacerbations of patients with COPD (Geake et al, 2015). Furthermore, the evidence showed a combination of corticosteroids and LABAs could improve lung function and decrease the frequency of asthma attack, and this dual therapy also contributed to reduced symptoms of COPD than either drug alone (Chauhan and Ducharme, 2014; Nannini et al, 2012; Nannini et al 2013). There are several combination products in the current market, e.g. salmeterol and fluticasone (Seretide®), formoterol and budesonide (Symbicort®), formoterol and fluticasone (Flutiform®), vilanterol and fluticasone (Relvar Ellipta®).

1.6.2 Anticholinergics

The principle of this group of agents is to block the muscarinic (M) receptors that mainly exist on the airways to block acetylcholine effects. The first approved anticholinergic agent was ipratropium, it is short acting, and used for initiation treatment of acute asthma and COPD. Ipratropium mainly blocks the M2 also with the M1, and M3 receptors, but long-acting anticholinergics such as tiotropium and umeclidinium have selectivity for the M1 and M3 receptors and obtain longer pharmacodynamic affinity to these receptors. In the clinic, Spiriva Handihaler® (tiotropium) is the most frequently prescribed inhaler used to treat COPD on a regular basis. Evidence showed treatment with tiotropium could improve lung function and reduce exacerbations and hospitalisations in the *Understanding Potential Long-term Impacts on Function with Tiotropium* (UPLIFT) trial (Tashkin et al, 2008). As they have no direct effects on inflammatory mediators e.g. histamine, anticholinergics are less useful for asthma therapy (Celli, 2005; GOLD, 2017). Due to their common quaternary ammonium structure, anticholinergics show low bioavailability after oral administration and are currently only available by the

inhalation route. Ipratropium is administrated by a small volume liquid inhaler (SVLI), Respimat®. Or a combination therapy with fenoterol (Duovent®) and salbutamol (Combivent®). Tiotropium is used as tiotropium bromide, which is either formulated in dry powder format in Handihaler®, or solution in Respimat®. Umeclidinium is used by a DPI, e.g. Incruse Ellipta® containing umeclidinium alone or Anoro Ellipta® containing a combination of umeclidinium and vilanterol.

1.6.3 Corticosteroids

Inhaled corticosteroids (ICS) are regarded as the most effective anti-inflammatory drug for the management of asthma, as well as a standard treatment for other respiratory conditions e.g. cystic fibrosis and allergies (SIGN, 2016). However, there is no evidence of using ICS alone for improvement in preventing long-term decline of forced expiratory volume in one second (FEV1) and reducing mortality rate of patients with COPD (Yang et al, 2012). The mechanism of action of ICS is to suppress the inflammation by binding to glucocorticoid receptors, which are located mainly in the cytoplasm (Nicolaides et al, 2010). As glucocorticoid receptors are activated upon ligand binding, reduced production of mediators e.g. histamine and cytokine to induce the anti-inflammatory actions (Smyth and Hickey, 2011). Different ICS are currently marketed for treating asthma comprising of beclomethasone dipropionate (BDP), fluticasone propionate (FP), budesonide, mometasone furoate (MF) and ciclesonide. ICS are administrated by a variety of aerosol delivery systems comprising of pMDIs, e.g. Qvar® (BDP), Flovent® (FP) and Alvesco® (ciclesonide), DPIs, e.g. Pulmicort Turbohaler® (budesonide) and Asmanex Twisthaler® (MF), and Nebulisers such as Respules® nebuliser suspension (budesonide). The combination products containing ICS and LABAs are also available for treating both asthma and COPD, which was described in Section 1.6.1.

1.7 Aerosol Delivery Systems

Aerosol inhalation is the preferred route for treating lung diseases rather than oral dosing as high local concentrations of the drug are achieved along with reduced systemic effects (Patton et al, 2010). However, a successful inhalation therapy depends on not only the pharmacologically active molecule and formulation but also the delivery device. Aerosol delivery devices can be broadly categorised into four types: nebulisers (NEBs), small volume liquid inhalers (SVLIs), dry power inhaler (DPIs), and pressurised metered dose inhalers (pMDIs).

1.7.1 Nebulisers

Nebulizers are some of the oldest devices for drug administration and make use of a compressed air-jet of high velocity air to shear a bulk suspension or solution of drug into a liquid film at the spray nozzle. The driving gas in the nebulizer is forced through a very small opening called a venturi, which creates a low pressure zone. The pressure difference leads to the “sucking up” of liquid from the reservoir through a capillary system and forms solution droplets (Figure 1.2). Nebuliser formulations are solutions or occasionally suspensions, and physical properties of the formulation such as pH, viscosity and surface tension, play vital roles in the performance of the formulation (Labiris and Dolovich, 2003a). As potential contamination can cause respiratory infection, the formulations are normally packaged in sterile containers (Monforte et al, 2005).

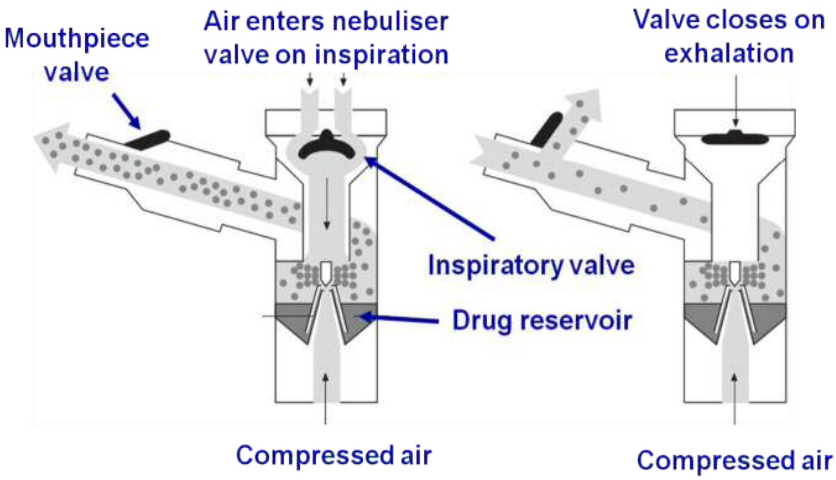

Figure 1. 2 Schematic Diagram of a Nebuliser
(Pari LC Star/Plus)

Nebulisers have advantages as inhalation devices such as delivering drugs that require a large dose, which could not be delivered through alternative means. Children and the elderly find using the device relatively easier than other inhalation devices, albeit through the assistance of a mouthpiece or facemask. However, the nebuliser device does demonstrate key disadvantages such as variability in delivered dose, even using the same nebuliser and compressor. Additionally, low delivery efficiencies require a longer duration of drug administration. The longer duration of drug delivery causes problems such as change of drug concentrations during aerosolisation. During nebulisation, there is a tendency for the diluents to evaporate causing an increase in concentration of the drug and up to 50% of the drug may remain in the reservoir at the end of dosing. In addition, at the end of administration a significant amount of solution generally remains on the baffles and the reservoir wall often referred to as “dead volume” (Beaucage and Nesbitt, 2002). These disadvantages along with the bulkiness of the device make nebulisers impractical for daily portable use. More recently, electronically controlled nebulisers such Aeroneb® Go (Aerogen), Omron MicroAir® (Evergreen) and eFlow® (Pari) have been developed and offer advantages in terms of efficiency compared to traditional air-jet nebulisers.

1.7.2 Small Volume Liquid Inhalers

Small volume liquid inhalers include Respimat® (Boehringer Ingelheim), (which is also described as a "soft mist" inhaler) and AERx® (Aradigm Corporation). These devices aerosolize drugs by a number of different mechanisms e.g. spring compression (Respimat®) and hydraulic extrusion (AERx) but have in common the feature of delivering the aerosol in small (microlitre) volumes inhaled by the patient during one breath (Rubin, 2011). The Respimat® (Figure 1.3), as a new generation, propellant-free inhaler, was designed to overcome inherent disadvantages of pMDIs (e.g. high velocity aerosols), DPIs (e.g. requirement for adequate patient inspiratory flow rate) and nebulizers (e.g. inconvenient daily use). As a multi-dose inhaler, the drug solution is stored in a double walled, plastic and collapsible bag in a drug cartridge (Dalby et al, 2004). Depending on a spring mechanism, a metered volume of the solution is withdrawn from the cartridge through the capillary tube into the pump. When the patient presses the dose release button, the metered solution is released through a two-channel nozzle, "Uniblock" to form a slow aerosol plume (Spallek et al, 2002).

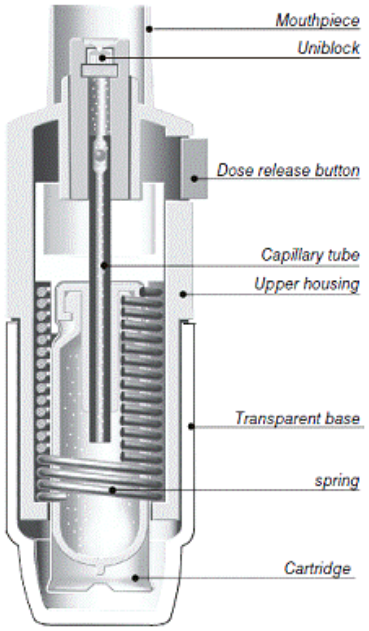

Figure 1. 3 Schematic Diagram of the Respimat®
(Boehringer Ingelheim)

1.7.3 Dry Powder Inhalers

Dry powder inhalers (DPIs) are devices that deliver active drugs in dry powder form for local or systemic effects. Since the first device Spinhaler® was introduced into the market in 1970, a wide range of DPIs have been developed including single dose, multi-dose and multi-unit dose devices (Islam and Gladki, 2008). In general, DPI formulations consist of either pure micronised drug particles (some Turbohaler® formulations) or drug particles blended with larger "carrier" particles (e.g. in Handihaler®) to facilitate uniform delivery of the drug (Newman and Busse, 2002). The formulations within DPIs can be individually preloaded into the device before use, such as hard gelatin (e.g. in Handihaler® and Aerolizer®) or hydroxypropylmethylcellulose (HPMC) capsules (Deboeck et al, 2010). The bulk can also be preloaded into multi-dose reservoirs within the device (e.g. Turbohaler® and Twisthaler®), or individually sealed in blisters for multi-unit dose deliveries (e.g. Diskus®/Accuhaler®). Most DPIs are passive inspiratory systems, and the airflow generated by the patient's inhalation causes fluidization of the powder bed and

entrainment of the powder into the airflow (Shur et al, 2008). Drug particles are required to de-agglomerate from large agglomerates of drugs or detach from the carrier particles by induced turbulence by the patient's airflow or passing through a screen equipped with the device (Islam and Gladki, 2008). As a passive delivery system, DPIs have overcome the coordination issue of actuation and inhalation normally associated with pressurised metered dose inhalers (pMDIs). Propellant is also not required to aerosolise the drug. As a sealed dry powder form, the formulation is at a low energy state, and reduces the probability of chemical degradation and contact with leachable compounds from device components (Telko and Hickey, 2005). However, due to the intrinsic resistance of the inhaler, DPIs in general require high inspiratory flows to efficiently aerosolise the formulation from the device, which if not achieved can provide variable delivery patterns (Newman and Busse, 2005; Cegla, 2004).

1.7.4 Pressurised Metered Dose Inhalers

Pressurised metered dose inhalers (pMDIs) are pressurised vessels containing drugs that are either dissolved or suspended in a liquefied propellant. Since the first pMDI was marketed in 1956 by Riker Laboratories (Medihaler-Epi™ and Medihaler-Iso™), pMDIs have become the most widely used devices for control and treatment of asthma and COPD (Myrdal et al, 2013). A pMDI consists of several key components: the canister, metering valve, actuator, drug formulation, and propellant as illustrated in Figure 1.4. The micronised or dissolved drug and excipients are maintained in compressed propellant gases in a pressure-proof and sealed environment. There is equilibrium between the liquefied propellant and its saturated vapour pressure, which can maintain a constant pressure during the product's lifetime (Smyth, 2003). Although there are various pMDI products in the market, most of them share a similar discharge mechanism. The pMDI device is used in an inverted position, and the formulation is pre-filled in the metering

chamber of the valve. With depression of the valve stem, the liquefied propellant containing the formulation is exposed to the atmosphere and due to the pressure differences, the propellant rapidly expands through the valve stem into the expansion chamber of the actuator, where the propellant starts to boil. The mixture of liquid/vapour (solution formulation) or liquid/solid/vapour (suspension formulation) is atomised through the actuator orifice into an aerosol plume containing various droplets. The droplets leaving the orifice is highly dynamic, as the evaporation of the propellant, the droplet size and composition are changed (Stein and Myrdal, 2006). The generated solid particles are then inhaled by the patients in a slow and deep breathing pattern.

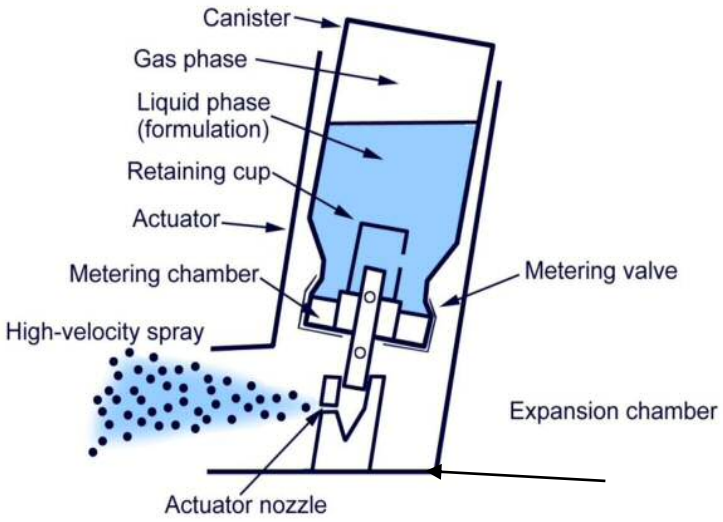

Figure 1. 4 Schematic Diagram of a pMDI
(Buddiga, 2015)

1.7.4.1 Components of Pressurised Metered Dose Inhalers

The canister is required to withstand the high pressure generated by the propellant. The volume typically ranges from 10 to 20 mL, e.g. 10, 14, and 19 mL, to accommodate the volume of each dose and the total dose number per canister. Typically, canisters are made from anodised aluminium, which is light, inert, robust and light proof, stainless steel or glass (Stein et al, 2014). The inner surfaces of the canister may be coated to prevent drug adhesion and chemical degradation, and such materials include fluorinated ethylene propylene, perfluoroalkoxyalkane and anodized aluminium (Ashurst et al, 2000; Lewis et al, 2000). The metering valve is the heart of the pMDI system, which ensures the consistent metering of doses from the bulk formulation and delivers the measured amount through the valve stem to the actuator. The typical volume of a metering valve ranges from 25 - 75 µl, e.g. 25, 50, and 63 µL. Generally, based on valve design strategies, metering valves are categorised into two types, the retention valve and "fast fill, fast empty" valve (FFFE) (Stein et al, 2014). Each metering valve operates under the same basic principles to ensure a precise dose is delivered. The valve is used in an inverted position, and following the release of the dose, the connection between the valve and environment is closed. In a retention valve, which contains a retention chamber, the metering chamber is immediately re-filled by the formulation driven by the vapour pressure and gravity. In a FFFE valve as shown in Figure 1.5, the gasket is made of elastomeric materials i.e. ethylene-propylene diene monomer (EPDM), the spring is made of metal, and the remaining components are either metal or moulded plastic (Gelotte and D'Silva, 2000; Stein et al, 2014). The metering chamber is sufficiently open to the bulk of the formulation, and just re-filled by gravity as the valve is depressed (Fradley and Hodson, 2008).

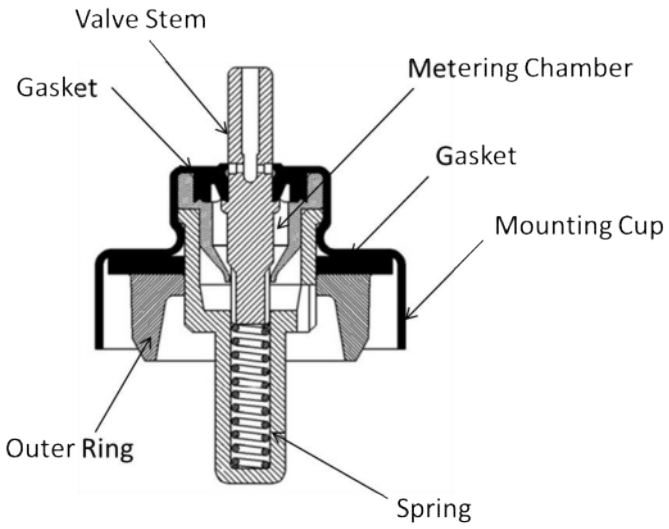

Figure 1. 5 Schematic Diagram of a VARI® KHFA Metering Valve

The pMDI actuator is a key component of the pMDI system as illustrated in Figure 1.6. The canister fitted with a valve fits into an actuator, which is used to prime the canister, atomise the formulation and direct the spray into the respiratory tract. Several factors related to the actuator design affect the performance of the pMDI including actuator materials, orifice length and diameter, mouthpiece design and configuration. Significantly, the orifice diameter has been identified to affect the spray formation and further the lung deposition (Newman, 2005). An in-vitro study using the Andersen cascade impactor indicated that small orifices gave the most efficient delivery of a HFA 134a formulation containing a solution formulation of beclometasone dipropionate (BDP) (Stein et al, 2014). Orifice diameters can range from 0.14 mm to 0.6 mm but are typically between 0.25 mm to 0.45 mm.

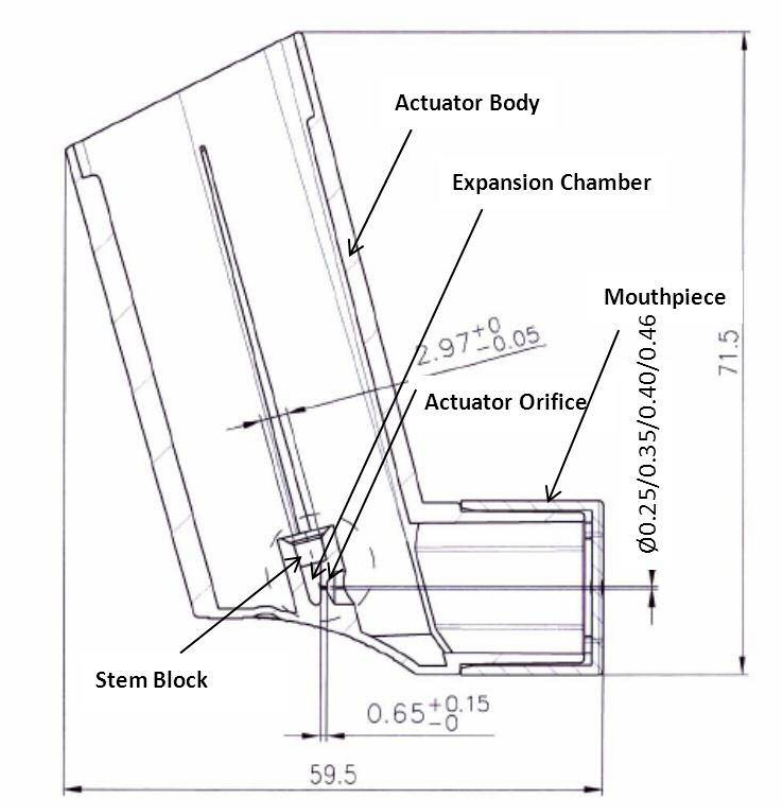

Figure 1. 6 Schematic Diagram of an H&T Presspart® Actuator

Liquid propellants are used to disperse solid particles and generate aerosols of drug formulations in pMDIs. Chlorofluorocarbons (CFCs) were traditionally used as medicinal propellants. As highlighted in Table 1.1, the most commonly used CFCs were CFC-11 (CCl_3F), CFC-12 (CCl_2F_2) and CFC-114 (CCl_2F_4), which show different physical properties. The strong carbon-chlorine and carbon-fluorine chemical bonds in CFC prevent their biological degradation and make them safe for pharmaceutical use. To generate adequate aerosol velocity, formulations generally contained a blend of CFC-11 and CFC-12, or CFC-11, CFC-12 and CFC-114, which give an intermediate vapour pressure (around 4.2 Bar). However, Molina and Roland (1974) reported that photodegradation of CFC could release chlorine atoms that destroyed the stratospheric ozone in catalytic cycles. Under the Montreal Protocol (1987), it was shown that several groups of halogenated hydrocarbons including CFCs were indeed involved in the depletion of the ozone layer. All ozone-

depleting CFC substances were phased out of use progressively from 1 January 1989. Furthermore, commencing on 1 January 1996 and thereafter, it was planned that the consumption and production of CFCs should be completely discontinued. However, where no acceptable substances could be found, "essential use exemption" clauses within the treaty allowed the continued use of CFCs in medicinal inhalers. Until a suitable replacement could be found, use of CFCs in pMDIs was permissible.

1.7.4.2 Replacement of CFC Propellants

Alternatives to CFCs were required for pMDIs. One possibility was the use of hydrocarbons such as butane but there were deemed unsuitable because of toxicity and inflammability. Another possibility, compressed gas, was non-toxic but could not maintain constant pressure through can life. Hydrofluoroalkanes (HFAs) such as HFA-134a ($C_2H_2F_4$) and HFA-227 (C_3HF_7) (Figure 1.7) are deemed suitable, as they are non-ozone depleting and have less global warming potential. They have broadly similar physical properties e.g. vapour pressure and density to CFC-12 but there is currently no commercially available alternative to CFC 11, which is liquid at room temperature and has relatively high solvency powers. Consequently, there is a challenge to prepare HFA mixtures with similar vapour pressure and density to CFC mixtures. Furthermore, HFAs show more polarity than CFCs but they exhibit poor solubilisation capacity for both hydrophilic and hydrophobic molecules, which lead to solubility issues for most drugs and excipients used in CFC systems (Smyth and Hickey, 2011).

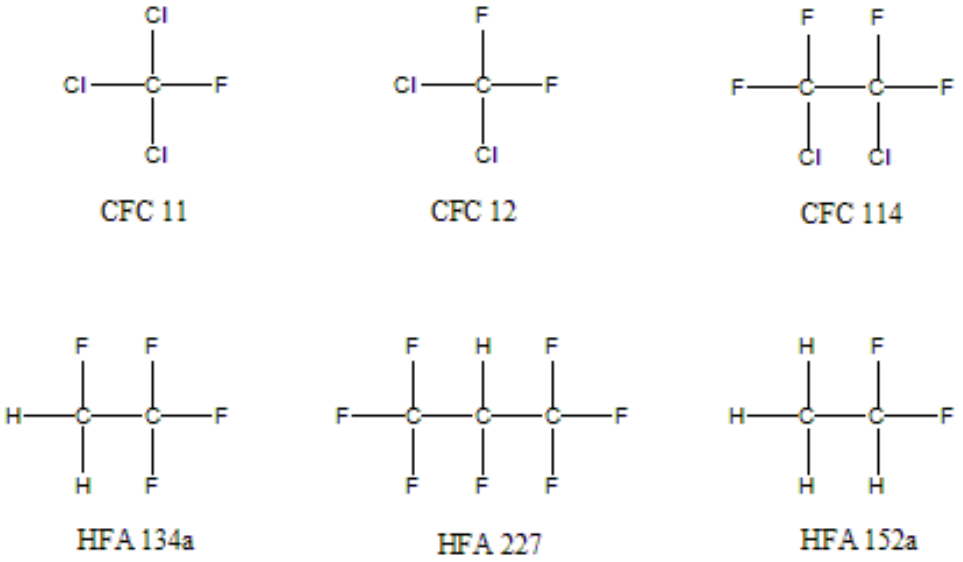

Figure 1. 7 Chemical Structure of CFCs (CFC 11/12/114) and HFAs (HFA 134a/227/152a)

In traditional CFC inhalers, most of the formulations were suspensions, in which the micronised drug particles were suspended with CFCs containing certain amount of surfactants. Three types of surfactants were approved by FDA to use in pulmonary delivery including; oleic acid, sorbitan trioleate and lecithin. Due to physiochemical differences, all of these surfactants were practically insoluble in HFAs. Although the addition of co-solvents e.g. ethanol, did alter the solvency strength and increase their dissolution rate, only oleic acid had been approved to use in HFA systems. Due to the relative higher vapour pressure in HFAs, the design of high pressure-proof device components e.g. the valve, was required, and actuator design was also critical to obtain similar performance profile (Myrdal et al, 2014).

The replacement of CFC with HFA propellants in inhalers was a huge task for the pharmaceutical industry. The first HFA inhaler in the USA, Proventil® HFA inhaler, was not available until 1996, a decade after the Montreal Protocol, whilst other drug formulations have not been commercially replaced (Hendeles et al, 2007). The aerosol performance of Proventil® was comparable to its CFC inhalers, with similar emitted dose and particle size distribution. In the USA, albuterol CFC pMDIs were no longer available after 2008. In the current USA market, there are three FDA-

approved albuterol HFA pMDIs, Proventil®, ProAir® and Ventolin®. Several CFC pMDI products including those containing triamcinolone and pirbuterol were phased out in 2011 and 2013 respectively. There are no equivalent HFA pMDIs or other devices for triamcinolone and pirbuterol, so patients needed to find alternative medicines for treating asthma. However, as HFA 134a and HFA 227 still contribute to the greenhouse effect, the Kigali Amendment (2016) was added to the Montreal Protocol, which required the replacement of HFA propellants with more environment-friendly alternatives. Although provision was made for continued usage in pMDI products, other alternative propellants such as 1,1-difluoroethane (HFA 152a) are investigated to minimise the environment impact. The structure and some properties of HFAs are illustrated in Figure 1.7 and Table 1.1. Although HFA 152 demonstrates a lower density than HFA 134a, pre-clinical studies have shown an improvement in suspension settlement and identical aerosol performance to existing products e.g. salbutamol sulphate in HFA 134a. Furthermore its low carbon footprint will have less impact on the environment, which shows a potential as a future pMDI propellant (Noakes and Corr, 2016).

Table 1. 1 Some Properties of CFC and HFA Propellants

(Noakes and Corr, 2016)

Propellant	Formula	Density (g/mL, 20°C)	Vapor Pressure (Bar, 20°C)	Boiling Point (20°C)	Ozone Depletion Potential	Global Warming Potential (CO_2=1)
CFC 11	$CFCl_3$	1.49	0.88	23.7	1	4660
CFC 12	CF_2Cl_2	1.33	5.66	-29.8	1	10800
CFC 114	CF_2ClCF_2Cl	1.47	1.80	3.5	1	8590
HFA 134a	CF_3CFH_2	1.23	5.72	-26.2	0	1300
HFA 227	CF_3CFHCF_3	1.41	3.89	-16.5	0	3350
HFA 152a	CH_3CF_2H	0.91 (25°C)	4.99 (25°C)	-24.7		

1.7.4.3 Secondary Particles

The suspension is a thermodynamically unstable system, and particles tend to collide to decrease the surface energy. In previous CFC systems, surfactants were used to reduce the particle surface free energy to prevent particle aggregation (Pilcer and Amighi, 2010). Since the production of CFC was phased out, the new HFA pMDIs have faced issues with low surfactant solubility, and variable safety and efficiency profile of alternative surfactants. In practice, the performance of the new product should be therapeutically equivalent to an existing CFC inhaler. In pMDIs, the drug, depending on its solubility, is either dissolved or suspended in propellant(s). In general, drugs practically insoluble in HFAs are formulated as suspension formulations and the problems associated with suspension formulations such as agglomeration, particle growth and caking need to be considered in their design (Myrdal et al, 2014).

Physical stability is the main concern in the reformulation process due to different solvency properties of propellants, and the aggregation behaviour of micronised particles (McDonald and Martin, 2000). The addition of surfactants contributes to the suspension stability by dispersing particles and lubricating the valve to facilitate consistent dose delivery during the life span of products. Surfactants are usually included at concentrations between 0.1% w/w and 2.0% w/w in CFC systems, and co-solvents such as ethanol are needed in HFAs to increase surfactant solubility. However, the incorporation of ethanol can decrease the fine particle fraction and increase the mass median aerodynamic diameter (Smyth and Hickey, 2002). In addition, the volatility of HFAs may be modified by the addition of a less volatile component e.g. glycerol to modify the aerosol performance closer to the corresponding CFC products. Other approaches to increase the physical stability of a formulation include hollow porous particles, low-density lipid-coated microcrystals, biocompatible microparticles e.g. polyvinylpyrrolidone and polymeric

nanocarriers (Dellamary et al, 2000; Tarara et al, 2004; Jones et al, 2006; Bharatwaj et al, 2010).

Small particles have a high degree of surface free energy and an increased tendency to agglomeration, which leads to poor physical stability. When particles adhere to each other after sedimentation, the weight of the sediment forces particles together, in close contact the potential energy of attraction due to Van der Waals force is strong. Consequently, a high level of energy is required to re-suspend the particles by overcoming the attractive force. With time, particles could eventually fuse together into a non-dispersible cake. The assumption of the secondary particulate theory is that in the propellant, the second particle (excipient) attracts the first particle (drug) to the surface by Van der Waals force and/or electrostatic attraction. This intervention prevents the first particle's collision and aggregation with each other (Figure 1.8). The second particles could sediment or float at an adequate rate. A loose cluster is expected to form without caking, and is easily re-dispersed by agitation. Consequently, as a canister is shaken, a homogenous suspension could be formed and metered accurately by the valve to give a reproducible delivery of the therapeutic agents. The generated droplets are assumed to contain drug/excipient, drug, excipient or propellant only, and with evaporation of the propellant, the drug is de-aggregated from the excipient. With the addition of suitable second particles, it is expected to improve dose reproducibility and maintain aerosol performance.

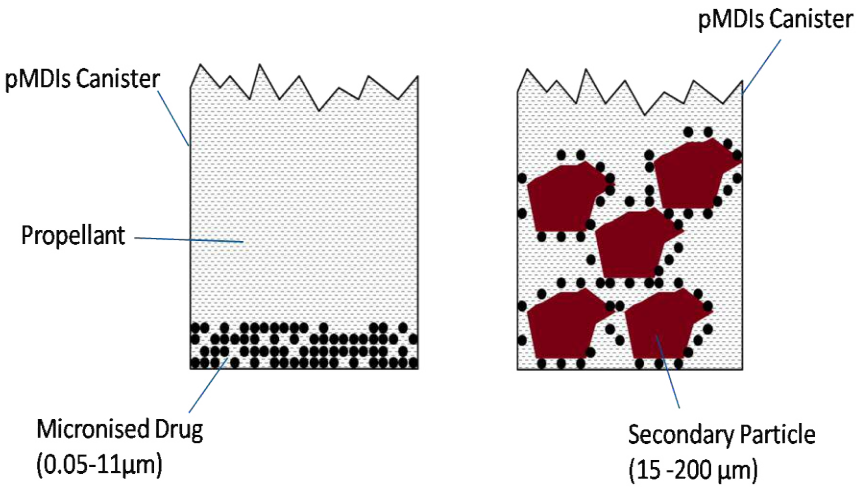

Figure 1. 8 Secondary Particulate Theory Based on Suspension System

In previous study by Dickson and Warren (2004), a patent was published regarding use of second particles to formulate a pMDI suspension particularly in liquid propellant. In this patent, the second particle should have a diameter in the range of around 25 to 125 µm. The weight ratio of the first and the second particles should be somewhere in the range of 1:1 to 1:50. For the purpose of sustainable use of the pMDI device during the whole canister life, which is normally more than 100 actuations, the second particle should be a sufficiently soft material of less than 6.5 Mohs hardness and preferably within the range 2 and 3 to prevent damaging the valve. Low solubility of both particles is essential for the stability of the formulation where their solubility is preferably less than 1%. Suitable second particles could include carbohydrates (e.g. lactose and glucose) and the reduced forms of carbohydrates (sorbitol), amino acids (leucine), peptides and proteins (Dickinson and Warren, 2004). The second particle could be used as an alternative to surfactant to prevent the aggregation of drug particles to achieve a good dispersion characteristics and valve performance.

1.8 Particle Sizing Techniques and In Vitro Measurement

Since the respiratory airways function as a particle size-selective sampling system, measurement of the aerodynamic particle size distribution in an aerosol cloud is currently the standard and compulsory method in development and evaluation of pharmaceutical products for inhalation. In this research, the physical stability and performance of novel secondary particle formulations are determined and predicted through in vitro measurements. Several kinds of techniques have been applied in practice including inertial classification, laser diffractometry, particle time-of-flight (TOF), and phase Doppler anemometry (PDA) (De Boer et al, 2002). In this research, the size distribution of raw materials is analysed by Mastersizer 2000 using laser diffractometry, and the aerodynamic size distribution of particles delivered from inhalers is determined by cascade impactor using inertial classification.

In the theory of laser diffractometry, an expanded laser beam is used to produce a parallel monochromatic light and the diffraction pattern generated by particles entering the measurement zone is collected by a Fourier transform lens (Mitchell and Nagel, 2004). Laser diffraction analysers can determine the whole particle distribution in the aerosol cloud together with some speed information, and the dynamic range of the size measurement could cover particle sizes from 0.02 μm to a few millimetres with good repeatability (Kippax, 2005).

The principle of inertial classification is to capture those particles with sufficient inertia when they cross gas streamlines. The impaction technique can provide information about inertial size fractionation e.g. the fine particle fraction (FPF) of the formulation, together with direct measurement of aerodynamic diameter, and can quantify mass of the drug by appropriate analytical techniques (Mitchell and

Nagel, 2003). Cascade impactors have been specified and used for regulatory approval for pharmaceutical tests. Two options include the Andersen Cascade Impactor (ACI) and the Next Generation Impactor (NGI). Both types of equipment offer the required degree of resolution and varied test range of particle sizes from around 0.1 to 10 µm (Copley, 2007). The main concerns on their use include particle bounce, overloading of plates, electrostatic effects and inter-stage losses. Hence the physical properties of the particles and the operation of the equipment need to be considered (Copley, 2010; Bonam et al, 2008). The ACI apparatus consists of 8 stages and was originally designed for air sampling and analysis at a flow rate of 28.3 L/min. With conversion kits, the device can be calibrated at flow rates from 15 L/min to 90 L/min. The NGI apparatus consists of seven stages and a micro-orifice collector (MOC), and can be operated over the flow rate range of 15 L/min to 100 L/min.

1.9 Hypothesis and Research Rationale

Suspensions are thermodynamic unstable systems, and physical stability is one of the main concerns in the development of a suspension drug delivery system. In an ideal suspension, suspended particles should remain uniformly distributed with adequate settling velocity, maintain constant particle size without aggregation through the long-term storage, and be easily re-dispersed by gentle agitation. In a non-polar pMDI suspension, micronised drug particles are suspended in liquefied hydrofluorocarbon, and physical instability of this system consists of aggregation, loss of active on device surfaces and a possibility of caking. Due to the high degree of surface energy, micronised particles tend to aggregate to form larger agglomerates or deposit on the surface of device components, which lead to non-uniform distribution of particles and loss of active over the canister life.

It is believed that with the addition of a large and inert particle, micronised drug particles are attached to the binding points of the inert particles by Van der Waals force and/or electrostatic attraction. As a steric obstruction, the inclusion of inert particles could prevent drug-drug and drug-device component interactions. Due to the shape and larger size of inert particles or the neutralisation of electrostatic charges, drug and inert particles combine as an agglomerate and form a loose floc by weak physical interaction e.g. Van der Waals force. The floc is readily dispersed to form a uniform suspension by agitation. The uniformity of a drug substance dispersed in a continuous phase ensures the same delivered dose each time a volume of dispersion is metered by a pMDI. This suspension can be used as an ideal pMDI formulation, as a physically stable and re-dispersible system can maintain the homogeneity of the suspension, uniformity of dose delivery and consequently consistent aerosol performance.

During the atomisation, the suspension is atomised into a plume by expansion force of the propellant, which contains a mist of droplets. The assumption of this dynamic process is demonstrated in Figure 1.9. The composition of the initial droplet could include propellant only (A), first particle and propellant (B), secondary particle and propellant (C), or first and secondary particles and propellant (D). Droplet A will evaporate off, Droplet B and C will form single-composition particles and Droplet D will form a dual-composition particle. The shear force could cause breaking of this weak interaction between the first and secondary particles. Smaller first particles are expected to penetrate into the lungs, and the remaining large first and secondary particles will deposit on the oropharynx. The change of droplet diameter is considered to affect the drug deposition and delivery efficiency (Sheth et al, 2017).

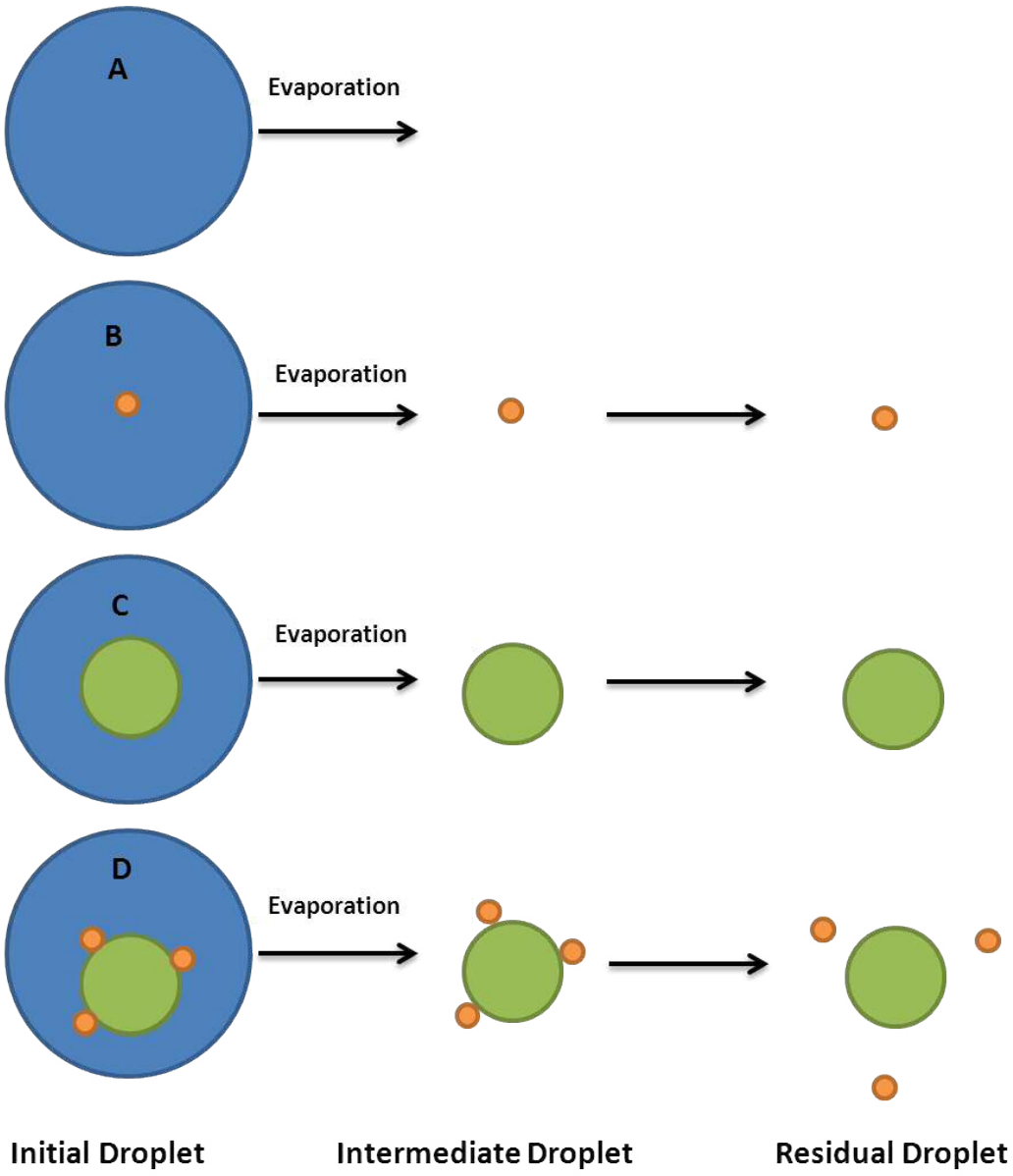

Figure 1.9

Figure 1. 9 Evaporation Process for Suspension pMDI Containing First and Secondary Particles (A: Propellant, B: First particle and propellant, C: Secondary particle and propellant, D: First, secondary particles and propellant)

A novel aerosol composition in the form of a suspension contains a HFA propellant, a first particulate material as the therapeutic agent and a second particulate material as the excipient, which is described in Patent US 6737044B1 by Dickinson and Warren (2004). In this patent, the second particle, which is expressed as secondary particle in following paragraphs and chapters, is used as an alternative to surfactants to prevent the aggregation of the drug particle, provide good dispersion characteristics and maintain good valve performance. In the previous research by Jones (2004), Taylor et al (2008), Tran et al (2011) and Tran et al (2012), several LABAs e.g. salmeterol, glucocorticoids e.g. fluticasone and budesonide, and some combination formulation e.g. salmeterol/fluticasone and budesonide/formoterol, were investigated as the model drugs, and the addition of secondary particles e.g. lactose and L-leucine, demonstrated promising aerosol performance and several key parameters e.g. stage-by-stage deposition, similar to the commercial comparators. However, suitability of formulating these drugs with leucine were evaluated based on factorial experiments, but limited exploratory work has been done regarding the factors affecting the formulation performance and particle association between drug and secondary particles. All the studied formulations contained relatively high amounts of active pharmaceutical ingredients. For highly potent drugs, small amounts of drugs are filled in each canister and it is necessary to understand whether the addition of a secondary particle can improve the physical stability, the aerosolisation process and aerosol quality. Furthermore, the secondary particles could act as bulking agents that could increase the powder uniformity and ease the dispensing process of small amounts of the drug. In this research, tiotropium was selected as the model drug, which is used medicinally in the form of tiotropium bromide monohydrate (Figure 1.10).

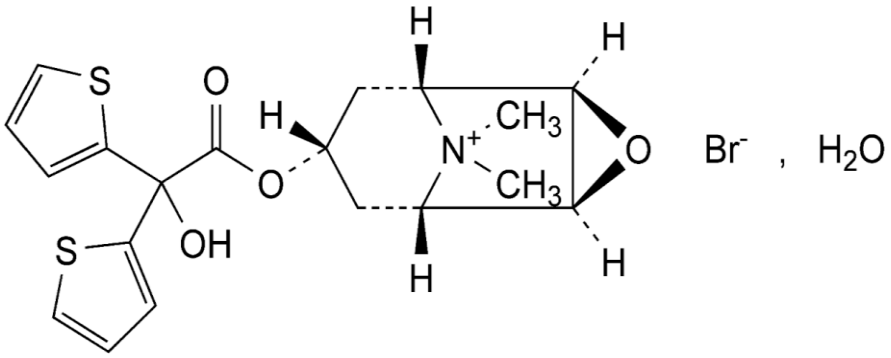

Figure 1. 10 Tiotropium Bromide Monohydrate Structure
(British Pharmacopoeia, 2017c)

The use of Tio delivered by the Spiriva Handihaler® device was approved by the FDA in 2004, and was mainly used for long-term maintenance treatment of COPD (Koumis and Samuel, 2005). The Handihaler® is a dry powder inhaler device and not deemed suitable for patients with restricted inspiratory flow e.g. elderly and children. Currently, Tio is administered as an inhalable dry powder using Spiriva Handihaler® 18 µg and inhalable solution using Spiriva Respimat® 2.5 µg (both expressed as tiotropium base). Spiriva Respimat® was assumed to have the same safety profile as Spiriva Handihaler®, but a 46% relative increase in risk of mortality in patients using Spiriva Respimat® compared with placebo has been reported (Singh et al, 2011). A call for the worldwide withdrawal of Spiriva Respimat® was announced by Singh et al (2012) following a meta-analysis of clinical trials. However, a follow-on trial, the *Tiotropium Safety and Performance in Respimat* (TIOSPIR), demonstrated the similarity of mortality and major cardiovascular adverse events between Spiriva Respimat® and Spiriva Handihaler® (Wise et al, 2013). The safety of Spiriva Respimat® is considered to be relevant to the delivery efficiency of the device (Cates, 2011), and therefore it is necessary to investigate aerodynamic performance of these two devices by in-vitro methods.

Currently, there is no available Tio pMDI marketed in Europe and the USA. Consequently, reformulation of tiotropium could not only increase the medical options but also benefit the patients with poor inspiration flow rate. Based on previous research, the incorporation of a secondary particle into a suspension formulation offers a potentially high in vitro aerosol performance. Consequently, in the first series of studies, the drug tiotropium was investigated as the candidate for this novel approach of pMDI formulations. The possibility of formulating a suitable pMDI with the secondary particulate technology is assumed with the following characteristics: comparable to commercialised products, consistent dose delivery, efficient delivery and good physical stability. With the advantages as a pMDI formulation, re-formulation of Tio is investigated with a number of formulation and hardware variable. Particle interaction between drug particles and associated secondary particles in situ and aerodynamic behaviour of airborne particles investigated indirectly using these in-vitro methods. The research will improve our understanding of particle interactions through the screening of the physical and physicochemical nature of secondary particles, propellant and device components. The variables involved in this process are manifold and determining some of the critical factors will be investigated.

1.10 Project Aims and Objectives

The aim of this research is to investigate the particle interaction between suspended drugs and inert excipient particles (secondary particles) in liquefied hydrofluoroalkanes through the in vitro evaluation of novel delivery systems. The high-potency drug, tiotropium, is used as the model drug, which is investigated under the scope of the secondary particulate theory. Gaining a better understanding of the mechanism and factors affecting the performance of this type of delivery system, will be useful for formulation design of novel inhaled medicines for both local and systemic delivery.

The specific research objectives are as follows:

(a) To assess the feasibility of formulating a highly potent drug, tiotropium with secondary particles as a pMDI suspension formulation.

(b) To investigate the effects of formulation variables e.g. excipient type, particle size, particle ratio and propellants on the in vitro performance of the novel formulation.

(c) To study the effects of device components e.g. volume of metering valves, actuator orifice diameter, and volume and coating of canister on the in vitro performance of the novel formulation.

(d) To determine the particle interaction between drug particles, secondary particles, propellants and device components through accelerated stability and aerosol performance studies.

(e) To evaluate the influence of the external environment e.g. moisture and temperature on the physical stability of suspension during long-term storage.

(f) To compare the performance of commercialised Tio inhalable products with our novel Tio pMDI formulations by in-vitro tests.

2 Method Development and Validation

2.1 Introduction

This chapter describes the common methods used in Chapter 3, 4, and 5, and any deviations to these methods are specified in each chapter. In this research, a novel pMDI formulation containing tiotropium and a secondary particle is developed and the factors that affect the performance and stability of the formulation are further investigated. The commonly used methods include the sample preparation process, the manufacture process for pMDIs, the performance testing for pMDIs and DPIs, and quantitation of tiotropium either as the raw material or in the formulation. The validation information consists of the methods used for quantification of the raw materials and pharmaceutical products, and evaluation of the canister performance.

As mentioned in Chapter 1, preliminary research was carried out by Dickinson and Warren (2004) and Jones (2004). It was reported that particulate excipients e.g. lactose or leucine were used to stabilise suspension pMDI formulations containing inhaled corticosteroids, alone and in combination with a long-acting β2-adrenergic agonist (Tran et al, 2011; Tran et al, 2012). Other investigations were mainly focused on less potent drugs e.g. salmeterol xinafoate, salbutamol sulphate or budesonide with doses from 50 µg to 200 µg, whereas tiotropium shows high potency, with a delivered dose of 22.5 µg (Spiriva Handihaler®) or 6.25 µg (Spiriva Respimat®), and thus is needed in very low amounts in an equivalent pMDI formulation. Variability of the emitted dose and drug deposition are certain intrinsic limitations caused by pMDI devices, which are fundamental for the highly potent drugs because of the induced variable therapeutic results and severe adverse effects (Jinks, 2003). Secondary particles were therefore investigated on the influence of the physical stability and delivery efficiency of this high-potency drug. The preparation of formulation containing tiotropium and secondary particles

e.g. lactose and leucine were modified accordingly with the concern of the small delivered dose of tiotropium.

As a suspension system, micronized drug particles must be physically stable and avoid particle growth. The potential dissolution of the drug could induce crystal growth, so-called Ostwald ripening, and further affect particle size distribution. To test the feasibility of tiotropium to be used in a suspension system, the solubility of the drug in propellant was initially investigated. When formulating a powdered dosage form, powders need to be mixed uniformly to obtain a consistent delivered dose. As small batches were required in this research, the raw material including the drug and excipients were mixed manually by trituration and blended by a Turbula® mixer. The degree of mixing was then evaluated by the ability of the mixture to meet the specification limits. Several kinds of methods were used for particle size analysis. For example, the particle distribution of the secondary particle was monitored before and after the mixing process by laser diffractometry using a Malvern® Mastersizer, and the aerodynamic diameter of the drug particle, along with the fine particle fraction was determined by inertial classification using a Next Generation Impactor (NGI). Small-scale canisters (< 100) were manufactured using the pressure fill technique. To avoid the waste of propellants when switching the filling equipment between different propellants, cold fill technique was applied when the batch size was less than 10 canisters. Water content in the canister was relevant to the solubility and chemical stability of the drug, which was analysed by a Metrohm® KF coulometer. The performance of the canister was investigated according to standardised pharmacopeia procedures, e.g. the dose uniformity test and particle size distribution test using dosage unit sampling apparatus (DUSA) and Next Generation Impactor (NGI). The quantification of the drug was performed by high performance liquid chromatography (HPLC).

As there are no available pMDI products containing tiotropium used for treating chronic obstructive pulmonary disease (COPD) and asthma in Europe and American markets, the initial design of the novel formulation was comparable with the marketed tiotropium dry powder inhaler (DPI) product, Spiriva Handihaler®, in respect of the particle size distribution and fine particle dose. The dosage and in-vitro performance of the novel formulation and Spiriva Handihaler® is expected to equivalent. Spiriva Handihaler® is designed as a pre-filled-capsule dry powder inhaler used as a maintenance bronchodilator to treat patients with COPD. Each capsule contains 22.5 μg tiotropium bromide monohydrate as one therapeutic dose, which is equivalent to 18 μg tiotropium base. In this thesis, tiotropium bromide monohydrate, the salt form, is expressed as tiotropium or Tio, except where specified as tiotropium base or Tio base. The target pMDI ex-valve dose per actuation was set at 11.25 μg, so two actuations would provide a therapeutic dose equal to that from the Spiriva Handihaler®. Each pMDI canister contained 120 puffs with 30% or 40% overage of Tio, and in- total 60 doses for two-month usage.

The pMDI system is aiming to provide accurate and reproducible delivery of the active pharmaceutical ingredient (API) to the lung, with adequate formulation stability during transport and storage (Williams and Hu, 2011). The delivery mechanism is different with the marketed dry powder inhaler and small volume liquid inhaler (SVLI). The in-vitro testing of these reference products was discussed in Chapter 3, and the general methods applied were recorded here. In contrast with other drug products, factors affecting the performance of pMDIs are more complicated. The formulation, container, closure, manufacturing, in-process and final controls, and stability all need to be considered during the development programme (FDA, 1998). Apart from the influence of manufacturing, the factors can be divided into the nature of the delivery systems such as formulation strategies and the production of inhalation devices. In terms of formulation strategies, factors such as excipient types, ratios, sizes and propellant types and in

terms of the production of inhalation devices, factors such as valve volumes, canister volumes, canister coatings and actuator orifices were investigated and described in Chapter 4, with the applied methods involving the preparation, manufacture and aerosol performance testing explained in this chapter. Following the addition of secondary particles, the optimised novel products were compared against control and reference products in Chapter 5, using methods including moisture, canister content, and stability testing under accelerated conditions as described here. Any deviation is further discussed in the corresponding chapter.

2.2 Materials and Equipment

2.2.1 Materials

Tiotropium Bromide Monohydrate (Tecoland); Tiotropium Bromide Monohydrate CRS European Pharmacopoeia Reference Standard (Council of Europe); Respitose® SV003 Monohydrate Lactose (DMV-Fonterra Excipients); Respitose® SV010 Monohydrate Lactose (DMV-Fonterra Excipients); L-Leucine (Sigma-Aldrich); HFA 134a (Mexichem); HFA 227 (Mexichem); Benzyltriethyl Ammonium Chloride (Sigma); Potassium Dihydrogen Orthophosphate (Fisher); Hydranal Water Standard 0.1 (Honeywell Fluka); Polyethylene Glycol Laboratory Reagent Grade (Fisher); O-Phosphoric Acid (Sigma); Methanol HPLC grade (Fisher); Water HPLC grade (Fisher); pH Buffer Solution (Hanna Instruments); 14 mL Plain Aluminium MDI Canisters (H&T Presspart); 25 µL KHFA Metering Valve (Valvole Aerosol Research Italiana); Mircrolance® 3 Needles (Becton Dickinson); Sterile Evacuated Elution Vials (Amersham Health); Minisart® RC25 Syringe Filter (Sartorius); 0.2 µm Nylon Membrane Filters (Whatman)

2.2.2 Equipment

Agilent 1200 Series HPLC System consisting: Quaternary Pump G1311A, Multiple Wavelength Detector G1314B, Autosampler G1329A, Thermostatted Column Compartment G1316A, Degasser G1322A, Chemstation LC software Rev. B.03.02 (Agilent Technologies); Agilent 1200 Series System consisting: Isocratic Pump G1310A, Variable Wavelength Detector G1314B, Autosampler G1329A, Thermostatted Column Compartment G1316A, Chemstation LC software Rev. B.04.02 (Agilent Technologies); Genesis C-18, 150 × 4.6, 5 µm Column; Two Stage Filling Equipment (Pamasol) with laboratory scale propellant pump (X2008-00) and crimping head (X2002-0043/013); Minor M200 Sieve Shaker (Endecotts) with Laboratory Test Sieve (Aperture: 180/150/125/106/90/63/38 µm); Turbula Mixer

(Willy A. Bachofen AG Maschinenfabrik); Mastersizer 2000 Particle Size Analyser (Malvern); HPC SM12 Rotary Screw Air Compressor (Straightset); 5799-S Stability Storage Cabinets (Vindon Scientific); Next Generation Impactor (Copley Scientific); Dosage Unit Sampling Apparatus (Copley Scientific); NGI Gentle Rocker (Copley Scientific); 831 KF Coulometer (Metrohm); Adjustable Pipe Cutter (Wickes); Circulaire 900 Fume Cabinet (Monmouth Scientific); U300 Sonicator (Ultrawave); PH300 pH Meter (Hanna Instrument); Adventurer Pro AV264CU Digital Balance (Ohaus); MC410S Analytical Balance (Sartorius); 7006 Ionizing Bar (Exair); SM26 Magnetic Stirrer Hotplate (Stuart Scientific); TLP 2844 Printer (Zebra); Nalgene® desiccator cabinet (Thermo Scientific)

2.3 Solubility Determination

The drug in a pMDI formulation is present as either a solution or a suspension dependent on the solubility of the drug in propellants. In the early stage of pMDI formulation development, solubility is an important physical property to determine the feasibility of the drug used in solution or suspension systems (Gupta and Myrdal, 2005). For a suspension pMDI, any dissolved drug could result in crystal growth that leads to aggregation of the suspended drug particles (Dalby et al, 1991). Poor physical stability of the suspension, such as crystal growth, may affect fine particle dose, reproducibility of emitted dose and may even clog the valve and actuator. Ideally, the drug should be totally insoluble in HFA propellants. However, in a pressurised environment, it is difficult to determine the extent of the drug dissolved in propellants by conventional methods. A method for solubility test in HFA propellants was adapted from Williams et al (1999).

The set-up of the device includes a rubber sealed evacuated elution vial, two syringe needles and a syringe filter as shown in Figure 2.1. One syringe needle was used for the alleviation of pressure within the vial, whilst the second needle was used for introduction of the sample from the pMDI canister as described in the following. The pMDI canister was shaken for 10 seconds in an inverted position and primed by firing through an actuator four times. The canister was shaken for another 5 seconds before being fired through a RC 0.20 µm syringe filter coupled with a plastic adapter into the receiving vial under ambient temperature. A 5-second shake was used between each actuation, and in total, 40 actuations for each canister were discharged. Following the actuation of the pMDI, the pressure within the vial was allowed to equilibrate before recovering the sample. Each vial was washed and recovered with 5 mL recovery solution containing an internal standard (Section 2.10.2). The drug concentration in each receiving vial was determined by HPLC and solubility of the drug in HFA propellant was calculated by

dividing the dissolved Tio mass by the propellant volume. The solubility of the drug in HFA system may be dependent on water content, storage temperature and propellant composition (Williams and Hu, 2011). Therefore, sample canisters are stored under accelerated conditions, according to Section 2.9, and the influence of ingress of water, and elevated temperature are further tested in Chapter 5.

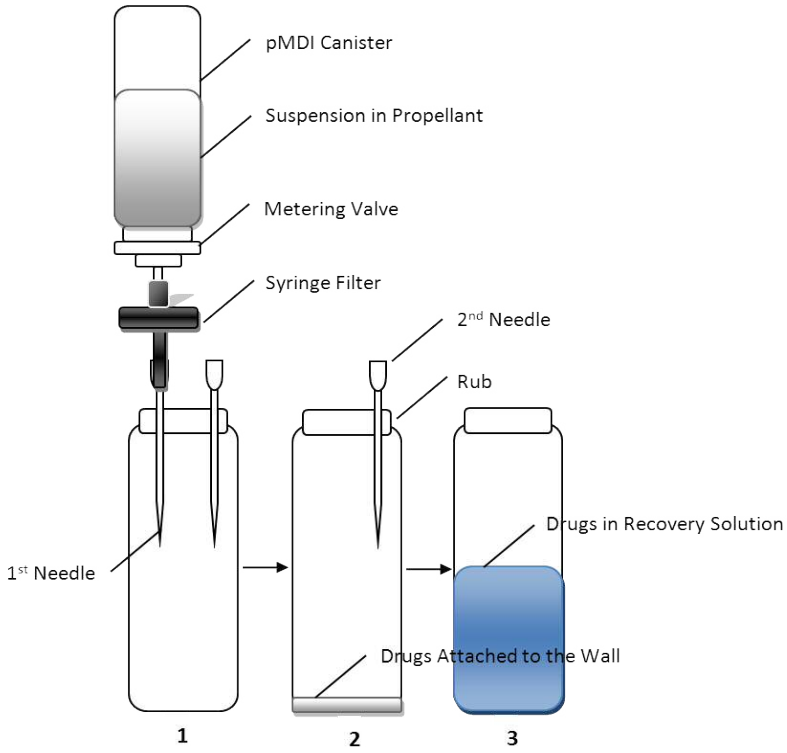

Figure 2. 1 Schematic illustration of determination of the drug solubility test in propellants. The set-up of this system includes a canister, a syringe filter, two needles and a receiving vial. 1: the canister is fired through the syringe filter into the receiving vial. 2: the canister and syringe filter with 1st needle are removed from the receiving vial. 3: the attached drugs on the wall of the receiving vial are recovered by the recovery solution.

2.4 Excipient Preparation and Sieving Process

Pharmaceutical excipients especially in DPI systems are used to improve the dispersing and metering of the API, and increase the powder flow properties (Pilcer and Amighi, 2010). The particle size of carrier excipients is normally in the range of 50 - 200 µm for DPI formulations (Pilcer and Amighi, 2010). According to Dickinson and Warren (2004), the secondary particle size used in pMDI formulation was preferable in the range of 25-125 µm, so inhalable excipients were purchased and sieved into this range. The sieving process could affect the physical characteristics of the powders, e.g. solid state, shape, surface area and density. The different properties of secondary particles could further affect the particle aerosolisation characteristics, e.g. aerodynamic particle size and fine particle mass. Due to the as yet unclear mechanism of secondary particles, the influence of particle size was investigated on the performance of pMDIs.

In this research, several types of excipients were used including L-leucine and lactose (e.g. Respitose® SV003 and SV010). Powdered L-leucine was purchased from Sigma and different fractions were prepared by mechanical vibrating using a M200 minor sieve shaker. The required sieves were stacked on top of the receiver and raw materials were placed on the top sieve of which aperture size was 250 µm. From the top to the bottom, sieves with smaller mesh size were used, e.g. 180 µm, 150 µm, 125 µm, 106 µm, 90 µm, 63 µm and 38 µm. Obtained particle fractions included 250-180 µm, 180-150 µm, 150-125 µm, 125-106 µm, 106-90 µm, 90-63 µm, and 63-38 µm. The operation speed was 3000 vibrations per minute at a frequency of 50 Hz. Each process was operated for 30 min, and several runs were needed until enough powder mass of required sizes had been obtained. Coarse lactose was purchased from DFE Pharma as commercialised products such as SV003 and SV010. SV003 has a narrow particle size distribution (19-106 µm) while SV010 has a relatively broad particle size (35-190 µm). Both types of lactose were

inhalation grade and no further mechanical vibrating was conducted. In the powder blending process described in Section 2.5, excipients were manually sieved through different-size meshes, and this process further reduced the particle size and increased particle numbers. The mesh sizes consisted of 90 µm, 63 µm, 38 µm and 20 µm, and the obtained particle fraction included <90 µm, <63 µm, <38 µm and <20 µm. All the powders generated were stored in glass jars and placed in a Nalgene® desiccator cabinet.

2.5 Dry Mixing and Blending

As two powdered substances were combined as a uniform mixture, the drug homogeneity in powder blends was controlled to assure consistency of the delivered dose, especially for highly potent drugs. Pre-treatment of Tio was necessary to reduce aggregation of micronised particles, and the de-aggregation process was handled by manually sieving through Endecotts® steel sieve with specific mesh sizes e.g. 20 µm, 38 µm or 63 µm. To assure adequate mixing, powders were mixed by trituration, and blended by tumbling. The de-aggregated Tio with excipients was initially mixed on a weighing paper using a spatula, and transferred into a mortar. Equal weight of excipients to the mixture was added and mixed by trituration. The same process was repeated and then the whole blend was tumbled by a rotating chamber (Allen et al, 2006). The specific process was as follows:

To compensate for the potential loss of Tio during the sieving process, Tio was measured out with an overage of 5-7%. The mass of the drug for one batch of pMDIs was measured out, followed by measuring of the mass of the excipient. A weighing paper was placed on the bottom of sieve pan. An equal amount of the excipient to the drug was added on the sieve top and pushed through by a spatula. This process was to de-aggregate large Tio particles and prevent the loss of Tio during the sieving process. A thin film of the excipient was laid on the sieve. The weighed total mass of the drug was added and forced by the same amount of the excipient, followed by another portion of the excipient. The mixture was initially mixed on the weighing paper by a spatula. As the mechanical sieving process or the dry environment could produce static electricity that could lead to adhesion between particles, attraction of dust or loss of the drug, an Exair® ionizing bar was used during the weighing, sieving and mixing processes. Then the mixture was transferred to a glass mortar, and several portions of the excipient equal to the

total mixture mass were added separately and mixed with the mixture until all the excipient had been used. The whole mixture was then transferred to a stainless steel screw cap jar surrounded by bubble paper and mixed by Turbula® mixer at the speed of 46 rpm for 30 minutes initially.

In-process controls of each batch were conducted by a content uniformity test to ensure the effectiveness of the mixing and blending process. Content uniformity testing is required when a drug product contains less than 50 mg of API per dosage form unit or the API is less than 50% of the dosage form unit by weight (FDA, 1999). Five samples equal to the weight of the entire canister content were weighed out in 10 mL beakers under an Exair® ionizing bar. Each beaker was filled with 5 mL methanol, covered by Parafilm® and sonicated for 3 min. The solution was transferred into a 200 mL flask, and the beaker was rinsed with 25 mL methanol and made up to 200 mL with methanol. An aliquot of 0.5 mL of the solution was diluted with 20 µg/mL benzyltriethyl ammonium chloride internal standard solution (BACIS) to make up to 10 mL. Samples were then tested by the HPLC to calculate the amount of tiotropium in each whole-canister dose. Dose uniformity was calculated based on the relative standard deviation (RSD) of drug amounts of the five samples. Extra runs of blending were repeated for batches where the uniformity was over 5%. Content uniformity was preferably less than 3%.

2.6 Particle Size Analysis

There are several particle-sizing techniques such as laser diffractometry, inertial classification, and TOF aerodynamic particle analyser. For the purpose of initial evaluation of raw materials, a Malvern® Mastersizer laser particle size analyser was used, which was based on laser diffraction technology. A He-Ne laser source was applied to generate a monochromatic and parallel beam, which passed through the sample in the sample cell. The beam detector then captured the remaining light beam to measure the scattering patterns, e.g. the intensity of scattered laser beam at different angle. The detected signals were input into a computer for analysing the equivalent volumetric particle size and particle size distribution (Ma et al, 2000). In this research, all the powdered samples including the raw materials, sieved raw materials and powder mixtures, were analysed by a Mastersizer 2000 (Malvern Instruments, UK) in a dry dispersion cell. As the dry powder forms tend to segregate due to differences in particle size, shape and density, which could lead to inaccurate measurement, all the raw materials were thoroughly mixed by inversion of the containers, and mixture materials were blended uniformly by Turbula® mixer before measurement. A minimum of three measurements were required for each sample under the standard operating procedure (SOP) of pre-set dry dispersion mode. Background measurement or blank runs were conducted 3 times in between different samples to avoid background electrical noise and scattering signals from dusts and contaminations. The measurement settings are shown in Table 2.1. The optical properties of the particles, e.g. particle refractive index and absorption values, were set as the default values, and the Scirocco 2000 module was utilised for dry dispersion.

Table 2. 1 Measurement Settings for Laser Diffraction Test by Mastersizer 2000

SOP	Module	Particle RI	Absorption	Size (µm)	Result Units
Dry Dispersion	Scirocco 2000	1.52	0.1	0.02-2000	Volume

(SOP: standard operating procedure as a dry dispersion, Module: a dry powder dispersion module of Scirocco 2000 is applied. Default values set for particle information e.g. refractive index (RI) as 1.52 and absorption as 0.1.)

Table 2. 2 Particle Size Information from the Certificate of Analysis (COA)

Sample	d(0.1) (µm)	d(0.5) (µm)	d(0.9) (µm)
Tiotropium	N/A	N/A	6.8 (*d0.97)
Respitose® SV003	29	57	88
Respitose® SV010	54	112	182

(d (0.1): particle size under which 10% of particles lie, d(0.5): particle size under which 50% of particles lie, d(0.9): particle size under which 90% of particles lie.* d(0.97): particle size under which 97% of particles lie)

The particle size information of the raw materials including tiotropium, Respitose® SV003 and Respitose® SV010 from the certificates of analysis (COA) is shown in Table 2.2. The measured values are illustrated in Table 2.3. The d(0.9) of Tio particle size is 6.16 µm, and there is not much difference with the production value as the COA document stated 97% < 6.8 µm. It was noted that d(0.5), or the mass median diameter (MMD) of Tio was 2.80 µm, i.e. in the range of respiratory delivery of 1 - 5 µm. The d(0.9) of SV003, 90.55 µm, is comparable to the COA value, 88 µm, but the d(0.1) of 15.38 µm was over 45% lower than the COA value. In the case of SV010, it is noted that the d(0.5) of 94.72 µm is nearly double the size of SV003 at 48.38 µm. Weighted residuals obtained from the particle size distribution of all these samples are less than 1%, which imply the correct use of the default refractive index and absorption values, and the calculated data is relevant to the measurement data. The span is a measurement of the width of the particle size distribution, and calculated by equation (5):

$$Span = \frac{d(0.9) - d(0.1)}{d(0.5)} \quad (5)$$

As shown in Table 2.3, the span of SV010, 1.62, indicated a wider distribution than SV003, 1.55. The level of signals is monitored by the obscuration of the laser beam, which represent the fraction of laser light loss from the mean beam due to the introduction of the sample. The obscuration level demonstrates the feed rate of the sample but due to the limited amount of samples, the obscuration values are all lower than 3%.

Table 2. 3 Particle Size Analysis of Raw Materials (n=3; mean ± SD)

Sample	d(0.1) (µm)	d(0.5) (µm)	d(0.9) (µm)	Obscuration	Span	Residual
Tiotropium	1.02 ± 0.01	2.80 ± 0.01	6.16 ± 0.14	1.46%	1.84	0.65%
Respitose® SV003	15.38 ± 0.15	48.37 ± 0.04	90.55 ± 0.01	1.09%	1.55	0.81%
Respitose® SV010	29.89 ± 0.58	94.72 ± 0.29	183.33 ± 0.48	1.13%	1.62	0.87%

(Obscuration: Loss of the mean beam caused by samples, Span: the width of the distribution, Residual: weighted residual which is defined by the manufacturer to indicate the relevance between the calculated and measured data)

The respiratory size range of particles is 1 to 5 µm, and the recommended diameter of the secondary particles is in the range of 25 – 125 µm. It is found that all these raw materials fulfil the criteria of particle size for pulmonary drug delivery. SV010 was identified with a larger particle size and wider particle size distribution than SV003. Furthermore, the settings of the dry dispersion measurement are qualified for the evaluation of the raw materials used in this research. The same procedure and instrument settings are employed in Chapter 3, 4 and 5 and any deviation is notified in specific sections.

2.7 Filling Process

There are two basic types of filling processes for manufacturing and filling pMDIs, e.g. pressure fill where propellants liquefy under pressure and cold fill where propellants liquefy under low temperature. In this research, HFA 134a and HFA 227 were mainly filled by pressure fill, and some batches of HFA 227 by cold fill, which was specified in the methods section of the relevant chapters. For pressure fill, a simple two-stage pressure filling process was applied in manufacturing of pMDIs on a laboratory scale. Each testing canister was labelled and labels were printed by Zebra® TLP2844 printer. Weights of the valve and canister, drug mixture, propellant, and final canister weight were recorded in each filling process. Drugs or blends of the drug and excipient were weighed manually under an ionizing bar and filled into a vessel, e.g. H&T Presspart® aluminium canister and polyethylene terephthalate (PET) bottles. Crimp height and diameter were adjusted depending on the size of the vessel and the valve. Valves were then crimped on the vessel and liquefied propellants were filled through the valve under pressure by two-stage Pamasol® filling equipment (Figure 2.2). As the density of the propellant is affected by the room temperature, filling weight of the propellant was monitored in process. Each canister was weighed before and after propellant filling, and the propellant weight should be kept in the range of ± 1% of the target filling weight (HFA 134a: 4.75 g/ HFA 227: 5.46 g). A cold filling technique was used as an alternative filling option when a small batch (<10) of canisters required a different propellant with the currently applied propellant in the filling line, as described in Section 4.4.3. Vessels filled with the drugs or blends and vessels containing liquefied propellants were cooled down by the cooling agent, e.g. a mixture of dry ice and acetone. Under low temperature, liquefied propellants were transferred into the empty vessels and valves crimped as quickly as possible to avoid the loss of propellants. Due to the condensation of moisture at low temperature, nitrogen gas was normally used to prevent the moisture increase in the formulation. Once pMDIs were manufactured, post-production weights were recorded, and seal properties were determined by

weight difference test between post-production weight and pre-test weight. pMDIs were placed in inverted positions at room temperature for at least three days before testing to allow the swelling of the valve materials, e.g. elastomers and fit with the propellant environment.

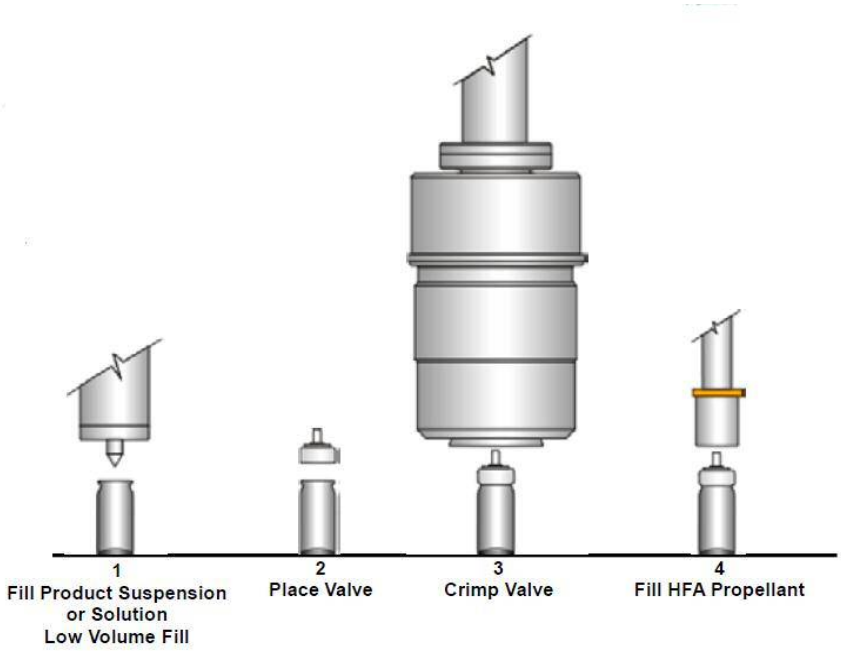

Figure 2 2 Schematic illustration of two-stage filling process. 1: product suspensions or solutions are filled through the product filler. As no co-solvent is used in this novel formulation, the dry powder is filled into canisters manually instead of through the product filler. 2: the valve is placed on the canister, and the height of crimp head is adjusted accordingly. 3: the valve is crimpled on the canister by the crimp head. 4: the HFA propellant is filled into the canister through the valve.

2.8 Performance Tests of pMDIs

The performance of pMDIs is highly dependent on the consistency of emitted dose and particle size distribution, especially the fine particle fraction (FPF), from each actuation. According to the British Pharmacopeia (2017a, 2017b), for pMDI products, uniformity of delivered dose can be analysed by the dose collection apparatus (DUSA), and the fine particle characteristics of the aerosol clouds can be analysed by the Next Generation Impactor (NGI). These performance tests were validated by testing samples spiked with known amounts of Tio.

2.8.1 DUSA/NGI Validation

The aim of the spike test was to verify the consistent measurement of the recovery method for uniformity and distribution tests. No drug interaction with the equipment surface should happen that could affect the recovery process. Known amounts of Tio were coated onto the surface of DUSA and NGI components, and this needed to be recovered by the same volume of recovery solution to that of the in-vitro tests described in Section 2.8.2 and 2.8.3. The Tio stock solution (200 µg/mL) was prepared by dissolving 0.1 g of Tio in 100% methanol, sonicated and filled to the volume in a 50 mL volumetric flask. Certain volumes (Table 2.4) of the stock solution were transferred by Gilson® pipette to give an either 10 µg or 4 µg deposition on the surface of testing devices. For the control group, the stock solution was spiked in a 25 mL flask and dried off in a drying cabinet at 50°C. For equipment components, the stock solution was spiked on the surface of DUSA cap or tube cup (Figure 2.4), DUSA tube or collection tube (Figure 2.4), and NGI plates including both standard and large plates, and dried off in drying cabinet at 50°C. All the flask and components were cooled down to room temperature, and an amount of recovery solution, referred to Section 2.10.2, was applied to recover the deposited drug.

Table 2. 4 Spike Volume and Recovery Volume for Different Equipment Components

Sample	Control	DUSA Cup	DUSA Tube	NGI Plate S	NGI Plate L
Spike Volume (µL)	50	50	50	20	20
Recovery Volume (mL)	25	25	50	10	10

(NGI Plate S: standard NGI plate, NGI Plate L: large NGI Plate. 50 µL of the stock solution equivalent to 10 µg Tio is spiked on DUSA components and 20 µL of the stock solution equivalent to 4 µg Tio is spiked on NGI plates. 25 mL of the recovery solution are used to recover Tio from DUSA cup, while 50 mL for DUSA tube and 10 mL for NGI plates.)

The recovered Tio was analysed by HPLC and data are shown in Figure 2.3. The percentage of recovery was calculated by dividing the recovered Tio mass with the spiked mass. The control group was shown to recover 99.42 ± 0.57% of the spiked Tio which is in the range of ±1% of the target. Although relatively high standard deviations were found in the DUSA tube (98.00 ± 2.13%) and the large NGI plate (100.64 ± 1.87%), component groups could recover 98% to 101% of the spiked Tio. There was no significant difference between control group and component groups (ANOVA, $P > 0.05$). It can be assumed that there is no interaction between Tio and the surface material of the equipment components, and the recovery volumes and recovery process for each component are workable, both of which assure the recovery accuracy.

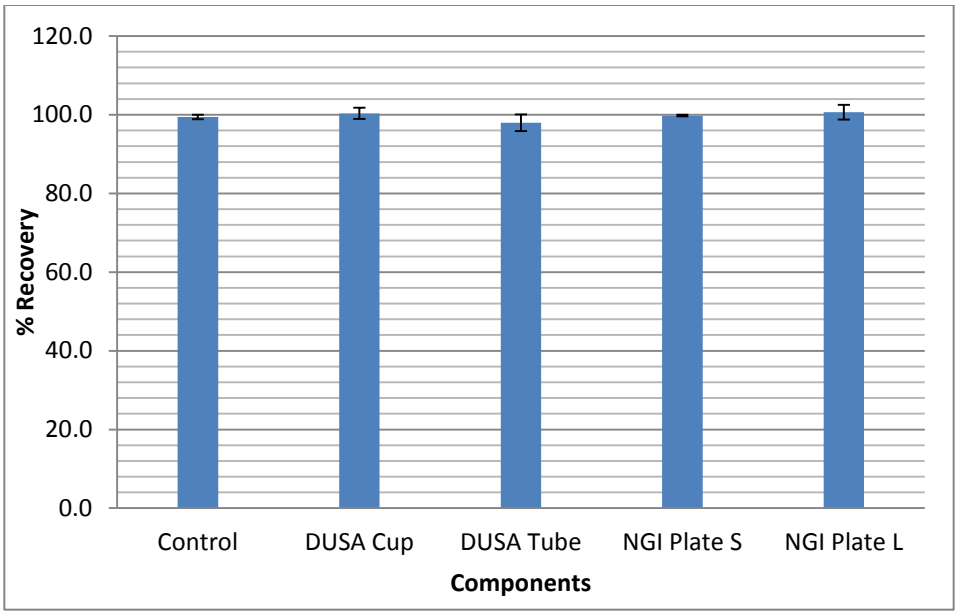

Figure 2. 3 Recovery efficiency of Tio from different components. Control: 99.42 ± 0.57%, DUSA Cup: 100.36 ± 1.44%, DUSA Tube: 98.00 ± 2.13%, NGI Plate S: Standard NGI plate 99.78 ± 0.20%, NGI Plate L: Large NGI Plate 100.64 ± 1.87%. (n=3; mean ± SD)

2.8.2 Emitted Dose Uniformity Test

For pulmonary delivery, batch-to-batch consistency is crucial. Suspension formulations are prone to heterogeneity where particle interaction, phase separation, flocculation, agglomeration or moisture ingress can occur over the life of the pMDI (O'Donnell and Williams, 2013; Myrdal et al, 2014). Dose inconsistency between batches and in batches over canister life can raise the concern of variability in clinical efficacy. Consequently, uniformity of delivered dose by pMDIs is required to be measured by a DUSA. The DUSA comprises a collection tube, a mouthpiece and mouthpiece adaptor, a filter support base and a vacuum connector (Figure 2.4). The apparatus was connected to a vacuum source and a flow rate regulator and meter. The continuous and consistent airflow was kept at 28.3 L/min within a 5% range (British Pharmacopoeia, 2017a). When the system was ready, each inhaler was shaken vertically using a stroke length of 6 inches and a frequency of 3 shakes per second for 5 seconds. One actuation was discharged into the fume hood using a separate actuator, and the inhaler was hold in the actuated position for 5 seconds. Quickly release the canister, and repeat the actuation cycle for further 3 actuations. . The valve stem was cleaned by methanol and the canister was seated into a new actuator. The canister and the total device including the actuator were weighed before and after discharging. The inhaler was shaken again by the same manoeuvre above for another 5 seconds and set into the mouthpiece adapter. The inhaler was discharged once with the valve depressed for 5 seconds to ensure a complete discharge, and then the pump was switched off. The drug was recovered from the actuator, the DUSA and the filter separately. 25 mL of recovery solution was needed for each of these three parts. For the canister life uniformity test, each canister was discharged at the beginning (shots 5, 6, 7), middle (shots 59, 60, 61, 62) and end (shots 118, 119, 120). In between the beginning, middle and end of the canister life, the canister was discharged to waste following the sequence that one shot was actuated every 10 seconds of continuous shaking. The canister weight was recorded every 20 actuations.

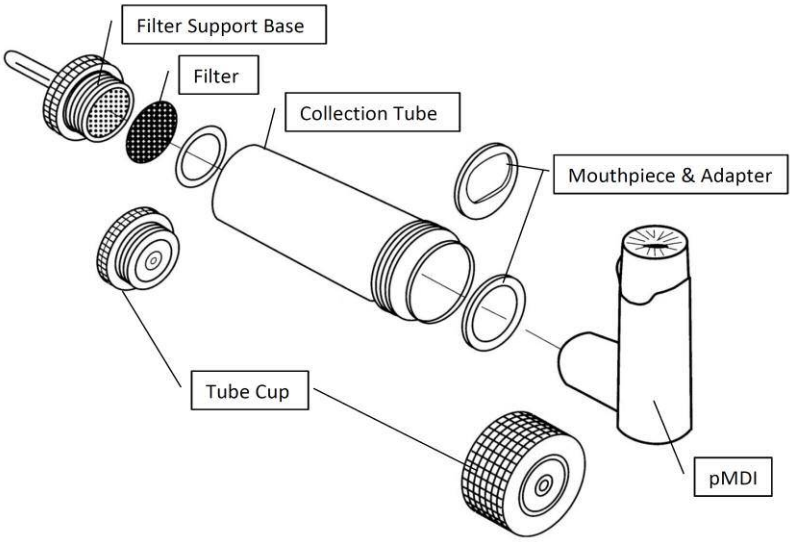

Figure 2. 4 Modified configuration of DUSA (British Pharmacopoeia, 2017a). A DUSA system consists of a DUSA tube or collection tube, mouthpiece. Adapter, filter and filter tube. The DUSA cup or tube cup is applied during the recovery process.

2.8.3 Aerosol Particle Size Measurement

Various sizing techniques, e.g. inertial classification, laser diffractometry and time-of-flight aerodynamic particle size analyser are available. The European Pharmacopoeia (Ph. Eur.) and United States Pharmacopoeia (USP) approved method of inertial classification most widely uses cascade impaction, e.g. the Andersen Cascade Impactor (ACI) and the Next Generation Impactor (NGI). In this research, an in-vitro deposition test of pMDIs was performed using the NGI, which was designed specifically for pharmaceutical inhaler testing (Marple et al, 2003b). It consists of an induction port (IP), a pre-separator, seal body (collection cups, a cup tray, a bottom frame and a lid), a clamp, an airflow outlet and filter holder (Figure 2.5). The pre-separator was applied for DPI products to retain large excipient particles without affecting the size distribution of the active pharmaceutical ingredients (API), which are specified in Chapter 3.

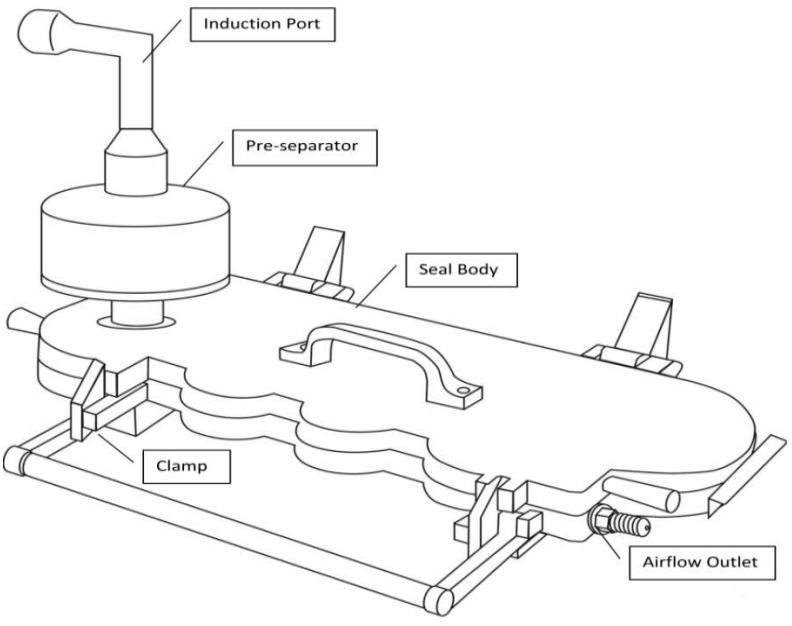

Figure 2. 5 Modified configurations of NGI apparatus (British Pharmacopoeia, 2017b). A NGI system includes the induction port, pre-separator, seal body and airflow outlet. The air flow passes through the induction port into the NGI body and out from the airflow outlet. The particles are captured inside the NGI body and classified into different size fractions.)

The NGI operates on the principle of inertial impaction whereby particles are separated by particle size and velocity. Eight nozzle pieces are identified including seven size-fractionation stages and a micro-orifice collector (MOC) (Figure 2.6). During the operation, the flow rate through the whole impactor is constant. The aerodynamics in each impactor stages are defined by Reynolds number, nozzle-to-plate distance and cross-flow parameter, which determines the cut-off size of the impactor stage (Marple et al, 2003b). The nozzle diameters of the seven stages and effective cut-off diameters (ECD), which were expressed as d50 values to give a meaningful definition of the size distribution with minimal stage overlap, are illustrated in Table 2.5. The NGI has been calibrated in the range of 30 L/min to 100 L/min, and the d50 value under certain volumetric flow rates (Q) is calculated by equation (6):

$$d_{50,Q} = d_{50,60L/min} * \left(\frac{60}{Q}\right)^{X} \quad (6)$$

$d_{50,Q}$: d50 value at the flow rate of Q; Q: the volumetric flow rate, X: factors described in Table 2.5 (Marple et al, 2003a)

Table 2. 5 Parameters for Calculating d50 Values between 30 L/min and 100 L/min (d50 values under certain volumetric flow rate is calculated by equation (6), which is based on the d50 values at 60 L/min.)

Stage	d50 at 60 L/min (μm)	X
1	8.06	0.54
2	4.46	0.52
3	2.82	0.50
4	1.66	0.47
5	0.94	0.53
6	0.55	0.60
7	0.34	0.67

An extension of calibration of the NGI was also undertaken at 15 L/min and d50 values are listed in Table 2.6 (Marple et al, 2004). According to the British Pharmacopoeia (2017b), pressurised inhalers are required to be measured at 30 L/min ± 5%. The calculated d50 values at 30 L/min are also shown in Table 2.6. Extreme fine particles are collected on the MOC and there is no specified diameter for this stage (< 0.5 μm).

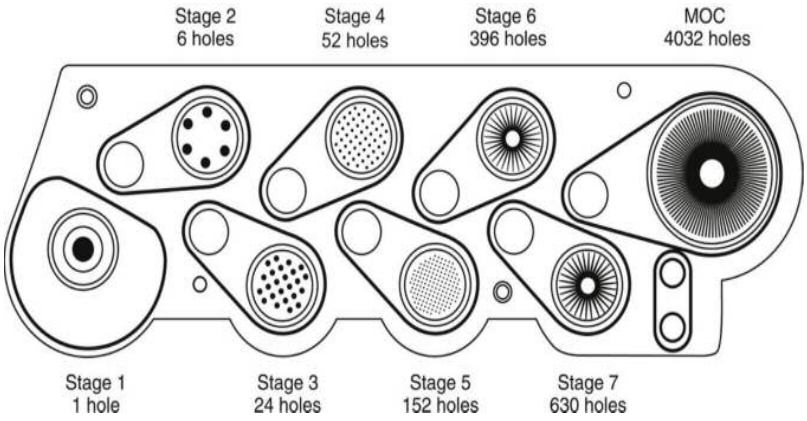

Figure 2. 6 Configurations of NGI Apparatus

(British Pharmacopoeia, 2017b)

Table 2 6 Effective Cut-off Diameter for NGI at Different Flow Rates (Marple et al, 2004)

Stage	Nozzle Diameter (mm)	d50 at 15 L/min (μm)	d50 at 30 L/min (μm)
1	14.3	14.1	11.72
2	4.88	8.61	6.40
3	2.185	5.39	3.99
4	1.207	3.30	2.30
5	0.608	2.08	1.36
6	0.323	1.36	0.83
7	0.206	0.98	0.54

Before the operation, impactor cups need to be coated by a high viscosity liquid to avoid the particle bounce and ensure the efficient capture, in this research 0.2%

(v/v) 400-grade PEG in acetone was applied. The coated impactor cups were placed in the cup tray and set into the bottom frame, and the impactor was then locked. The airflow outlet was connected with a pump, and the pump was switched on. The flow rate was kept at 30 L/min within a 5% range, and the system was ready to use (British Pharmacopoeia, 2017b). Each inhaler was shaken for 5 seconds as described in Section 2.8.2 and one actuation was then discharged into a fume cabinet using a separate actuator, and this process was repeated for a further 3 times to prime the canister. A suitable mouthpiece adaptor was placed into the induction port. An inhaler was set into the adapter, and discharged for 5 seconds. Then the pump was switched off, and switched back on when the flow rate dropped to zero. The procedure was repeated a further 4 times. Then the tray containing receiving cups was placed onto the NGI gentle rocker. 5 mL recovery solution was poured in Stage 1, 2, 6, 7 and MOC, and 10 mL in Stage 3, 4, 5 separately. Then the tray cups on the rack were placed on the NGI gentle rocker to shake for 5 minutes and the sample solutions were filled into HPLC vials. The induction port and the actuator were recovered by 50 mL and 25 mL recovery solution respectively.

2.9 Stability Test

According to regulatory authority guidance for industry, stability studies should be performed under certain conditions that a drug substance is susceptible to change during the storage (FDA, 2003). The choice of validated analytical procedures, e.g. storage conditions and storage length is specified in Table 2.7.

Table 2. 7 General Storage Conditions and Length of Conditions Specified by Regulatory Authorities (FDA, 2003)

Study	Storage Condition	Minimum Time Period Covered
Long Term	25°C ± 2°C/60% RH ± 5% RH Or 30°C ± 2°C/65% RH ± 5% RH	12 Months
Intermediate	30°C ± 2°C/65% RH ± 5% RH	6 Months
Accelerated	40°C ± 2°C/75% RH ± 5% RH	6 Months

In this research, the stability study is performed under accelerated conditions. The pMDI products were stored in an inverted position in Vindon® stability storage cabinets under accelerated storage conditions, e.g. 40°C/75% RH for periods up to six months. At the time points such as 0, 1, 2, 3 and 6 months, six canisters were randomly selected for testing. In-process controls included moisture and canister content testing. Three canisters at each time point were used for a canister content test. The remaining three canisters were used for the emitted dose uniformity test referred to in Section 2.8.2 and aerosol particle size measurement referred to in Section 2.8.3. The canister content test was conducted to ensure the dose consistency and indicate degradation of Tio under accelerated conditions. The procedure is further described in Section 5.4.3 in Chapter 5. Moisture testing was conducted using a Metrohm® Karl Fisher coulometer at the beginning of canister life. Each canister was shaken for five seconds and fired into the titration vessel five times in rapid succession. Moisture values were calculated in parts per million (ppm)

automatically, and each canister was tested in triplicate, where the RSD of the three readings was higher than 10%, further repetitions would be performed.

2.10 HPLC Assay of the Drug

HPLC is designed for providing high resolution and efficient analysis of target substances in mixtures, even similar analytes, in a short time. As a tool for both qualitative and quantitative analysis, target peaks can be identified, and peak area and height of each signal are proportional to the amount of the corresponding substance (Meyer, 2013). In this research, HPLC (Agilent, 1200 Series) was applied to quantify the tiotropium recovered in canister content tests, uniformity tests and particle size measurements. The method was based on published analytical methods (Ding et al, 2008; Amighi and Guerra, 2011). Method validation was subject to the International Council for Harmonisation (ICH) guideline Q2 (R1) for validation of analytical procedures text and methodology (ICH, 2005).

2.10.1 Chromatographic Conditions

The HPLC method (Table 2.8) was modified from the HPLC/ESI/MS method for Tio determination in human plasma (Ding et al, 2008) and the HPLC method for Tio determination in spray-dried formulations (Amighi and Guerra, 2011). The optimisation aims for modifying the HPLC method were to shorten the total sample analysis time, decrease the size of the solvent front peaks, and achieve good resolution between peaks of solvent, internal standard and Tio. The final sample run time was controlled at 8 min. An internal standard was used in this research to compensate for condensation of Tio recovery solution caused by solvent evaporation during time-consuming tests. An internal standard should have similar chemical characteristics with the target compounds and not be expected to exist in the sample. In these experiments, benzyltriethyl-ammonium chloride (BAC) was used and the optimised HPLC parameters are illustrated in Table 2.7 and a sample of the HPLC chromatogram of Tio and BAC is presented in Figure 2.7.

Table 2. 8 HPLC Parameters

HPLC System	Quaternary Pump G1311A, Multiple Wavelength Detector G1314B, Autosampler G1329A, Thermostatted Column Compartment G1316A, Degasser G1322A Isocratic Pump G1310A, Variable Wavelength Detector G1314B, Autosampler G1329A, Thermostatted Column Compartment G1316A
HPLC Column	Genesis SB-C18, 150 × 4.6 mm, 4 µm Column Temperature: 35°C
Mobile Phase	100 mM Potassium dihydrogen orthophosphate buffer: Methanol (65:35, v/v), adjust to pH = 4 by Phosphoric acid
UV Wavelength	238 nm
Flow Rate	1.0 mL/min
Injector	Injection Volume: 100 µL Injector Air Temperature: 5°C
Run Time	8 min
Retention Time	Benzyltriethyl ammonium chloride: 2.4 min Tiotropium: 6.5 min

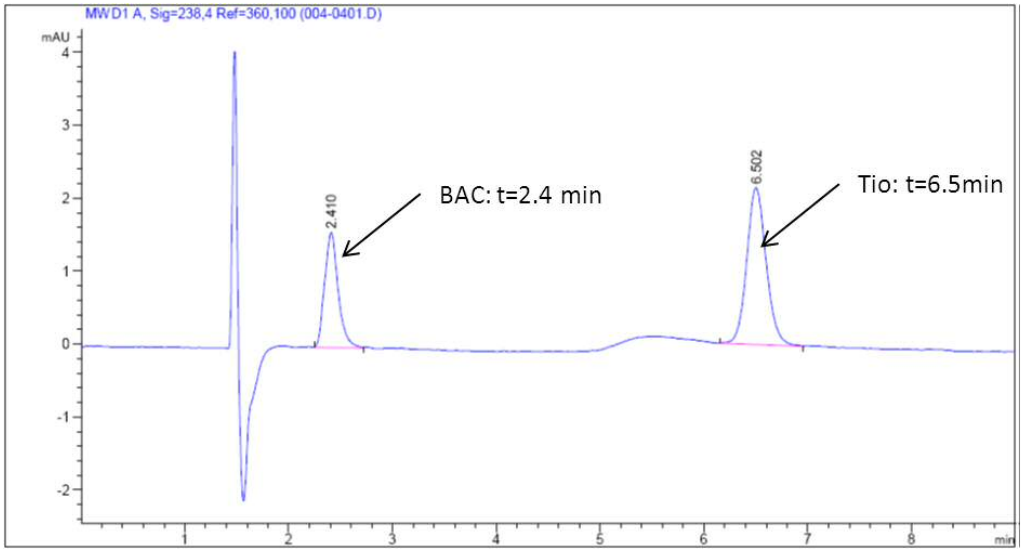

Figure 2. 7 A sample of HPLC chromatogram of the solution containing 0.16 µg/mL Tio and 20 µg/mL BAC **(BAC: t=2.4 min, Tio: t= 6.5 min)**

2.10.2 Stock and Recovery Solution Preparation

The stock of BAC (2 mg/mL) was prepared by dissolving 0.1 g of BAC in 100% methanol, sonicating and adjusting the volume to 50 mL in a volumetric flask to produce a concentration of 2 mg/mL. The recovery solution, benzyltriethyl ammonium chloride internal standard solution (BACIS), was prepared by diluting the stock of BAC, to produce a concentration of 20 µg/mL in 65:35 (v:v) water: methanol. The required amount of the BAC stock (10 mL for 2L BACIS) was measured by a graduated glass pipette fitted with a quick release pipette filler. The stock solution was transferred into a 1000 mL cylinder, and methanol was added to make up to 700 mL. The total content was transferred into a 2 L reagent bottle. 1300 mL of HPLC grade water was then measured by the same cylinder and transferred into the reagent bottle. The solution was completely mixed by inversion and kept at 2-4°C for no more than one week after preparation. BACIS was used to prepare Tio standard and calibration solutions, and as the recovery solution to retrieve Tio from canisters, DUSAs and NGI plates.

2.10.3 Standard Stock and Standard Solution Preparation

The Tio standard stock (200 µg/mL) was prepared by dissolving 0.01 g of Tio in 100% methanol, sonicated and completed to the volume in a 50 mL volumetric flask to produce a concentration of 200 µg/mL. Tio standard solution was then prepared by diluting the Tio standard stock with BACIS to produce a Tio concentration of 0.40 µg/mL and a BAC concentration of 20 µg/mL. All the Tio stocks and Tio standard solutions were kept at 2-4°C for up to one week.

2.10.4 Buffer and Mobile Phase Preparation

100 mM potassium-dihydrogen-orthophosphate buffer was prepared by dissolving 17.69 g potassium-dihydrogen-orthophosphate in 1300 mL of HPLC grade water, which was adjusted with o-phosphoric acid to pH 4 within 1% limit. The buffer was mixed with 700 mL methanol, and was further filtered and degassed through 0.2 µm-nylon membrane filter.

2.10.5 Validation of HPLC Method

2.10.5.1 Specificity

Specificity indicates the discrimination between drug candidates and potential compounds with related structures and critical separation should be achieved with suitable resolution between compounds (ICH, 2005). There are no available impurity samples and degradation standards. The specificity can be supported by the comparison of the chromatogram of the standard solution and the pharmaceutical formulation (Elkady and Fouad, 2011). The recovery solution was prepared according to Section 2.10.2. The tiotropium European Pharmacopoeia reference standard is prepared as the reference standard solution, where 10 mg of the reference Tio was dissolved in 50 mL methanol as the stock solution, and 100 µL of the stock is diluted by the recovery solution to make a Tio concentration of 0.4 µg/mL. The novel formulation containing Tio: SV003 (w/w) 1: 5 was blended as described in Section 2.4 and 2.5. The blended powder was weighed to give an amount of 10 mg Tio, and dissolved in 50 mL methanol and diluted by recovery solution to make a Tio concentration of 0.4 µg/mL. The identical chromatogram between the reference solution and the pharmaceutical formulation confirms the specificity of the adapted HPLC method (Figure 2.8).

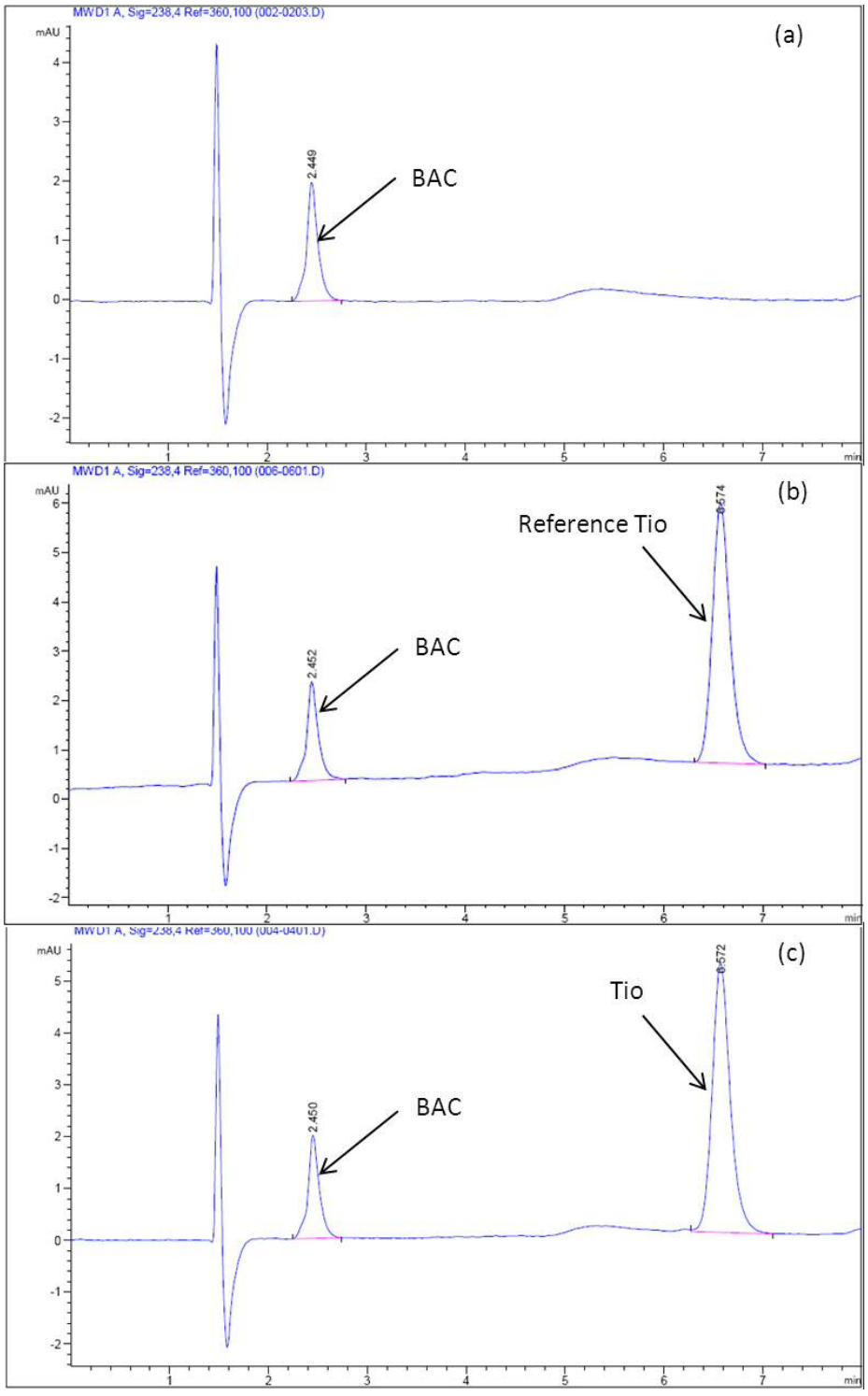

Figure 2. 8 Identification of Internal Standard and Tio Peaks in Reference Tio Standard Solution and Pharmaceutical Formulation ((a) BAC: t=2.45 min; (b) Tio Standard Solution: BAC: t=2.45 min, Tio: t=6.6 min; (c) Novel Formulation Tio: SV003 (w/w) 1:5: BAC: t=2.45 min, Tio: t=6.6 min)

2.10.5.2 Linearity

A linear relationship should be demonstrated by a plot of signals or signal ratios as a function of analyte concentration or contents, and a minimum of five concentrations is recommended (ICH, 2005). Eight concentrations of Tio solutions were prepared that covered the concentration range from 2.00 µg/mL to 0.02 µg/mL. 10 mg of Tio was weighed in a 10 mL beaker, and dissolved in 5 mL methanol, using sonication. The content was transferred to a 10 mL flask and methanol was then used to rinse the beaker and make up to 10 mL, to produce a concentration of 1 mg/mL. The stock solution was diluted with BACIS to make a concentration of 2.00 µg/mL, and this was further diluted with BACIS to produce concentrations of 1.20 µg /mL, 1.00 µg/mL, 0.80 µg /mL, 0.40 µg /mL, 0.16 µg /mL, 0.08 µg /mL and 0.02 µg /mL. For each concentration, three samples were prepared and run through the adapted HPLC method. To evaluate the linear response, the mean response ratio of Tio/BAC was plotted against the corresponding theoretical concentrations. Large correlation coefficient indicated good degree of linearity between response ratio of Tio/BAC and Tio concentration, and the analytical data of the calibration curve is illustrated in Figure 2.9.

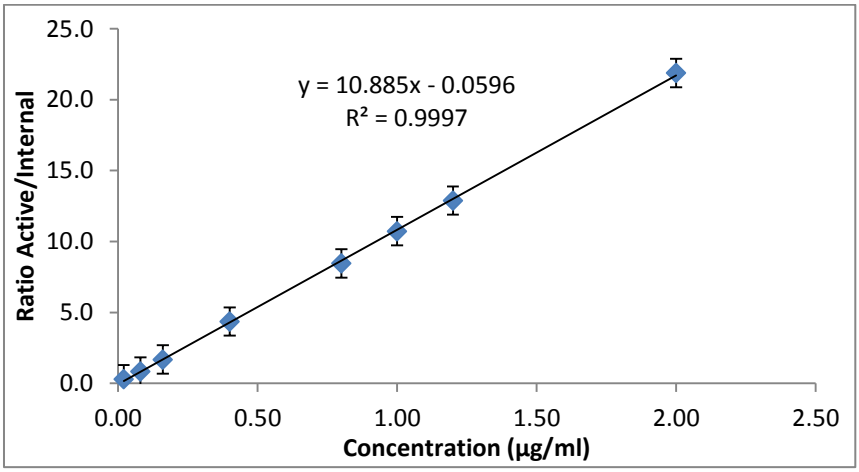

Figure 2. 9 Calibration curve of Tio standard solution (slope of linear regression: 10.89, y intercept: -0.06, correlation coefficient: 1.00) (n=3, mean ± SD)

2.10.5.3 Precision

The precision test aims at expressing the closeness of agreement between a series of measurements obtained from multiple sampling from the same homogenous sample or artificially prepared sample solution under the prescribed conditions (ICH, 2005). Three concentrations (0.04, 0.40 and 1.20 µg/mL) were chosen in the range 0.02 to 2 µg/ml. Each vial was injected six times by HPLC and the average peak-area ratio of Tio: BAC is illustrated in Table 2.9. The average ratios are 0.44 ± 0.01 (0.04 µg/mL), 4.27 ± 0.03 (0.40 µg/mL) and 12.83 ± 0.09 (1.20 µg/mL) separately, and relative standard deviations were all lower than 3%, the limit set for this method.

Table 2. 9 Precision Test Based on Three Selected Concentrations

Concentration (µg/ml)	Sample Peak Areas		Ratio Tio/BAC	Average Ratio	SD	RSD (%)
	Tio	BAC				
0.04	6.41	14.75	0.44	0.44	0.01	2.66
	6.44	15.46	0.42			
	6.71	14.81	0.45			
	6.37	14.61	0.44			
	6.40	14.73	0.43			
	6.47	14.70	0.44			
0.40	62.44	14.56	4.29	4.27	0.03	0.65
	63.67	14.88	4.28			
	62.14	14.56	4.27			
	63.00	14.63	4.31			
	62.90	14.77	4.26			
	62.02	14.68	4.23			
1.20	187.12	14.64	12.78	12.83	0.09	0.70
	187.03	14.46	12.93			
	187.03	14.66	12.76			
	186.69	14.57	12.81			
	187.19	14.46	12.94			
	186.81	14.66	12.75			

2.10.5.4 Accuracy

Based on a conventional true value or an accepted reference value, the accuracy means the closeness of agreement between that predetermined value and the value found (ICH, 2005). As the specificity, precision and linearity of this method have been established, accuracy can be inferred.

2.10.5.5 Detection Limits and Quantitation Limits

The limit of detection (LOD) represents the minimum amount of analytes that can be detected and the limit of quantitation (LOQ) represents the minimum amount that can be quantitatively determined. According to ICH guidelines (2005), the detection and quantitation limit can be determined by visual evaluation, signal-to-noise or based on the standard deviation of the response and the slope. In this research, the test for the LOD and LOQ were estimated based on the calibration curve. The calculation was based on the following Equation (7) and Equation (8). As shown in Table 2.10, the LOD and LOQ of the analytical procedure were 0.041 µg/mL and 0.126 µg /mL respectively. The quantitation range of this analytical method was therefore between 0.126 and 2.000 µg/mL. For pulmonary delivery, the dose is low and normally less than a few milligrams or even just several micrograms for potent drugs, e.g. the Tio dose is 22.5 µg as bromide salt form in the Spiriva Handihaler® capsule and only 6.25 µg in bromide form in Respimat®. For accurate quantitation of Tio during the in-vitro test, a minimum of 5 actuations were required for the NGI test. Though it was found that some samples recovered from Stage 7 and MOC of the NGI exhibited lower concentration than the LOQ, the small amount of the Tio (<1% of the total emitted mass) was considered negligible.

The LOD was expressed as:

$$LOD = \frac{3.3\sigma}{S} \quad (7)$$

Where σ = the standard deviation of the response

S = the slope of the calibration curve

The LOQ was expressed as:

$$LOQ = \frac{10\sigma}{S} \quad (8)$$

Where σ = the standard deviation of the response

S = the slope of the calibration curve

Table 2. 10 Calibration Statistics for Tio Assay

Slope Coeff	10.885	Intercept Coeff	-0.060
St.error of slope	0.076	St. error of intercept	0.072
R-Sqaured	1.000	St. error of regression	0.137
F-Test Overall	20707.317	Degrees of freedom	6
Regression SS	387.219	Residual SS	0.112
LOD (µg/ml)	0.041	LOQ (µg/ml)	0.126

2.11 Data Analysis

For manipulation of data generated from the dose uniformity test, two parameters are calculated, the ex-valve emitted dose and ex-actuator emitted dose. The ex-valve emitted dose refers to the dose metered by the metering valve, or the total emitted dose per actuation, which is calculated by addition of the mass recovered from each component. Ex-actuator emitted dose refers to emitted dose through the actuator, which is calculated by addition of the mass recovered from all the components except the actuator. Actuator deposition is calculated by the result of ex-valve emitted dose minus ex-actuator emitted dose divided by ex-valve emitted dose. For each batch of pMDIs, three or five randomly selected canisters were tested at the beginning, middle and end of the canister life. 10 actuations were discharged from each canister, and the average delivered doses including either the ex-valve dose or ex-actuator dose were plotted against the actuation number. For the purpose of quality control, if three canisters were selected for testing, and no more than 3 of the 30 values lie outside ±25% of the target value, and no values lie outside 35% of the target value (British Pharmacopoeia, 2017a). This criteria is used by regulatory authorities i.e. Medicines and Healthcare products Regulatory Agency (MHRA) for approval of inhalation products to determine the safety and efficacy, whist in this research, it is used as an indicator of the formulation stability and quality. The target ex-valve emitted dose is 11.25 µg, and the ex-valve dose distribution is plotted as shown in Figure 2.10. The ex-actuator emitted dose is 6.25 µg, and the ex-actuator dose distribution or canister life content uniformity is plotted as shown in Figure 2.11.

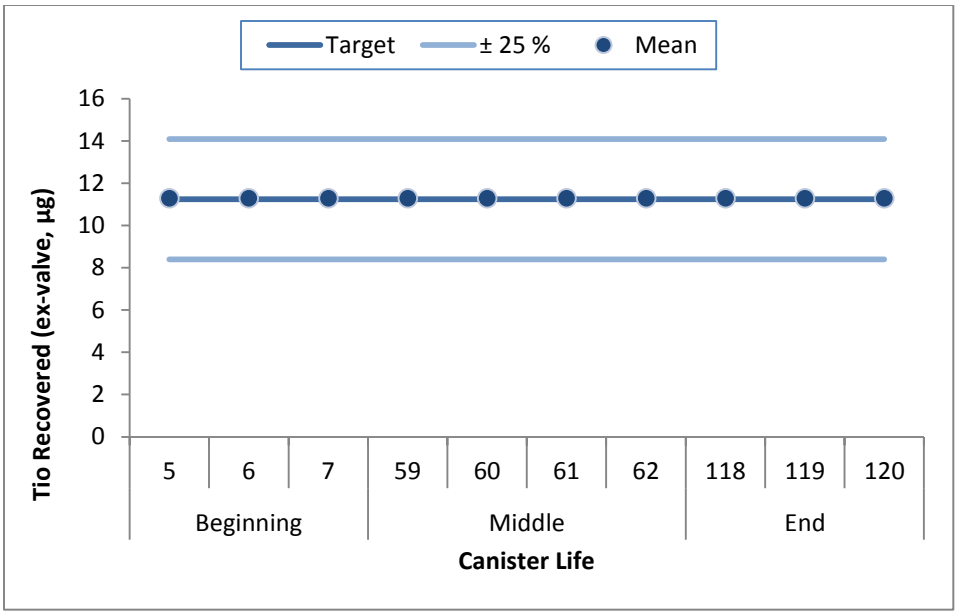

Figure 2. 10 Example of ex-actuator emitted dose distribution. The ex-valve dose is plotted against the actuation number. No more than 3 of the 30 values lie outside the ± 25% of the target, 11.25 µg.

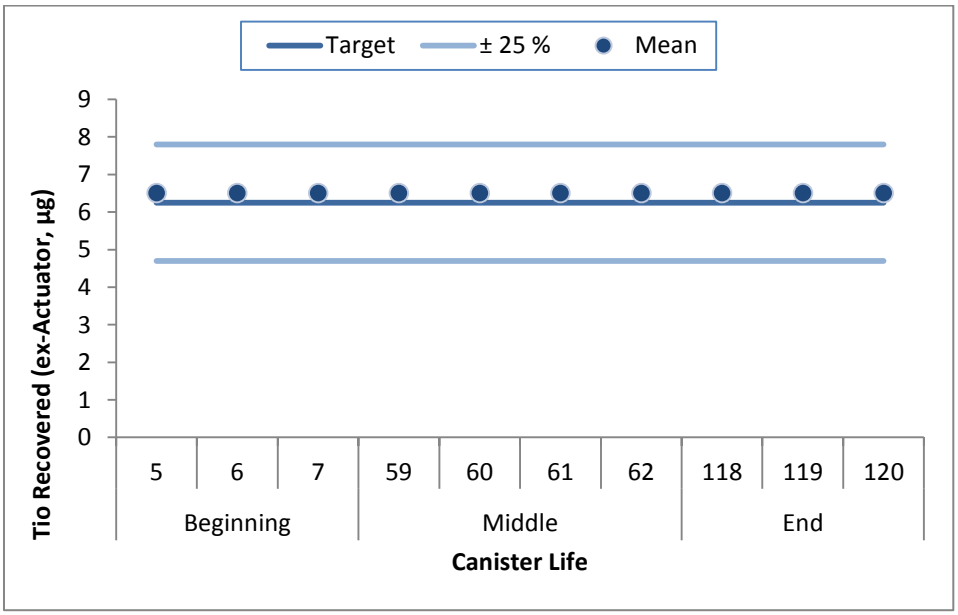

Figure 2. 11 Example of ex-actuator emitted dose distribution. The ex-actuator dose is plotted against the actuation number. No more than 3 of the 30 values lie outside the ± 25% of the target, 6.25 µg.

In in-vitro deposition test, several fundamental metrics were calculated based on the gathered raw data. The fine particle fraction (FPF) refers to the percentage of the mass of the active ingredient less than 5 μm. The fine particle dose (FPD) refers to the mass of the active ingredient less than 5 μm, which is the product of the FPF and ex-actuator dose. The mass median aerodynamic diameter (MMAD) refers to the mass diameter at which 50% mass of the total particles which reach the impactor body is lower or higher. MMAD is calculated from a log-cumulative mass percentage plot. A probit value of 5 is equal to 50% of the cumulative mass, and 2 data points above and 2 data points below probit 5 are regressed to calculate the MMAD value. An example is shown in Figure 2.12.

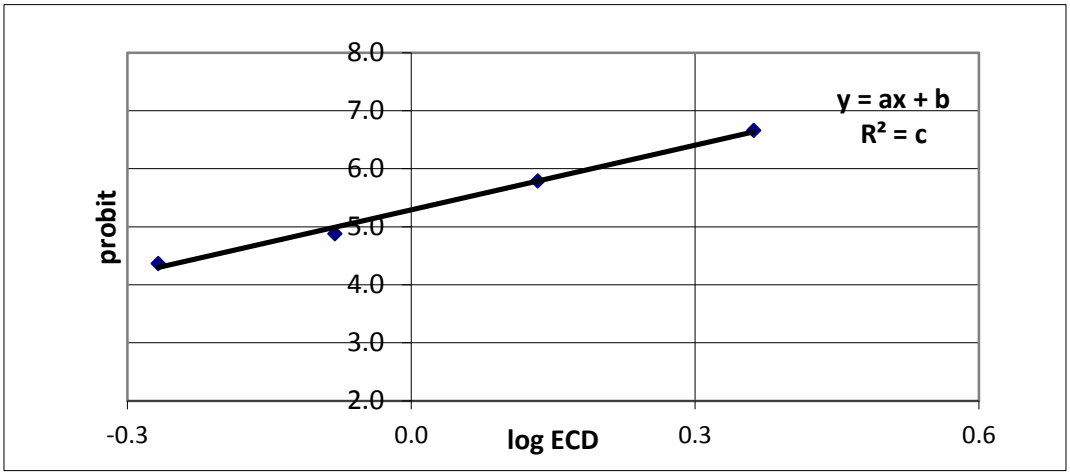

Figure 2. 12 Example of log-cumulative mass percentage plot (a: slope, b: intercept, c: coefficient of determination)

The geometric standard deviation (GSD) refers to the degree of heterodispersity of the distribution, which is determined from the log-cumulative mass percentage plot by the following equation (9):

$$\mathrm{GSD} = \sqrt{\frac{d_{84.1}}{d_{15.9}}} \quad (9)$$

($d_{84.1}$: diameter at 84.1% cumulative mass or Probit 6, $d_{15.9}$: diameter at 15.9% cumulative mass or Probit 4)

As the d84.1, d15.9 and MMAD sit in the same straight line of a perfect log-normal distribution, GSD can be calculated by the following equation (10):

$$\mathrm{GSD} = \frac{\mathrm{d}_{84.1}}{\mathrm{MMAD}} \quad (10)$$

($d_{84.1}$: diameter at 84.1% cumulative mass or Probit 6, MMAD: diameter at 50% cumulative mass or Probit 5)

The percentage of deposition of ex-actuator emitted dose is plotted against the different stages including induction port, Stage 1 to 7 and MOC. The particle size distribution graph or NGI Distribution Pattern (%) is illustrated in Figure 2.13.

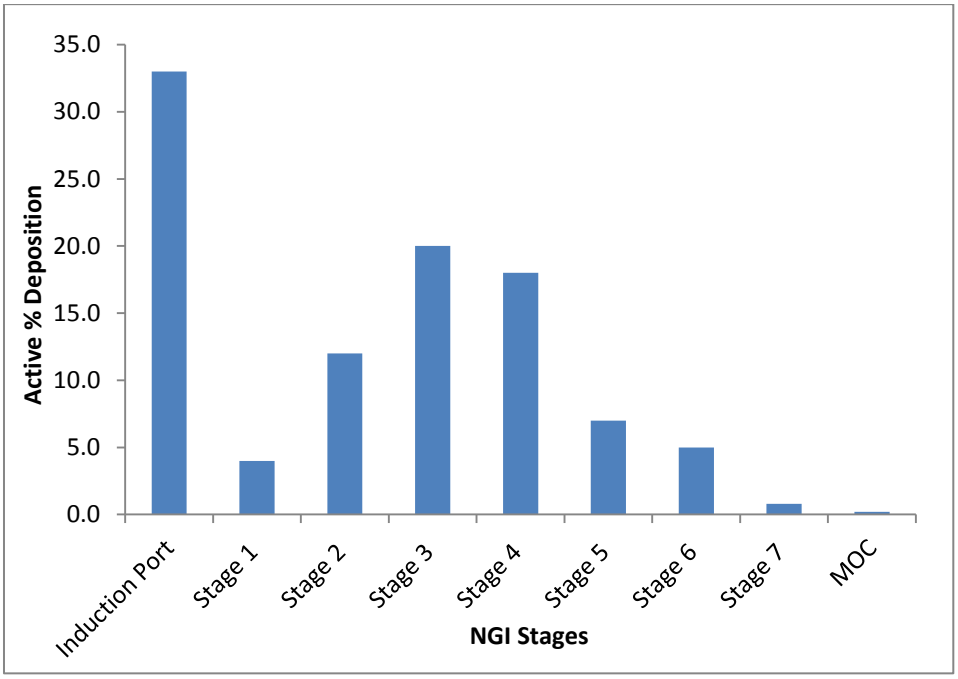

Figure 2. 13 Example of the NGI distribution pattern (%). The percentage of Tio deposition is plotted against the NGI stage. Stages includes induction port (IP), Stage 1 to Stage 7 and MOC, and the corresponding d50 value of each stage is calculated based on the flow rate.

Data of the dose uniformity test and in-vitro deposition test was further analysed by t-test using Excel Office and ANOVA test with post-hoc test i.e. Tukey HSD using SPSS software. Data for validation of HPLC method was expressed as variance, standard deviation (SD), relative standard deviation (RSD), detection limit (LOD), quantitation limit (LOQ), and analysed under the guidelines of ICH.

3 Comparisons of Tiotropium Inhalation Products

3.1 Introduction

In this research, secondary particles are introduced into a pressurised metered dose inhaler (pMDI) suspension system, with their influence on drug stability and aerodynamic performance being determined to better understand the potential of the secondary particulate theory. The feasibility of developing a suspension pMDI system is dependent on the solubility of the active pharmaceutical ingredient (API). An adapted classical method was applied to determine the drug solubility in HFA 134a and HFA 227. Tiotropium was selected as the model drug, with the high potency dictating the small amount required for each canister. Accordingly, the addition of a small amount of selected secondary particle, L-leucine, was applied in a preliminary study. This study aims to assess the practicability of the secondary particle with the high potency drug at a low concentration. As no Tio pMDI products are currently marketed in the EU and the US, a dry powder inhaler (DPI), Handihaler®, a 'small volume liquid' inhaler (SVLI), Respimat® and a pMDI product marketed in other territories, Tiova®, were tested as the reference products.

Understanding solubility of the API in propellants is essential for formulating into a suspension system. Due to the dissolution of suspended particles, at a concentration higher than the API equilibrium solubility, dissolved API will deposit on the larger API particles and lead to particle growth, which is called Ostwald ripening (Mullin, 1972). Thus, the size change of suspended particle affects the size of API aerosolised particles during the atomisation process. The difference in aerodynamic behaviour will change the particle distribution and the therapeutic effect. As Tio has already been formulated in aerosol formulations as dry powder and aqueous solution forms, the pre-formulation study is mainly focused on solubility testing of Tio in HFA propellants. Due to the pressurised environment, it is difficult to determine the solubility in HFA propellants. A filtration apparatus was

applied to capture the suspended particles and transfer the dissolved API into a receiving vial. The testing method was adapted from Williams et al (1999) as shown in Section 2.3. Tio (as tiotropium bromide monohydrate), is a hydrophilic substance, which exists as a quaternary ammonium and is sparingly soluble (2.5% w/v) in water, independent of pH (FDA, 2014). Consequently, monitoring of moisture and Tio concentration inside the canister over a storage period is also necessary to understand suspension stability.

In previous research by Jones (2004), L-leucine was found to improve the aerosol performance when added in a suspension formulation with salbutamol sulphate in HFA 134a. As follow-on research in this chapter, L-leucine was added into the Tio pMDI formulation in HFA 134a to give an initial view about the effects of excipients on pMDI performance. Tio alone was filled with HFA 134a as the "Tio Control" group, and Tio was co-suspended with leucine in HFA 134a as the experimental group. Both groups were manufactured using pre-selected pMDI hardware components. The canisters were stored at accelerated conditions over a 3-month period, and the emitted doses monitored at different time points. The uniformity of emitted doses give a general understanding of the compatibility between the drug and the excipient. Once the feasibility has been established, further design and investigation will be conducted in following chapters.

Due to the existing quaternary ammonium group of tiotropium, it is hard to deliver through the gastrointestinal route with an absolute bioavailability of 2%-3% (Scholar, 2009). In the current EU and US market, Tio is administered by pulmonary delivery using a DPI device, Handihaler® or a SVLI device, Respimat® (Table 3.1). The Handihaler® is a dry powder inhaler, where the dry dispersed dose is released from a hard capsule during inhalation. The Handihaler® consists of a dust cap, mouthpiece, chamber, piercing button and base (Figure 3.1). During use, the dust

cap is released and one hard capsule is inserted into the chamber. Once the capsule is pierced by pressing the piercing button the dose can be delivered through the mouthpiece upon inspiration (Chodosh et al, 2001). As a passive DPI system, performance of the formulation is highly dependent on the pattern of inspirations. The flow resistance of the device requires higher flow rate, compared with pMDIs, to rapidly vibrate the capsule and eventually trigger the aerosolisation of the powder. Each capsule contains 22.5 µg of Tio, and two inhalations of one capsule provide an ex-device delivery of 12.5 µg of Tio (Table 3.1).

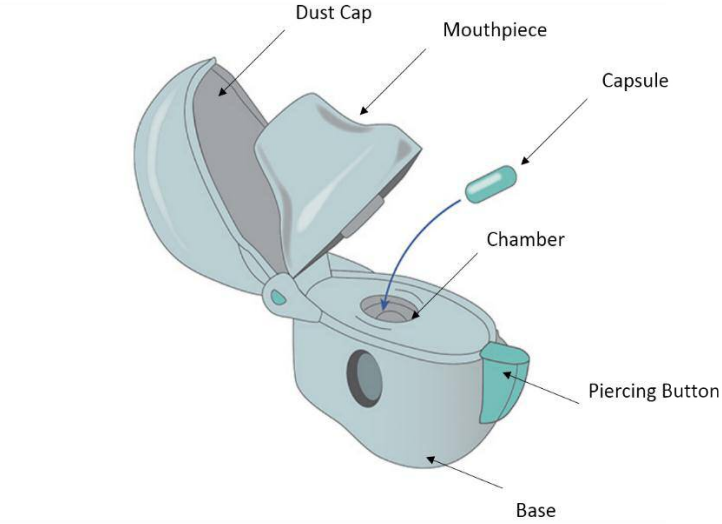

Figure 3 1 Configurations of Handihaler®

Respimat® is categorised as a small volume liquid inhaler (SVLI) or "soft mist" inhaler, which consists of a cap, mouthpiece, dose release button, cartridge, clear base and safety catch (Figure 3.2). The cartridge is set into the inhaler, and covered by the clear base. Twisting the clear base 180° compresses a spring and loads the dose into pump cylinder. As the dose release button is pressed, the compressed spring provides the mechanical power to generate a slow mist of drug solution by a nozzle system, which is called a "uniblock" (Anderson, 2006). Two jets of the drug

solution are formed in the uniblock and impinge at a predetermined angle to generate a fine mist of droplets (Spallek et al, 2002). Each puff delivers 3.124 µg of Tio outside the mouthpiece, and two puffs comprise one medicinal dose of 6.25 µg of Tio (Table 3.1).

Figure 3. 2 Configurations of Respimat®. During usage, the clear base is taken off and the cartridge is inserted into the inhaler. The clear base is put back with a half twist. Once the cap is opened the dose release button can be used deliver a dose.

A new DPI product, the Braltus®, has been recently marketed in the EU, following the same DPI delivery mechanism as Handihaler®. The Braltus product was not available at the time of conducting the experiments presented in this chapter. A pMDI formulation under the trade name Tiova® is available in certain unregulated territories but not in EU or US markets. However, there are no published references relevant to the formulation of Tiova®. The information of all the four types of Tio inhalation devices are summarised in Table 3.1. In this chapter, the Handihaler®,

Respimat® and Tiova® were analysed under different conditions as reference products for the novel pMDI formulations.

Table 3. 1 Information of Marketed Tiotropium Products

Product	Device	Type	Formulation	Dose	Ex-device	Frequency
Spiriva®	Handihaler®	DPI	Dry Powder	22.5 μg	12.5 μg	Once a day
Spiriva®	Respimat®	SVLI	Solution	n/a	6.25 μg	Once a day
Braltus®	Zonda®	DPI	Dry Powder	16 μg	10 μg	Once a day
Tiova®	n/a	pMDI	Suspension	11.25 μg	n/a	Once a day

(DPI: Dry powder inhaler; SVLI: small volume liquid inhaler; pMDI: pressurised metered dose inhaler; Dose: weight of tiotropium as salt per medicinal dose; Ex-device: weight of tiotropium in salt form delivered out of the device.)

3.2 Chapter Aims and Objectives

The aims of this chapter were to determine the feasibility of formulating Tio with secondary particles in HFA propellants, and gain an understanding of the variability in aerosol performance between Tio marketed products including Handihaler®, Respimat® and Tiova® (in EU or India).

The objectives of this chapter are as follows:

1) To determine the Tio solubility in HFA 134a and HFA 227, and monitor Tio solubility over long-term stability test at 40°C/75% RH.

2) To evaluate the feasibility of formulating Tio with a secondary particle, L-leucine, and test dose uniformity against Tio only as the control group over 3-month storage at 40°C/75% RH.

3) To analyse the in-vitro performance of reference products, e.g. Handihaler® dry powder inhaler (DPI), Respimat® 'Small Volume Liquid' Inhaler (SVLI) and Tiova® pMDI.

3.3 Materials and Equipment

3.3.1 Materials

Tiotropium Bromide Monohydrate (Tecoland); L-Leucine (Sigma-Aldrich); HFA 134a (Mexichem); HFA 227 (Mexichem); Benzyltriethyl Ammonium Chloride (Sigma); Spiriva Handihaler® (Boehringer Ingelheim) which was described as Handihaler® in the content; Spiriva Respimat® (Boehringer Ingelheim), which was described as Respimat® in the content; Tiova® (Cipla); 14 mL Plain Aluminium MDI Canisters (H&T Presspart Manufacturing); KHFA 25 µL Metering Valve (Valvole Aerosol Research Italiana); 0.25 mm Actuator (H&T Presspart Manufacturing); Microlance® 3 Needles (Becton Dickinson); Minisart® RC25 Syringe Filter (Sartorius); Sterile Evacuated Elution Vials (Amersham Health); 0.2 µm Nylon Membrane Filters (Whatman);

3.3.2 Equipment

The HPLC system and Pamasol filling system were described in Chapter 2; Next Generation Impactor (Copley Scientific); Dosage Unit Sampling Apparatus (Copley Scientific); Dry Powder Controller Model TPK (Copley Scientific); NGI Gentle Rocker (Copley Scientific); 5799-S Stability Storage Cabinet (Vindon Scientific) (40°C/75%); Minor M200 Sieve Shaker (Endecotts) with Laboratory Test Sieve (Aperture: 38 µm); Turbula Mixer (Willy A. Bachofen); Circulaire 900 fume cabinet (Monmouth Scientific)

3.4 Methods

Methods including solubility test, particle size analysis, preparation of canisters, dose content uniformity test and distribution test are described in Chapter 2. Modifications of any method are specified in specific chapters.

3.4.1 Tio Control Preparation and Solubility Determination

To determine Tio solubility in HFA propellants, an amount of Tio without excipients is suspended in HFA 134a and HFA 227 in 14 mL plain aluminium canisters (H&T Presspart®). The formulation compositions are shown in Table 3.2. Each canister contains 156 doses, 120 doses plus 30% overage, crimped with 25 µL KHFA metering valves (VARI®). Nine canisters of each formulation were prepared and kept in the inverted position for up to 3 days to allow adequate equilibration of the valve components that were exposed to the formulation (Stein et al, 2014). Three canisters of each formulation were randomly selected and kept for 3 days at ambient temperature before testing. The remaining canisters were stored at the accelerated stability condition of 40°C/75% RH in a Vindon® stability storage cabinet for up to 6 months. Three of each formulation were randomly selected at time points 1 month and 6 months separately. These selected canisters were used to determine the solubility of Tio in propellants at predetermined intervals of 0, 1 and 6 months separately. The methods were referred to in Section 2.2, and prior to testing, the canisters were allowed to equilibrate to room temperature. The moisture level of each canister was also determined before the solubility test according to Section 2.9.

Table 3. 2 pMDI Formulation of Tio Only in HFA 134a and HFA 227

	Per actuation		Per Canister		% w/w
	µL	mg	mL	g	
HFA 134/227	25	30.45/35.00	3.90	4.75/5.46	99.96/99.97
Tiotropium		0.0113		0.0018	0.04
Totals	25	30.4613/35.0113	3.90	4.7520/5.4618	100.00

3.4.2 Preliminary Study of Secondary Excipients

In preliminary studies, a novel Tio formulation was initially prepared by dry-blending of micronised Tio particles with a model excipient, L-leucine, and co-suspended in a model propellant, HFA 134a, regardless of the influence of the formulation and device factors. According to Dickinson and Warren (2004), the particle size of the secondary particles is preferably in the range of 25-125 µm. The fraction (106-90 µm) of leucine was prepared by mechanical vibrating (Section 2.4). The experimental group was prepared at Tio: Leu (w/w) 1:5 for a 25-canister batch (Table 3.3). Tio and Leucine were mixed and blended according to Section 2.5 but used with a 38-µm mesh Endecotts® sieve. Due to the potential loss of drug particles during the sieving process, an empirical overage of 7% of Tio was measured, and in this case, 0.1053 g of Tio was calculated to fill 25 canisters while 0.1127 g was actually measured out for sieving (Table 3.3). The blend was then filled individually in 14 mL plain aluminium canisters (H&T Presspart®) and suspended in HFA 134a to target 156 doses. A batch of Tio control canisters were prepared by suspending Tio without excipients in propellants. The formulation of control canisters containing Tio without excipients is illustrated in Table 3.2. 1.8 mg of Tio was filled individually in three 14 mL plain aluminium canisters (H&T Presspart®), and then suspended in HFA 134a to target 156 doses. Both batches were crimped with 25 µL KHFA valves (VARI®), and all the generated aerosol data was based on 0.25 mm orifice actuators (H&T Presspart®).

Table 3. 3 pMDI Formulation of Tio: leucine in HFA 134a

	Per actuation		Per Canister		% w/w	25 Can Batch	Batch+7%
	µL	mg	mL	g		g	g
HFA 134a	25	30.45	3.90	4.75	99.78	285.01	285.01
Leucine		0.0563		0.0088	0.18	0.5265	0.5265
Tiotropium		0.0113		0.0018	0.04	0.1053	0.1127
Totals	25	30.5175	3.90	4.7607	100.00	285.6438	285.6512
Total Solids				0.0105		0.6318	0.6392

Three canisters of each batch were prepared according to Sections 2.4-2.7, and the emitted dose testing was performed according to Section 2.8. Modification to Section 2.8 was as follows: as the KHFA valves allowed optimised rapid-fill/ rapid drain properties, there was no need for priming of canisters (Tran et al, 2011). However, as the resting time according to the manufacturer' specification and requirement for priming were not validated in our lab, each canister was discharged for four waste actuations to prime the valve before testing. In this pilot test, 3 Tio control and 3 experimental canisters were kept in a stability cabinet at 40°C/75% RH over a 3-month period. At the time points of 0, 1, 2 and 3 months, three actuations per pMDI was collected individually and analysed by HPLC. The same canisters were used over the stability test and the emitted doses were only collected in the beginning of canister life. This method is conducted to evaluate the feasibility of secondary particles to stabilise a high-potency drug at a low concentration of both the drug and the secondary particle.

3.4.3 Handihaler® DPI

The Spiriva Handihaler® will simply be expressed as Handihaler® to distinguish it from the Spiriva Respimat® product. According to Section 2.8.2, the uniformity of delivered dose of Handihaler® was measured using a Copley DUSA. The device was connected with a critical flow controller Model TPK as shown in Figure 3.3. P1 stood for the pressure drop over inhaler, where 4 kPa, the average pressure drop in DPI devices to standardise energy input upon actuation, is required (Mohammed et al, 2014). P2 and P3 stood for the absolute pressure measurement, where P2 must be more than twice that of P3 to assure critical flow conditions.

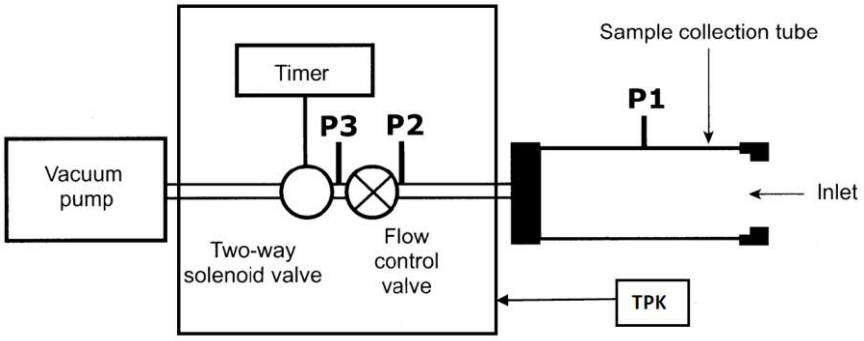

Figure 3. 3 Configurations of DUSA apparatus for the DPI (British Pharmacopoeia, 2017a). The DPI device is connected with a DUSA tube, a critical flow controller model TPK and a vacuum pump.

An inspiration volume of 4 L is used, which represents patients' (adult male, weighing approximately 70 kg) average forced inspiration volume. The test time (T) in seconds is calculated as the following equation:

$$T = \frac{4(L) \times 60(s)}{Flow\ Rate\ (L/min)} \quad (11)$$

The DUSA and the 2-way solenoid valve were connected to a vacuum pump. The flow control valve was adjusted until the pressure drop in the Handihaler® is close to 4 kPa. A flow meter was then connected to the DUSA and the flow rate indicated

as 46 L/min. Based on the test time equation (11), the sampling duration was set as T = 5.2 s. Two inhalations were conducted for each capsule, as required by the patient information leaflet (PIL) to provide the medicinal dose, and in total three capsules for each device were tested to give an average emitted dose. The Tio mass deposited on the DUSA tube and Handihaler device were recovered referred to Section 2.8.2, and the remaining drug inside the capsule was recovered by a further 25 mL recovery solution.

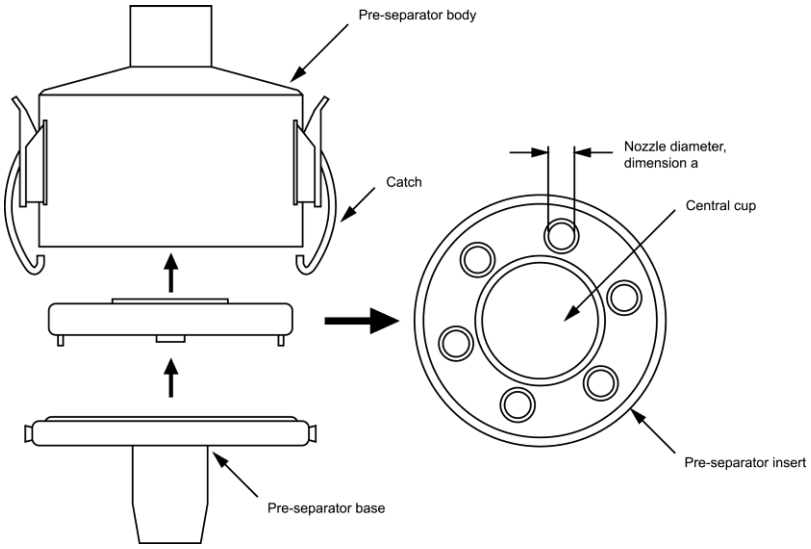

Figure 3. 4 Configurations of Pre-separator (British Pharmacopoeia, 2017b). The pre-separator base is inserted into the NGI body. Place the pre-separator insert and fill the central cup with 15 mL recovery solution. Install the pre-separator body and secure by the catch.

According to Section 2.8.3, the aerodynamic particle distribution of the Handihaler® was measured by a standard NGI with pre-separator and a critical flow controller. The configuration was similar to Figure 3.2, but with replacement of the sample collection tube by the NGI assembled with the pre-separator as shown in Figure 2.5. The pre-separator consists of the pre-separator base, the insert and the body, and the configuration is illustrated in Figure 3.4. The pre-separator base was inserted into the impactor inlet and 15 mL of the recovery solution was then added in the central cup, followed by securing the pre-separator body on the pre-

separator base. The same testing procedure was conducted as the DUSA. Three capsules, in total six inhalations were collected for each device to give an equivalent dose to 5 actuations of pMDI devices. The Handihaler® is reported to have a high device resistance (Chapman et al, 2011) and due to limitations of the power of the vacuum pump and the maximum flow rate, it was difficult to meet the 4 kPa pressure difference by our vacuum pump. As reported by Chodosh et al (2001), the flow resistance with the capsule was achieved by 4kPa at the flow rate of 39 L/min. Therefore, two flow rates were set for the NGI including 46 L/min and 39 L/min. The testing time was calculated based on Equation (10), which were T = 5.2 s (46 L/min) and 6.2 s (39 L/min) respectively. The corresponding effective cut-off diameter (d50) of NGI stages were calculated based on Equation (6) (Section 2.8.3), which were illustrated in Table 3.4.

Table 3. 4 Effective Cut-off Diameter (d50) for NGI at Different Flow Rates

Stage	d50 at 39 L/min (μm)	d50 at 46 L/min (μm)
1	10.17	9.30
2	5.58	5.12
3	3.50	3.22
4	2.03	1.88
5	1.18	1.08
6	0.71	0.65
7	0.45	0.41

3.4.4 Respimat® 'Soft Mist' Inhaler

The Spiriva Respimat® will simply be expressed as Respimat® to distinguish it from the Spiriva Handihaler® product. The aerodynamic particle distribution of Respimat® was measured by the same apparatus used for pMDI testing, a NGI. Due to a unique release mechanism, Respimat® is a solution formulation but not categorised as a nebuliser device. There was no standard pharmacopeia method available for in-vitro evaluation. If it was regarded as a nebuliser device, a flow rate of 15 L/min is recommended to be comparable to the mid-inhalation flow rate of a tidally breathing healthy adult (British Pharmacopoeia, 2017b). There was another report that a Respimat® device containing budesonide can be tested at an average flow rate of 30 L/mL using a four-stage multistage liquid impinger (Pitcairn et al, 2005). Therefore, two flow rates including 15 L/ min and 30 L/min were used to evaluate the in vitro performance of the Respimat® in this chapter. The corresponding effective cut-off diameter (d50) at 15 L/min was provide by Marple et al (2004), and 30 L/min were calculated based on Equation (6) in Section 2.8.3 (Table 3.5).

Table 3. 5 Effective Cut-off Diameter (d50) for NGI at Different Flow Rates

Stage	d50 at 15 L/min (µm)	d50 at 30 L/min (µm)
1	14.1	11.72
2	8.61	6.40
3	5.39	3.99
4	3.30	2.30
5	2.08	1.36
6	1.36	0.83
7	0.98	0.54

Furthermore, according to Stapleton and Finlay (1999), as the solution based aerosol droplets transits through the impactor, particle size can be undersized due to temperature-induced droplet evaporation. Additionally, it was recommended by Berg et al (2007) to cool the impactor to a lower temperature than the entrained

air, to ensure 100% relative humidity (RH) inside the impactor. Therefore, the Respimat® assessments were performed at both ambient and cooled conditions of the NGI for analysing the particle size distribution. For the cooled condition, the assembled NGI including the induction port (IP) was placed in a refrigerator under 5°C for 1 hour and used for test within 5 min after removing from the refrigerator. Due to the fine mist generated by Respimat®, a Nylon membrane filter was applied in the filter holder after the micro-orifice collector (MOC) to collect any extreme fine particles. The recovery method was referred to in Section 2.8.3, with modification of recovery volume of Stage 5 by 5 mL and filter by 10 mL, and all the samples were analysed and quantified by HPLC.

3.4.5 Tiova® pMDI

The patient information leaflet (PIL) for Tiova® inhaler indicates that Tio is formulated in HFA 227 with 11.25 µg Tio in each dose and 200 doses per canister. As a pMDI system, the in-vitro performance of Tiova® was analysed by the standard pharmacopeia method. The uniformity of delivered dose was measured by a DUSA at a flow rate of 28.3 L/min, according to Section 2.8.2. Three Tiova® pMDIs were used for canister-life uniformity test, and emitted doses at the beginning, middle, and end of canister life, in total ten actuations, were collected and analysed by HPLC. The aerodynamic particle distribution of Tiova® was measured by a standard NGI at the beginning of canister life with a flow rate of 30 L/min, according to Section 2.8.3. An accelerated stability testing was performed to evaluate the variability of aerodynamic performance of the product over time. Three Tiova® pMDIs were then stored in a Vindon® stability storage cabinet at 40°C/75% RH conditions for 6 months. At time points of 0, 1, 3, and 6 months, each canister was removed from the cabinet and equilibrated at room temperature. Then the canisters were discharged for four waste actuations, and a further five actuations were collected by the NGI. The same canisters were used during the stability test, and all the samples were analysed and quantified by HPLC.

3.5 Results and Discussion

3.5.1 Solubility in HFA 134a and HFA 227

The filtration apparatus was adapted from the method used for determination of steroids in hydrofluoroalkane propellant (Williams et al, 1999). Tio was suspended in HFA 134a and HFA 227 at concentrations of 0.0369 (% w/w) and 0.0321 (% w/w). 11.25 µg Tio is targeted per actuation, with a total of 40 actuations releasing approximately 450 µg of Tio from the metering valve. The solubility of Tio in HFA 134a and HFA 227 is illustrated in Table 3.6. Based on the HPLC data, the Tio peaks are either undetectable in some cases or the peak area is lower than the LOD level (0.41 µg/mL), and cannot be calculated based on the calibrated HPLC methods. The peak area is lower than the reference concentration of 0.02 µg/mL. The specific solubility is not clarified but is found to be < 0.1 µg/mL, with < 0.02% of the total Tio mass per canister dissolved in propellants, which is a negligible amount. According to the solubility scale, Tio is considered to be practically insoluble in both HFA 134a and HFA 227, which implicates the possibility of formulating Tio as a suspension with HFA propellants.

Table 3. 6 Solubility of Tio in HFA 134a and HFA 227

Propellant Type	HFA134a	HFA227
Tio Dissolved Mass in 40 Actuations (µg)	< 0.10	<0.10
Propellant Volume in 40 Actuations (mL)	1.00	1.00
Solubility (µg/mL)	< 0.10	<0.10
Tio Total Mass in 40 Actuations (µg)	450.0	450.0
% w/w Dissolved	< 0.02	<0.02

Physical stability of a formulation for a given propellant system can be indicated by monitoring drug concentrations over time. Any significant change of the solubility could suggest the occurrence of crystal conversion (Gupta and Myrdal, 2004). Each canister is stored at the condition of 40°C/75% RH for up to a 6-month period. The moisture level and solubility is summarised in Table 3.7. It was observed in the HFA 134a system that water content in canisters increased from 188.28 ppm at 0 month

to 517 ppm at 6 months, and in HFA 227 system, water content increased from 246.83 ppm at 0 month to 429.84 ppm at 6 months. Under the same storage conditions, the rate of moisture uptake is dependent on the propellant types and the sealing material of valves' gaskets (Williams and Hu, 2000). The water ingress rate of HFA 134a is higher than HFA 227, which is due to higher water solubility in HFA 134a than HFA 227 (Myrdal et al, 2014). The ingress of water could be correlated with the alteration of solvency of the propellant and lead to an increase of Tio solubility. However, as unquantified amounts of Tio are found in all the samples, it is assumed the moisture level gained in the canister has no measurable influence on the Tio solubility.

Table 3. 7 Solubility of Tio in HFA 134a and 227 over 6 Month Storage (n=3, mean ± SD)

Propellant	HFA 134a			HFA 227		
Time Points	0 Month	1 Month	6 Month	0 Month	1 Month	6 Month
Moisture (ppm)	188.28 ± 3.52	299.90 ± 13.22	516.76 ± 14.25	246.83 ± 5.02	288.32 ± 3.97	429.84 ± 24.28
Solubility (µg/mL)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1

3.5.2 Preliminary Study of Secondary Excipients

As reported by Jones (2004), blended mixtures of L-leucine and salbutamol sulphate demonstrate particle association in both dry format and suspension in HFA propellants. Furthermore, superior aerosol characteristics and consistent dose delivery throughout canister life were observed with the addition of L-leucine. However, a large dose of salbutamol sulphate (120.45 μg) is delivered per actuation associated with 5-fold or 10-fold of the amount of L-leucine. As a follow-on study, the emitted dose of Tio was investigated as 11.25 μg per actuation with a 5-fold of L-leucine added as the secondary particle. This section was a pilot study to demonstrate the feasibility of adding secondary particles to high potency drug in low concentrations. The main question in this preliminary research is whether L-leucine can maintain or improve the physical stability of Tio pMDI suspension. Consequently, two pilot batches were manufactured, one of which contained Tio only as the control group, and the other a co-suspension of Tio and L-leucine as the experimental group.

Three canisters of the Tio Control and the Tio: Leu formulations were tested at the beginning of canister life throughout the 3-month storage. The distribution of emitted dose (ex-valve) is illustrated in Figure 3.5. According to ANOVA and Tukey HSD test, the ex-valve dose of the control group is similar between 0 and 1 month ($P > 0.05$), but significant decreases are found in 2 and 3 months ($P < 0.05$). In Tio: Leu, the ex-valve dose is reproducible with no statistic difference ($P > 0.05$). The emitted dose (ex-actuator) is shown in Figure 3.6, and the distribution pattern is similar to the emitted dose (ex-valve) regardless of influence of the actuator. In the Tio Control group, the emitted dose at 1 month is comparable to 0 month ($P > 0.05$), but is significantly lower ($P < 0.05$) at 2 and 3 months; 3 month is also statistically less than 2 months. Comparing between Tio Control and Tio: Leu by unpaired t-tests, the ex-actuator doses show the same results. At the time point of 0 ($P > 0.05$) and 1 month ($P > 0.05$), the emitted dose of Tio control is comparable to Tio: Leu.

However, at the time point of 2 and 3 months, the emitted dose of Tio control decreases obviously and becomes significantly lower than Tio: Leu ($P< 0.05$).

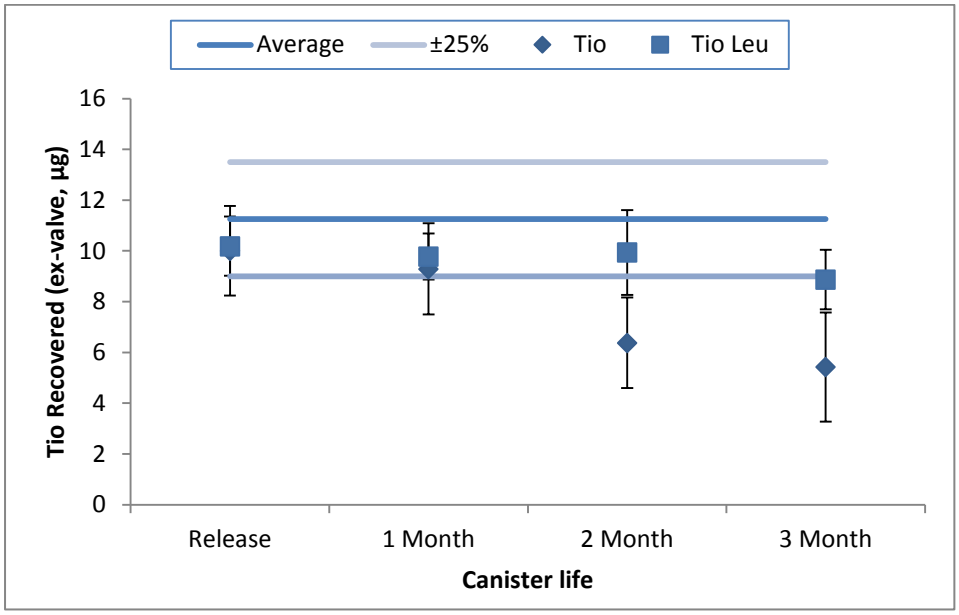

Figure 3. 5 Distribution of emitted dose (ex-device) of Tio Control and Tio: Leu pMDI formulations in 3-Month Storage (n=9; mean ± SD). Tio Control: 0 Month: 10.01 ± 1.77, 1 Month: 9.29 ± 1.80, 2 Month: 6.38 ± 1.78 µg, 3 Month: 5.43 ± 2.15 µg; Tio: Leu: 0 Month: 10.19 ± 1.16 µg, 1 Month: 9.78 ± 0.91 µg, 2 Month: 9.94 ± 1.67 µg; 3 Month: 8.87 ± 1.17 µg. According to ANOVA and Tukey HSD, the ex-valve dose of the control group is similar between 0 and 1 month ($P> 0.05$) but significant decrease is identified in 2 and 3 months ($P< 0.05$). The Tio: Leu showed reproducible ex-valve doses with no statistic difference ($P> 0.05$).

The obvious trend of dose (ex-valve and ex-actuator) reduction at 2 and 3 months in the Tio Control group and the reproducible dose in Tio: Leu suggests that the secondary particle improves physical stability of the suspension over the storage period. The decrease of the Tio dose in the control group could be due to degradation or absorption of Tio particles on the inner surface of the device, which leads to a decrease of Tio concentration over time. The addition of leucine as the excipient could reduce the dose variability and improve the dose consistency up to 2 month storage. In this initial study, L-leucine, as a secondary excipient has exhibited some potential to increase the physical stability of the Tio suspension as

less variability of the metered dose is identified. Emitted doses are consistent throughout the storage under accelerated conditions, which indicates leucine could prevent the interaction of drug particles in low concentrations with the device components even in uncoated aluminium canisters.

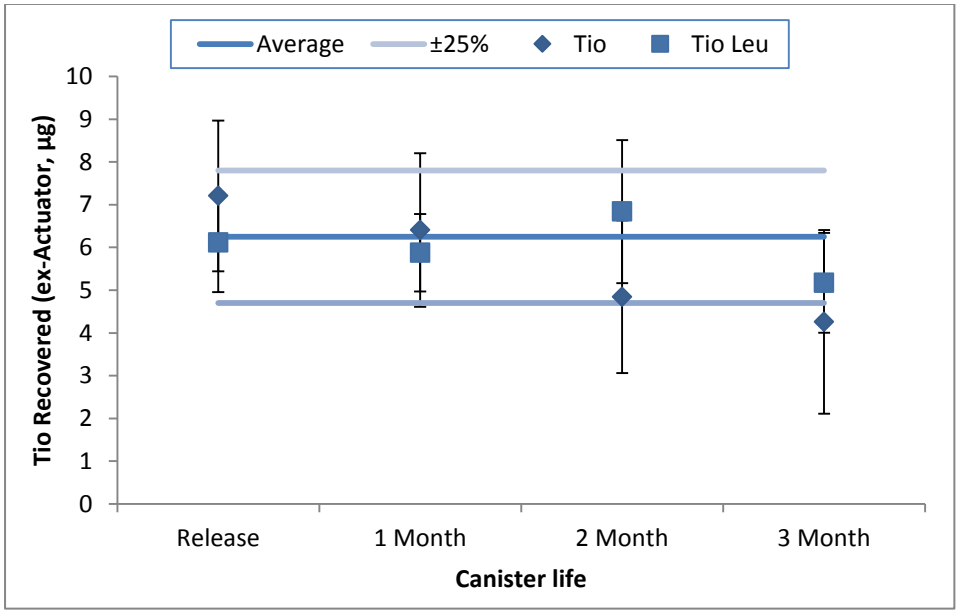

Figure 3. 6 Distribution of emitted dose (ex-actuator) of Tio Control and Tio: Leucine pMDI formulations in 3-Month Storage (n=9; mean ± SD). Tio Control: 0 Month: 7.21 ± 0.81 µg, 1 Month: 6.40 ± 1.41 µg, 2 Month: 4.85 ± 1.07 µg, 3 Month: 4.26 ± 1.80 µg; Tio: Leu: 0 Month: 6.12 ± 0.90 µg, 1 Month: 5.98 ± 0.80, 2 Month: 6.84 ± 1.27 µg, 3 Month: 5.17 ± 0.62 µg.

3.5.3 In-vitro Testing of Handihaler®

Handihaler® is the most prescribed device for treating COPD. There have however been concerns of increased risk of cardiovascular death, myocardial infarction and stroke associated with the individual formulations of anticholinergic drugs (Singh et al, 2008). Since then, the *Understanding Potential Long-term Impacts on Function with Tiotropium* (UPLIFT) trial was conducted with a large patient number over 4-year period. The results indicated there was no significant increase in the risk of stroke, heart attack or cardiovascular death between the Handihaler® group and placebo group (Tashkin et al, 2008). Furthermore, the improvement in lung function and quality of life overcame the risk induced by Handihaler®. Thus, Handihaler® is used as a reference product to the novel Tio pMDI formulation, and in-vitro performance of Handihaler® is evaluated by the DUSA and NGI.

Table 3. 8 Dose Uniformity Test of Handihaler® (n=3; mean ± SD)

Tio Recovered from Device (µg)	1.03 ± 0.24
Tio Recovered from DUSA (µg)	10.61 ± 2.02
Tio Recovered from Capsule (µg)	8.92 ± 0.77
% Tio Delivered Ex-device	51.38 ± 7.19

(The DUSA test includes 3 capsule, and the Tio mass is recovered from the device, the DUSA and the capsule separately.)

According to the PIL, it is stated that each Handihaler® capsule contains 22.5 µg Tio and two inhalations of one capsule give one medicinal dose. The dose emitted from the mouthpiece of the device is about 12.5 µg theoretically, and about 45% of the total dose is trapped within the device and capsule. One capsule contains one medicinal dose in 5.5 mg lactose monohydrate. In dose uniformity testing, Tio was recovered from the Handihaler® device, the mouthpiece, the DUSA and the capsule separately as shown in Table 3.8). The total mass balance per capsule was 20.55 ± 1.33 µg, some 8.7% less than the theoretical dose. It is identified that 5% and 43% of the total dose were trapped in the mouthpiece and the capsule respectively. The

ex-device dose per capsule was 10.61 µg, 15% less than the theoretical dose. The effectively delivered mass accounts for only 51.38 ± 7.19% of the total mass of each capsule, and the relative high variance was found with Tio mass recovered from different compartments.

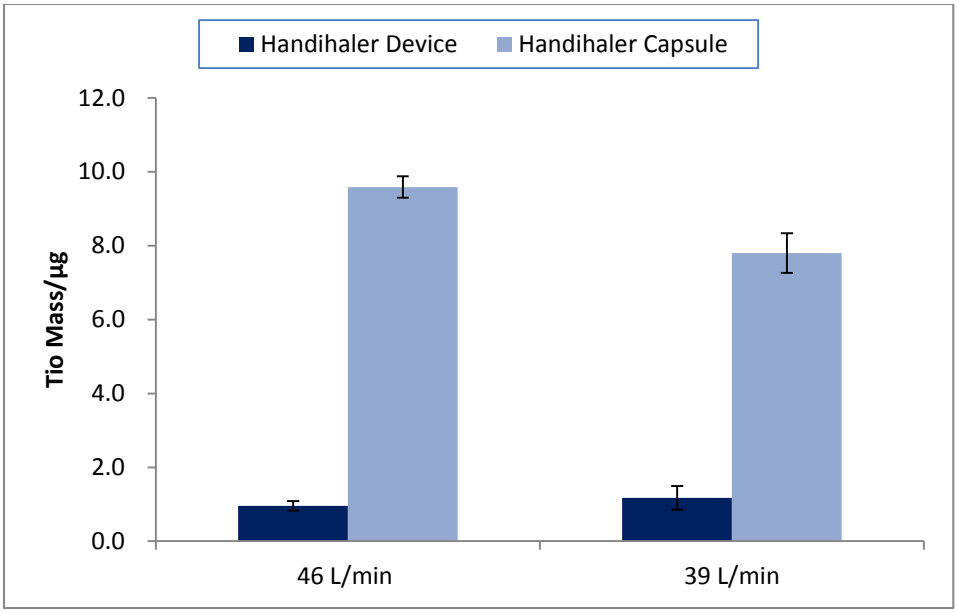

Figure 3. 7 Tio Mass Remaining in Handihaler ® Device and Capsule with Different Flow Rates (46 L/min: n=6; mean ± SD, 39 L/min: n=9; mean ± SD). According to t-test, the amount of Tio remained in the device is comparable regardless of flow rates (P> 0.05). The capsule retention, however, is significantly increased at higher flow rate of 46 L/min (P< 0.05).

In this research, the flow rate of 39 L/min, which was found to achieve a 4kPa flow resistance inside the Handihaler®, and the flow rate of 46 L/min, the maximum flow rate achieved by our vacuum pump, were applied to evaluate the aerodynamic performance of the Handihaler® (Chodosh et al, 2001). The Tio mass remaining in the Handihaler® device and capsule under different flow rates are shown in Figure 3.7. At 46 L/min, the amount of device retention, 0.96 ± 0.13 µg, is comparable to 39 L/min, 1.17 ± 0.32 µg (t-test, P> 0.05). However, the capsule retention is significantly increased (t-test, P< 0.05) at higher flow rate, where 46 L/min is 9.59 ±

0.29 and 39 L/min is 7.80 ± 0.54. It was found by Coates et al (2005) that the capsule emptying time was reduced at higher flow rate and the capsule retention was increased with the decrease of the flow rate in the range of 30 and 60 L/min by an Aerolizer® device. Similar results were found in a Cyclohaler® device, the drug mass deposited inside the capsule was reduced as the increase of flow rate from 20 to 55 L/min (Shur et al, 2012). Aerolizer® and Cyclohaler® are recognised as low-resistance devices, and the device performance is highly dependent on the inspiration flow rate (Dal Negro, 2015). The overall level of turbulence generated by the DPI device is increased with the increase of the flow rate, which could explain the decreased capsule retention. However, in high-resistance devices, i.e. Handihaler®, Shur et al (2012) found the minimum capsule retention was obtained at 39 L/min in the range of 20 and 55 L/min. In our research, a reduction in capsule retention of Handihaler® is also observed at 39 L/min when compared against 46 L/min. The release efficacy from the capsule is likely relevant to the intrinsic resistance of the device, and the shorter time need to generate a 4 L volume at 46 L/min could also contribute to higher capsule retention.

Table 3. 9 Aerosol Characteristics (ex-device) of Handihaler® with Different Flow Rates (46 L/min: n=6; mean ± SD, 39 L/min: n=9; mean ± SD)

NGI Flow rate	46 L/min	39 L/min
Tio FPF (% < 5 μm)	39.38 ± 1.55	40.60 ± 3.16
Tio MMAD (μm)	3.39 ± 0.17	3.64 ± 0.19
Tio FPD (μg < 5 μm)	3.91 ± 0.27	4.03 ± 0.54
Tio GSD	1.86 ± 0.08	1.83 ± 0.05

The aerosol characteristics are illustrated in Table 3.9. There is no significant difference between the NGI flow rates in FPF (t-test, P> 0.05) and FPD (t-test, P> 0.05). The higher flow rate was expected to improve the aerosolisation of particles and the emptying of APIs from the device as the increased turbulence could increase the intensity of particle-device interaction, which increases the de-aggregation potential of Tio from the carriers (Copley, 2010a, Coates et al, 2005). In previous research, it was reported that the product performance of Turbohaler®

(formoterol and budesonide) in terms of FPF was sensitive to change of flow rate in the range of 30 and 60 L/min, where a low flow rate could not generate a sufficient energy to de-aggregate both drugs from lactose carriers (Buttini et al, 2016). Coates et al (2005) also indicated that the FPF of Aerolizer® increased with the flow rate between 30 and 75 L/min. However, in this research, the in-vitro assessment of Handihaler® indicates the FPF and FPD are reproducible in the range of 39 L/min to 46 L/min. Chodosh et al (2001) also reported fine particle doses and fractions of Handihaler® using an ACI were consistent with flow rates ranging from 28.3 to 60 L/min. Similarly, Shur et al (2012) found that the fine particle mass of Handihaler®, which was defined as drug mass collected on Stage 2 to MOC of a NGI, was consistent in the range of 39 and 55 L/min. Therefore, the influence of flow rate on DPI products is device dependent. Furthermore, the GSD of particle size distribution was also comparable between 39 L/min and 46 L/min (t-test, $P > 0.05$). The MMAD of 39 L/min is 7% higher than 46 L/min with a statistical difference (t-test, $P < 0.05$) which indicates a better de-aggregation of Tio from carrier particles at high flow rate (Shur et al, 2012). Overall, due to the intrinsic design of Handihaler® device, a flow rate of 20 L/min can result in enough vibration of the capsule to release the Tio powder, no significant difference is found in delivery efficiency in a limited range of flow rates between 39 L/min and 46 L/min.

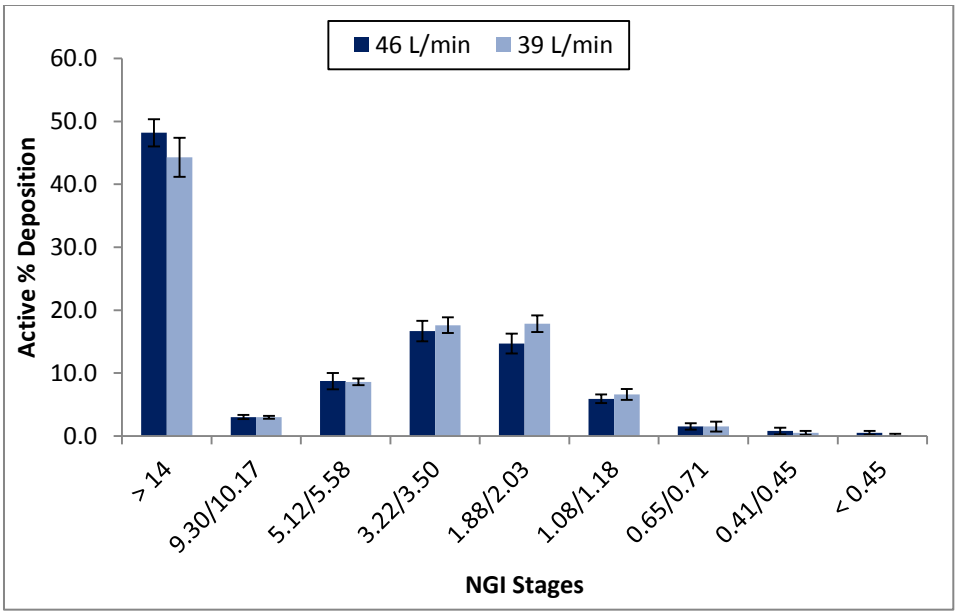

Figure 3. 8 NGI Distribution Pattern (%) of Handihaler® with Different Flow Rates of 46 and 39 L/min with associated cut-off diameters (46 L/min: n=6, 39 L/min: n=9; mean ± SD). According to t-test, more particles are deposited in IP, where particle size> 14 µm, at 46 L/min against 39 L/min (P< 0.05). Grouping of IP, Stage 1 and Stage 2 indicates significantly lower deposition at 39 L/min (P< 0.05), and accordingly, grouping of Stage 3 to MOC showed significantly higher deposition (P< 0.05).

In aerodynamic particle size distribution (APSD) testing, a pre-separator was assembled between the induction port (IP) and impactor body, mainly to retain the large carriers without affecting the size distribution of Tio, and capture carrier-bound or aggregated drug particles (Mohammed et al, 2012). When analysing the APSD, the IP and pre-separator are combined together as a group, where the particle size is over 14 µm. As Handihaler® is operated under different flow rates, the effective cut-off diameter (d50) of each stage is changed, which is stated in the Figure 3.8. The distribution pattern is similar between 39 and 46 L/min. However, more particles are found >14 µm in 46 L/min, 48.18 ± 2.17%, than 39 L/min, 44.29 ± 3.10%, which is statistically different (t-test, P< 0.05). When IP, Stage 1 and 2 are combined together as the non-respiratory fraction (particle diameter > 5.12 µm), then 39 L/min has a significantly lower deposition (P< 0.05). Correspondingly, when combining Stage 3 to MOC as the respirable fraction (particle diameter < 3.50 µm),

39 L/min has significantly higher deposition ($P< 0.05$). The increased flow rate could contribute to particle-particle and particle-device interaction, which cause more effective de-aggregation of drug de-association, which is indicated by decreased MMAD, but the increased velocity also leads to impaction and deposition of larger particles indicated by increased deposition of particles >14 µm . Therefore, in this research, the flow rate has no direct influence on the aerosolisation performance, e.g. FPF and FPD, of Handihaler® but the inverse relationship of MMAD and the flow rate is proven. The improved de-aggregation of Tio from carrier particles at higher flow rate is compensated by loss of large particles caused by the high momentum. In terms of Handihaler®, 39 L/min is sufficient and recommended as the reference flow rate.

3.5.4 In-vitro Testing of Respimat®

Respimat® contains Tio in the form of a solution formulation. The specific delivery mechanism leads to high delivery efficacy of Tio, which is considered relevant to the significantly increased risk of cardiovascular death than Handihaler® (Beasley et al, 2012). In the previously mentioned UPLIFT trial, patients with renal impairment, myocardial infarction or other cardiovascular diseases were excluded from the research (Tashkin et al, 2008). Another safety concern over Respimat® was raised about the increased risks of mortality and cardiovascular death by Singh et al (2011). Another *Tiotropium Safety and Performance in Respimat* (TIOSPIR) trial was initiated, with the findings supporting similar safety profiles and exacerbation efficacy between Handihaler® and Respimat® (Wise et al, 2013). In our study, the performance of Respimat® was determined by in-vitro methods to gain understanding of the difference of the delivery pattern and efficiency between different Tio delivery systems.

Respimat® is categorised as a soft mist inhaler or small volume liquid inhaler, and no standard testing method is available from Ph. Eur. and USP. From the PIL, the delivered dose or ex-device dose is 3.124 µg of Tio per actuation and two actuations comprise one medicinal dose. The other ingredients include benzalkonium chloride, disodium edetate, hydrochloric acid and water. As a SVLI, the performance of a solution formulation is highly dependent on the droplet size. To evaluate the influence of operating flow rate on the performance of Respimat®, two reported flow rates including 30 L/min and 15 L/min were performed at ambient conditions.

Table 3. 10 Aerosol Characteristics (ex-device) of Respimat® at Different Flow Rates at the Ambient Temperature (n=9; Mean ± SD)

Factors	30 L/min Ambient	15 L/min Ambient
Tio FPF (% < 5 μm)	53.47 ± 6.57	44.03 ± 6.55
Tio MMAD (μm)	2.72 ± 0.52	4.00 ± 0.91
Tio FPD (μg < 5 μm)	1.88 ± 0.29	1.49 ± 0.15
Tio GSD	3.31 ± 0.05	3.03 ± 1.20

At ambient conditions, the ex-device emitted doses were 3.51 ± 0.16 μg and 3.41 ± 1.16 μg under 30 and 15 L/min, which are 12% (30 L/min) and 9% (15 L/min) greater than the theoretical dose, respectively. As shown in Table 3.10, a flow rate decreases from 30 L/min to 15 L/min, resulted in significant decrease of FPF and FPD from 53.47% to 44.03% (t-test, P< 0.05) and 1.88 to 1.49 μg (t-test, P< 0.05) respectively, whist MMAD increases from 2.75 μm to 4.00 μm (t-test, P< 0.05). However, there is no significant difference in GSD of particle size distribution (t-test, P> 0.05). The aerodynamic particle size distribution is shown in Figure 3.9 with large variations observed in stage depositions. At increased flow rate, there is no indication of increase of IP deposition (t-test, P> 0.05) with increased particle velocity, and extra fine particles (< 0.5 μm) deposited on MOC are also comparable (t-test, P> 0.05). According to Zhou et al (2007), during inhalation, mist of droplets are mixed with the air to give a large inspiration volume, and the droplets size is affected by the proportion of the mixed air. At higher flow rates, e.g. 30 L/min, more air is inhaled and mixed with aerosolised droplets, which is likely to accelerate the evaporation of droplets and generate more fine particles. For a nebuliser, tidal breathing is required for the inhalation, where mid-inhalation flow rate of a healthy adult is around 15 L/min. However, the Respimat® requires a slow and deep breathing pattern to achieve optimal delivery efficacy, which is similar to a pMDI product. Therefore, a larger volume of air is required to inspire and mix with aerosols than the tidal breathing pattern of the nebuliser in clinic, where 30 L/min is recommended for in-vitro testing of Respimat® in this study.

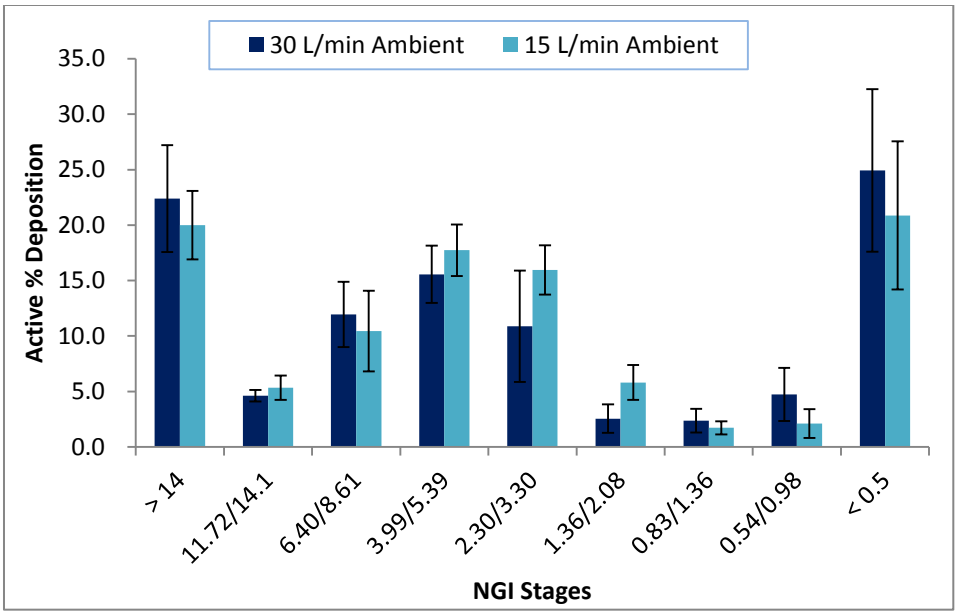

Figure 3. 9 NGI Distribution Pattern (%) of Respimat® at Different Flow Rates at the Ambient Temperature (n=9; Mean ± SD)

As a solution formulation, the aqueous droplets are more volatile in lower relative humidity and higher temperature when using a cascade impactor (Dennis and Ameen, 2003). During the in-vitro test, the environment conditions, i.e. temperature and relative humidity, could affect droplet generation and deposition, and therefore need to be well-controlled. It has been reported in nebuliser systems, the pattern of particle size distribution and MMAD of droplets were affected by relative humidity when using ACI at 28.3 L/min flow rate, as well as in cooled conditions of the NGI (Zhou et al, 2005). In previous studies (Pitcairn et al, 2005), Respimat® was recommended to be tested at 30 L/min, so to evaluate the influence of environment conditions, two batches of Respimat® were evaluated at cooled conditions at 30 L/min.

Table 3. 11 Aerosol Characteristics (ex-device) of Respimat® at Different Temperatures at 30 L/min (Ambient n=9; Mean ± SD, Cool n=3; Mean ± SD)

Factors	30 L/min Ambient	30 L/min Cool
Tio FPF (% < 5 μm)	53.47 ± 6.57	43.84 ± 0.49
Tio MMAD (μm)	2.72 ± 0.52	4.25 ± 0.17
Tio FPD (μg < 5 μm)	1.88 ± 0.29	1.59 ± 0.15
Tio GSD	3.31 ± 0.05	2.00 ± 0.02

The ex-device dose is 3.51 ± 0.16 μg (Ambient) and 3.63 ± 0.32 μg (Cool) with no statistic difference (t-test, P> 0.05). According to Table 3.11, compared with ambient conditions, FPF and FPD measured in cool conditions are significantly decreased (t-test, P< 0.05) whilst MMAD (t-test, P< 0.05) increased. There was no significant difference in GSDs (t-test, P> 0.05). The distribution of aqueous aerosols is dependent on clinical factors, e.g. patient inspiration patterns and physiological conditions of the respiratory tract, and the conditions used for in-vitro tests, e.g. flow rate, temperature and relative humidity, to reflect the equivalent pattern in-vivo. The generated droplets travel inside the impactor and deposit on different stages based on their particle size and velocity, but the possibility of droplet evaporation in vitro may lead to inaccurate predictions (Berg et al, 2007). Furthermore, the internal volume of the NGI with induction port (1245 mL) is greater than the ACI with induction port (975 mL) (Copley et al, 2005). Therefore, evaporation issues could be greater in the NGI. In cooled conditions, the air with higher relative humidity is mixed with the generated droplets inside the NGI, and it is found that the enhanced humidity decreases the evaporation of droplets, which contributes to smaller FPFs and FPDs, and larger MMADs. When comparing Respimat® (30 L/min, ambient conditions) and Handihaler® (39L/min, ambient conditions) as shown in Table 3.9 under similar environmental conditions, the FPF of Respimat® is 53.47 ± 6.57%, which is significantly higher than Handihaler®, 40.60 ± 3.16%. However, the FPD of Respimat® is 1.88 ± 0.08 μg and two puffs deliver a medicinal dose of 3.76 μg of Tio, which is similar to Handihaler®, 4.03 ± 0.54 μg of Tio. Therefore, the similarity of FPDs of Respimat® and Handihaler® in in-vitro data

may explain the comparable findings in the TIOSPIR trial regarding the safety profile and exacerbation efficacy (Wise et al, 2013).

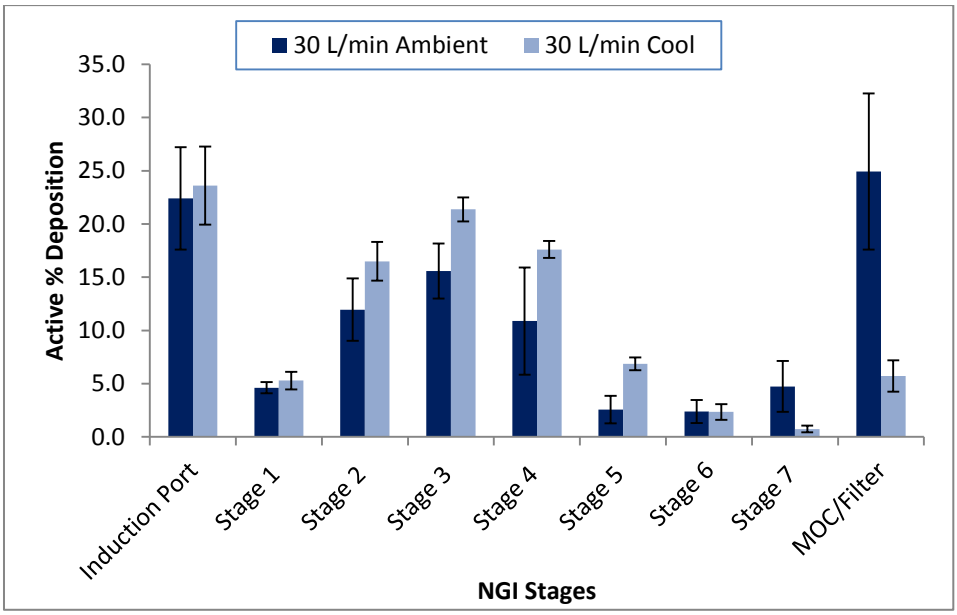

Figure 3. 10 NGI Distribution Pattern (%) of Respimat® at Different Temperatures at 30 L/ min (Ambient n=9; Mean ± SD, Cool n=3; Mean ± SD)

The distribution pattern under different environmental conditions are shown in Figure 3.10. There is no significant difference in the IP deposition regardless of the temperature and humidity (t-test, P> 0.05). The MOC and Filter represents ultra-fine particles < 0.5µm, and these two stages are combined together in the graph. Under ambient conditions, over 20% of the ex-device emitted dose is found less than 0.5 µm, and these extremely fine particles could be exhaled by patients without depositing in the respiratory tract. While in cool conditions, the percentage of extra fine particles decreases to less than 7%. Furthermore, statistically more drug particles deposit on Stages 2, 3, 4, and 5 (t-test, P< 0.05) in contrast with ambient conditions. Consequently, it is proven that under higher flow rate and low relative humidity, the droplets generated by Respimat® are more highly evaporated

inside the impactor which leads to changes in aerodynamic performance of the device. When the humidity is increased and the temperature decreased of the NGI body, the number of fine droplets are reduced significantly but compensate the delivery efficiency.

3.5.5 In-vitro Testing of Tiova®

Tiova®, as a pMDI formulation of Tio, has been marketed in certain "unregulated" territories (e.g. India). In vitro performance and stability of Tiova® was investigated as a reference to the novel Tio pMDI formulation. From the PIL, the medicinal dose of Tiova® is indicated as 22.5 µg from two actuations, but the label and information sheet provide no statement on whether 22.5 µg is the ex-valve or ex-actuator emitted dose. Based on our analyses, the ex-valve emitted dose per actuation was found to be 9.97 ± 1.60 µg, and the ex-actuator dose 8.96 ± 1.31 µg, with an average actuator deposition of 9.98 ± 0.52%.

Table 3. 12 Aerosol Characteristics (ex-actuator) of Tiova® over 6 Month Storage (n=3; mean ± SD)

Time Points	Release	1 Month	3 Month	6 Month
Tio FPF (% < 5 µm)	52.48 ± 0.57	55.92 ± 1.13	50.35 ± 0.73	50.50 ± 2.92
Tio MMAD (µm)	2.70 ± 0.09	3.13 ± 0.09	3.11 ± 0.10	3.08 ± 0.01
Tio FPD (µg < 5 µm)	4.85 ± 0.12	5.10 ± 0.23	4.57 ± 0.38	4.73 ± 0.19
Tio GSD	1.60 ± 0.03	1.57 ± 0.01	1.59 ± 0.04	1.62 ± 0.01

The aerosol performance characteristics of Tiova® over 6-months accelerated stability study are illustrated in Table 3.12. Based on ANOVA and Tukey HSD test, the 1 month sample shows the highest FPF with statistical significance ($P< 0.05$), and the rest are comparable ($P> 0.05$). However, there is no significant difference between FPD ($P> 0.05$) and GSD ($P> 0.05$). The Release (0 month) data exhibits the lowest MMAD ($P< 0.05$) where the remaining groups tested are similar ($P>0.05$). Consequently, the product performance of Tiova® has been established under the pharmacopeia method and the formulation shown to effectively maintain the physical stability and aerosol performance during the accelerated stability test. The aerosol performance of Tiova® at release was compared against the DPI product, the Handihaler®, tested at 39 L/min. Regarding the FPF, Tiova® (52.84 ± 0.57) (Table 3.12) was significantly higher than Handihaler® (40.60 ± 3.16%) (Table 3.9). With respect to FPD, Tiova® (4.85 ± 0.12 µg per actuation, with two actuations delivering about 9.70 µg of fine particle dose of Tio) was more than two-fold of the FPD of

Handihaler®, 4.03 ± 0.54 (Table 3.9). Therefore, the in-vitro data indicates a better aerosol performance of Tiova® when compared against Handihaler®.

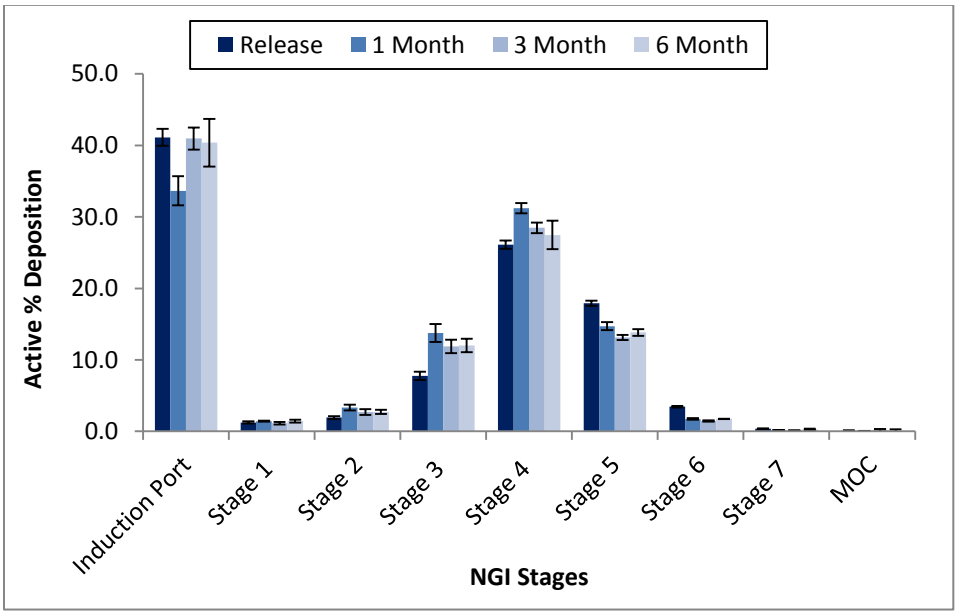

Figure 3. 11 NGI Distribution Pattern (%) of Tiova® over 6 Month Storage (n=3; mean ± SD)

According to Figure 3.11, similar distribution patterns were obtained during 6-months storage. The IP stage deposition was observed for all the time points to be in the range of 33.65% - 41.12%. According to ANOVA and Tukey HSD test, the lowest IP deposition ($P< 0.05$) was at 1 month, whilst the remaining groups tested were similar ($P> 0.05$). When IP, Stage 1 and 2 were combined together as the non-respiratory fraction (particle diameter > 6.4 µm), 1 Month data also showed the lowest deposition ($P< 0.05$), and the rest of the groups were comparable ($P> 0.05$). Correspondingly, when combining Stage 3 to MOC as the respiratory fraction (particle diameter < 3.99 µm), 1 Month has the significantly highest deposition ($P< 0.05$), which is also demonstrated by the greatest FPF in Table 3.12. In general, the aerosol performance of Tiova® is consistent over the 6-month storage, however the data is not robust where deviations are observed in the 1 month group.

The ex-valve dose uniformity is shown in Figure 3.12. The ex-valve dose was reproducible throughout the canister life except two outliers at Shot 59 and Shot 119. The two outliers are from Can01 and Can03 which indicates canister variability. The same pattern is observed in the ex-actuator dose uniformity in Figure 3.13. Ex-actuator emitted doses are consistent throughout the canister life except two high doses at Shot 118 and Shot 119, which are relevant to the high ex-valve dose regardless of the performance of the actuator. Due to the limited number of Tiova® products, three canisters were conducted for dose uniformity testing, and larger batches of products would be required for more extensive investigations. The average of the ex-actuator dose of Tiova® is 8.96 ± 1.31 μg per actuation, and two actuations deliver about 17.92 μg, whist the ex-device dose of Handihaler® is 10.61 ± 2.21 μg per capsule (Table 3.8). There is a lack of published data relevant to Tiova®, however based on this initial evaluation, Tiova® exhibits a high and consistent FPF and FPD, and the formulation maintained physically stable during the accelerated stability study.

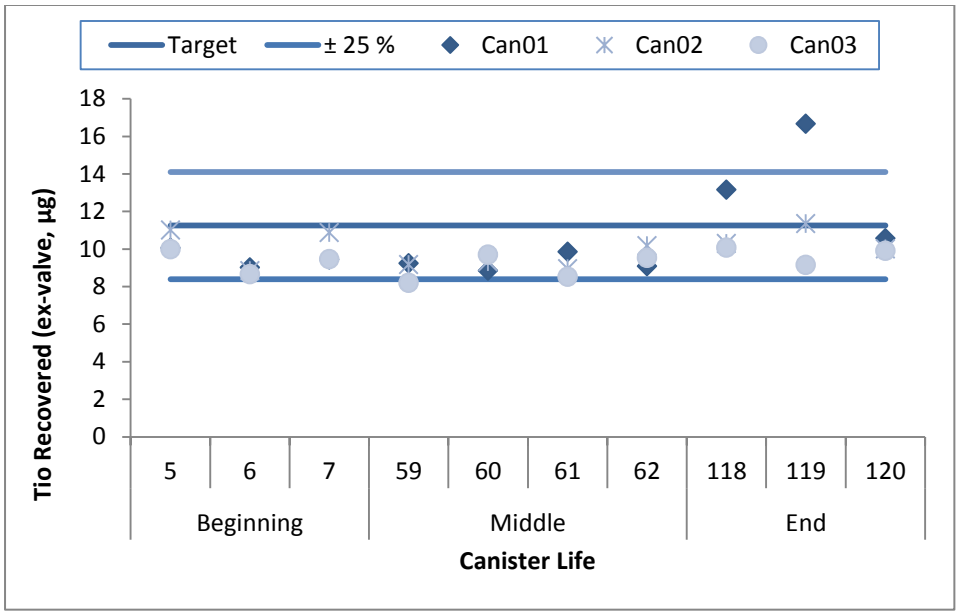

Figure 3. 12 Ex-valve Dose Uniformity of Tiova® over 6 Month Storage (n=3; mean ± SD)

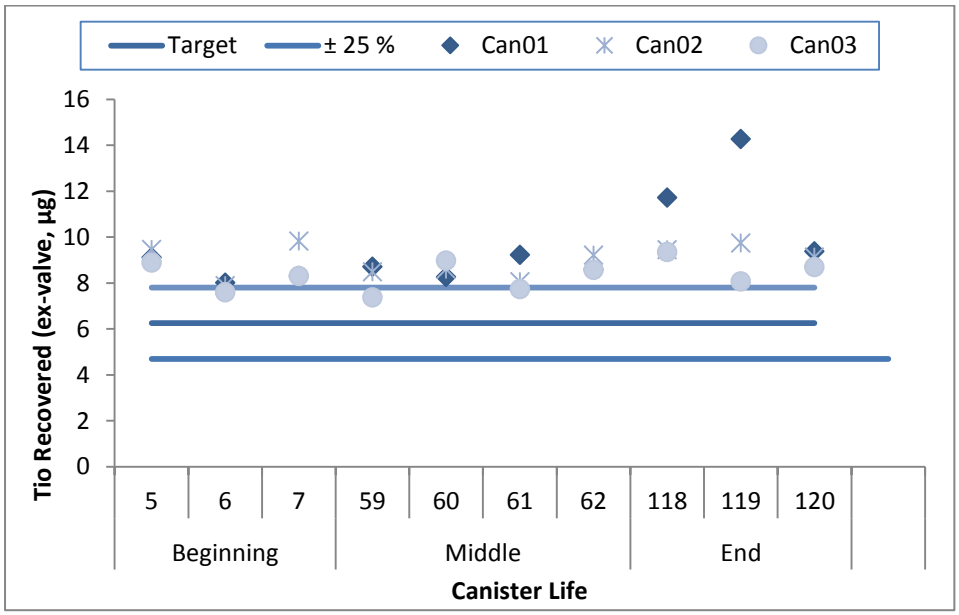

Figure 3. 13 Ex-actuator Dose Uniformity of Tiova® over 6 Month Storage (n=3; mean ± SD)

3.6 Conclusion

Micronised Tio was shown to be practically insoluble in both HFA 134a and 227 initially, and during accelerated storage at 40 °C/75 RH. There was no indication of an association between the ingress of water and dissolution of Tio particles when the moisture level was maintained at low levels of <550 ppm for HFA 134a and <450 ppm for HFA 227. No physical instability of the suspension caused by environmental factors was identified. A further investigation spiking a large amount of water into the formulation may be required to gain better understanding of the influence of increased water levels on Tio physical and chemical stability. Nevertheless, Tio is considered to be feasible to be formulated in suspension pMDI systems and the addition of leucine as a secondary particle demonstrates advantages to increase the physical stability of micronised Tio particles and maintain the suspension uniformity during the accelerated storage. The preliminary research suggests the secondary particle can effectively prevent the interaction and adherence between Tio particles and device components and maintain the consistence of emitted doses of pMDIs even at low concentrations.

Based on the initial evaluation of the commercialised products, the delivery efficiency and particle distribution patterns have been established as references to evaluate the performance of the novel Tio pMDI formulation. Handihaler®, the most popular device for treating COPD, delivers Tio in a dry powder form. Due to the inherent device resistance, 45% of the total dose is trapped in the device itself including the capsule and mouthpiece. The standard in-vitro aerosol performance tests for Handihaler® were performed at 46 L/min for dose uniformity testing, and 39 L/min for particle size distribution testing. Comparable FPF and FPD were identified at 39 L/min and 46 L/min, indicating that the change of flow rate between 39 L/min and 46 L/min had no influence on generation of fine particles in the respirable range. Although the increased flow rate could lead to an increase in

de-aggregation potential of Tio from carrier particles, where a smaller MMAD is found with 46 L/min, the generation of fine particle appears to be compensated by increased particle velocity.

Respimat®, as a novel and highly efficient inhaler, delivers Tio in solution form. Though Respimat® is reported to have potential to increase mortality relevant cardiovascular disease, the new clinical trial, TIOSPIR, has indicated comparable clinic performance with Handihaler® (Wise et al, 2013). During in-vitro testing, the flow rates and environmental conditions, e.g. temperature and relative humidity, were found to affect the formation of droplets and performance of the formulation. At increased flow rates an increase in temperature and decrease in humidity lead to more effective evaporation of droplets, resulting in higher FPFs and FPDs. Therefore, the environmental conditions for in-vitro testing of Respimat® need to be controlled at certain values. In this research, the recommended parameters for the NGI test of the Respimat® is 30 L/min at ambient conditions.

Tiova®, the equivalent pMDI product to Handihaler®, shows a high IP deposition due to the high velocity of the aerosol, but high FPFs (> 50%) were also observed. Furthermore, consistent aerosol performance was exhibited in the accelerated stability study.

Consequently, the aerosol performance characteristics of three commercialised Tio products have been well established. The investigation of factors affecting the novel pMDI formulation and the performance of the novel formulation will be further conducted and discussed in the following chapters.

4 The Influence of Novel Excipients and pMDI Components on Aerosol Performance

4.1 Introduction

In the development of pMDI systems, the research mainly focused on two aspects of the pMDI, the formulation and the hardware or pMDI component. Drugs in pMDIs were either completely dissolved in liquefied propellants as a solution system, often with other excipients such as co-solvents and preservatives, or suspended in propellants as a suspension system with excipients like co-solvents and surfactants (Myrdal et al, 2014). In this research, Tio is selected as the model drug and suspension formulation is our main focus. The formulation design is referred to the DPI product, the Handihaler®. As described in Chapter 3, each Handihaler capsule contains 22.5 µg Tio corresponding to a 12.5 µg ex-mouthpiece delivery within two inhalations. Accordingly, in the design of the pMDI formulation, the metered dose (ex-valve) per actuation contains 11.25 µg Tio targeting a 6.25 µg ex-actuator delivery, and two inhalations gives a medical dose of 12.5 µg. The role of excipients, including the secondary particles, e.g. lactose and leucine, and propellants e.g. HFA 134a and HFA 227, are investigated to give a clear understanding of effects of their physico-chemical properties on the in vitro performance of pMDIs. The formulation is contained in an airtight environment, the hardware, to keep consistent storage conditions avoiding influence of air, moisture and other environment factors, and maintain constant vapor pressure. Therefore, the pMDI components including the canister, the metering valve and the actuator are also evaluated to demonstrate their influence on the physical stability of the formulation and efficiency of the aerosol delivery.

Excipients are used in MDI and DPI products to maintain the safety, quality, stability, performance and effectiveness of the active pharmaceutical ingredients (APIs) (FDA, 1998). In powder form, due to the cohesive and hygroscopic properties, micronised

drugs tend to agglomerate or aggregate which leads to poor powder uniformity and compromised aerodynamic properties. Excipients or carriers are normally used in DPIs to improve the powder dispersibility and flowability, and increase the delivery efficiency. The selection of excipients is essential as physico-chemical and aerodynamic properties, particle size, shape and surface of the excipients directly affect deposition pattern of the drug. Excipients should also have adequate adhesion force with drugs so that the shearing force of the inspired airflow detaches the drug from the carrier particles, with the aim of achieving deep lung deposition (Telko and Hickey, 2005). Dickinson and Warren (2004) described a pharmaceutical composition wherein micronized drugs were combined with physiological acceptable excipients, described as secondary particles, within a pMDI formulation. The secondary particles, with larger particle size within the range of 15 to 200 μm, were used to keep the suspension homogeneous and reduce undesirable aggregation. The stability of a suspension system is dependent on the balance of two types of particle interactions including the attraction force (e.g. Van der Waals attraction force) and repulsion force (e.g. electrostatic repulsive force). Due to the large specific surface area, drug particles, in the range of 1 - 5 μm, tend to decrease their surface energy by attaching to each other. Merged large agglomerates could lead to reduced powder flowability and inaccurate metering of doses, and even block the valves or actuators. With the addition of secondary particles, it was hypothesised that drug particles could be adsorbed on the surface of the larger particles, and become stabilised by steric obstruction. The mixture of particles could sediment or float depending on the density of the secondary particles, and be easily re-dispersed by several shakings. To take the previous work by Dickinson and Warren (2004) forward, a new drug candidate with high potency was evaluated with the addition of secondary particles at a low concentration. As the feasibility of excipients used for Tio-containing pMDI had been initially established in Chapter 3, this was further investigated and described in this chapter. The influence of the formulation on the performance of pMDIs was mainly focused

in this chapter on the physico-chemical properties of the novel excipients and propellants e.g. excipient ratio, particle size, excipient and propellant types.

Aside from the formulation a pMDI device consists of a canister, a metering valve and an actuator. The performance of a pMDI device is dependent on the combined action of the formulation and hardware components. In a pMDI, the valve is pre-filled with the formulation from the bulk in the canister. As the valve is depressed, the liquefied propellant containing formulation starts to boil in the expansion chamber of the actuator. The liquid/vapor (solution) or liquid/solid/vapor mixture (suspension) is atomised into fine droplets by aerodynamic force at the spray orifice, forming a spray plume (Clark, 1996; Newman, 2005; Stein, 2008). The pMDI canister, as the containing device, is critical to the physical and chemical stability of the formulation. As a pressurised environment, the canister must be able to withstand the high pressure induced by the liquefied propellants. The inert canister materials include plastic, plastic-coated glass, tin-plated steel and more preferable aluminium. Inner coatings (e.g. anodized aluminium or perfluoroalkoxyalkane) are recommended due to the absorption of APIs on the canister inner surface, corrosion of the canister materials or catalytic degradation of APIs (Smyth, 2003). The valve, as the most critical component of a pMDI, consists of two main parts including the metering chamber and the valve stem. It is responsible for accurate and reproducible dosing, so the valve material must be compatible with the formulation without API loss during repeated use. The valve has direct contact with the formulation and the potential leachable compounds released from the valve material will be considered with significant safety concern (Ball et al, 2007). These compounds may also negatively influence the formulation stability, and therefore leachables from the elastomeric and plastic components should be minimised or eliminated (FDA, 1998). The actuator is a moulded plastic component, and essential for the formation of aerosol plume and directing the high-velocity aerosol to the

respiratory tract. Under pressing of the canister, which is seated into the actuator, the metered dose is connected to the expansion chamber of the actuator through the valve stem. The atomisation of liquefied propellants occurs at the actuator orifice, and the actuator features e.g. orifice size, length and sump depth are significant factors influencing the droplet size, plume exit velocity, plume geometry, and the final particle distribution (Smyth et al, 2006; Ganderton et al, 2003). Therefore, the influence of the pMDI components in this chapter were mainly focused on the physical properties of the device hardware, e.g. the actuator orifice, metering valve volume, canister volume and coating.

4.2 Chapter Aims and Objectives

The aims of the work described in this Chapter were to investigate the formulation and hardware variables for their influence on pMDI aerosolisation performance. The potential interaction of Tio particles with different types of inert particle excipients, e.g. lactose (Respitose® SV003, SV010) and leucine, and hardware components were evaluated by in-vitro testing.

The objectives of this chapter are as follows:

1) To determine the influence of canister volumes and canister coating materials on physical stability and aerosol performance of Tio pMDI suspensions, where two types of volume were used, e.g. 14 and 19 mL, and two types of canister coating materials were used, e.g. plain aluminium and FCP.

2) To determine the influence of valve volumes on physical stability and aerosol performance of Tio pMDI suspensions, where three types of valve volumes were used, e.g. 25, 35 and 50 µL.

3) To determine the influence of actuator orifices on physical stability and aerosol performance of Tio pMDI suspensions, where four types of orifices were used, e.g. 0.25, 0.35, 0.40 and 0.46 mm.

4) To determine the influence of propellant types on physical stability and aerosol performance of Tio pMDI suspensions, where two types of HFA propellants were used, e.g. HFA 134a and HFA 227.

5) To determine the influence of excipient ratios on physical stability and aerosol performance of Tio pMDI suspensions, where four types of excipient ratios were used, e.g. Tio: Excipient (w/w) 1: 2.5, 1: 5, 1: 10 and 1: 25.

6) To determine the influence of excipient types on physical stability and aerosol performance of Tio pMDI suspensions, where three types of excipients were used, e.g. Respitose® SV003, Respitose® SV010 and L-leucine.

7) To determine the influence of excipient sizes on physical stability and aerosol performance of Tio pMDI suspensions, where three types of excipient sizes were used, e.g. Sieve Fraction <63 µm, <38 µm and <20 µm.

4.3 Materials and Equipment

4.3.1 Materials

Tiotropium Bromide Monohydrate (Tecoland); Respitose® SV003 Monohydrate Lactose (DMV-Fonterra Excipients); Respitose® SV010 Monohydrate Lactose (DMV-Fonterra Excipients); L-Leucine (Sigma-Aldrich Co); HFA 134a (Mexichem); HFA 227 (Mexichem); 2H, 3H-decafluoropentane (Apollo); Benzyltriethyl Ammonium Chloride (Sigma); 14/19 mL Plain/FCP Aluminium MDIs Canisters (H&T Presspart Manufacturing); 25/35/50 µL KHFA Metering Valve (Valvole Aerosol Research Italiana Spa, VARI); 0.25/0.35/0.40/0.46 mm Actuator (H&T Presspart Manufacturing); 0.2 µm Nylon Membrane Filters (Whatman); Liquid Carbon Dioxide (BOC); Oxygen-Free Nitrogen (BOC); Polyethylene Glycol Laboratory Reagent Grade (Fisher); Brilliant Blue R (Sigama)

4.3.2 Equipment

Agilent 1200 Series HPLC System consisting: Quaternary Pump G1311A, Multiple Wavelength Detector G1314B, Autosampler G1329A, Thermostatted Column Compartment G1316A, Degasser G1322A, Chemstation LC software Rev. B.03.02 (Agilent Technologies); Agilent 1200 Series Isocratic System consisting: Isocratic Pump G1310A, Variable Wavelength Detector G1314B, Autosampler G1329A, Thermostatted Column Compartment G1316A, Chemstation LC Software Rev. B.04.02 (Agilent Technologies); Genesis C-18, 150×4.6, 5 µm Column; New Generation Impactor (Copley Scientific); Dosage Unit Sampling Apparatus (DUSA) (Metal & Copley Scientific); NGI Gentle Rocker (Copley); Turbula Mixer (Willy A. Bachofen AG Maschinenfabrik); Two Stage Filling Equipment (Pamasol) with laboratory scale propellant pump (X2008-00) and crimping head (X2002-0043/013); HPC SM12 Rotary Screw Air Compressor (Straightset); PH300 pH Meter (Hanna Instrument); Mastersizer 2000 Particle Size Analyzer (Malvern); Minor M200 Sieve Shaker (Endecotts); Adventurer Pro AV264CU Digital Balance (Ohaus); MC410S

Analytical Balance (Sartorius); Drying Cabinet (Genlab); SM26 Magnetic Stirrer Hotplate (Stuart Scientific; 7006 Ionizing Bar (Exair); TLP-2844 Printer (Zebra)

4.4 Methods

Methods including particle size analysis, preparation of canisters, dose uniformity and aerodynamic particle size distribution tests were as described in Chapter 2. Modifications of any methods are specified in this chapter.

4.4.1 Investigation of Canister Volume

Canisters are usually made of aluminium, although others, made of stainless steel or glass have been used. In this section, plain aluminium canisters were used to evaluate the effect of canister volume or equivalent inner surface area (CAN 14/19 mL) on the physical stability and in-vitro performance over the pMDI life. Two control batches containing Tio only without secondary particles were prepared with HFA 134a as the reference batches, and filled in either 14 or 19 mL plain aluminium canisters. Two other experimental batches were prepared at 1:5 (w/w) ratio of Tio: SV003 and suspended in HFA 134a in 14 and 19 mL plain aluminium canisters. The manufacture of a 20-canister batch of control canisters (Tio only) was prepared according to Section 2.7 without manufacture processes, e.g. excipient preparation, and dry mixing and blending. The manufacture of 60-canister batch of experimental canisters (Tio: SV003) was according to Section 2.4 to 2.7, wherein the particle size of SV003 was sieved to <38 µm. All the canisters were then crimped with 25 µL VARI® KHFA metering valves, and all the generated data was based on 0.25 mm H&T Presspart® actuators. Uniformity of delivered dose was measured according to Section 2.8.2, and the aerodynamic particle distribution was measured according to Section 2.8.3.

4.4.2 Investigation of Valve Volume

The metering valve works as the core component of the pMDI system, which ensures the accurate metering of uniform dosage from the bulk formulation. In this chapter, we mainly focused on the influence of valve metering chamber volume (VAL 25/35/50 µL) on the in-vitro performance of the novel formulation. Respitose® SV003 was used as the model excipient and HFA 134a used as the model propellant. Two batches of canisters including 1:25 (w/w) Tio: SV003, and 1:5 (w/w) Tio: SV003 were manufactured.

The formulation of 1:25 (w/w) of Tio: SV003 was prepared according to Section 2.4 to 2.7 wherein the particle size of SV003 was sieved to <38 µm. The formulation was suspended in HFA 134a in 19 mL plain aluminium canisters, and three batches of canisters were crimped with VARI KHFA metering valves with different metering volumes including 25 µL, 35 µL and 50 µL separately. The target emitted dose of Tio was 11.25 µg per actuation, and therefore each batch contained the same amount of Tio and SV003 but different volumes of the propellant. All the generated data was based on 0.25 mm H&T Presspart® actuators. The aerodynamic particle distribution was measured by a standard NGI (Section 2.8.3).

Another batch was prepared at 1:5 (w/w) ratio of Tio: SV003 according to Section 2.4 to 2.7 wherein the particle size of SV003 was sieved to <38 µm. The formulation was suspended in HFA 134a in 19 mL plain aluminium canisters, followed by crimping with 25 µL VARI KHFA valves. The target emitted dose of Tio was 11.25 µg per actuation, and each canister was equipped with a 0.25 mm H&T Presspart® actuator. The aerodynamic particle distribution was measured according to Section 2.8.3.

4.4.3 Investigation of Propellant Type

Propellants in the pMDI system are used as both the dispersed phase and the source of energy for generating aerosols (Pilcer and Amighi, 2010). The two approved pharmaceutical propellants, HFA 134a and HFA 227 differ in several physico-chemical properties, for example HFA 134a has a higher vapour pressure than HFA 227 (5.72 vs. 3.90 bar), while HFA 227 has a higher density (1.41 vs. 1.21 g/mL) and viscosity (0.26 vs. 0.22 mPa.s) than HFA 134a. It is important to investigate and understand the effects of differences in physico-chemical properties of these HFA propellants on the performance of the novel formulation. Two batches of the formulation 1: 5 (w/w) ratio of Tio: SV003 were suspended in HFA 134a and HFA 227 separately in 14 mL plain aluminium canisters. A 60-canister batch was prepared for each formulation according to Section 2.3 to 2.7, wherein the particle size of SV003 was sieved to <38 µm. All the canisters were crimped with 25 µL VARI KHFA metering valves, and equipped with 0.25 mm H&T Presspart® actuators. The canisters containing HFA 227 were filled by cold transfer according to Section 2.7 and the transfer percentages of HFA 227 was 98.4% ± 0.4% (n=3, mean ± SD). Uniformity of delivered dose was measured by metal DUSA (Section 2.8.2), and the aerodynamic particle distribution was measured by a standard NGI (Section 2.8.3).

4.4.4 Investigation of Actuator Orifice

The pMDI actuator is a key component in a pMDI system which determines the atomisation and delivery of the generated aerosol from the nozzle of the actuator. Initial studies investigated the effect of the size of actuator orifices (ACT 0.25/0.35/0.40/0.46 mm) on a Tio: Leucine formulation. Further studies explored the effects on Tio formulations containing Respitose SV003, the commercial carrier approved by regulatory authorities for use in inhalation dosage forms.

A formulation was prepared at 1:5 (w/w) ratio of Tio: Leu and suspended in HFA 227. According to the reference product, the Handihaler, the ex-valve dose was initially designed as 11.25 µg per actuation to give a 6.25 µg ex-actuator delivery. It was found that the pMDI formulation containing leucine suggested an increase of both the ex-valve and ex-actuator doses. Therefore, the ex-valve dose in the formulation containing leucine was reset as 9 µg to target the ex-actuator dose of 6.25 µg per actuation. The manufacture of this 60-canister batch was according to Section 2.4 to 2.7. In Section 2.5, bulk formulation powder was treated with 20 µm sieve. The 25 µL valve was noticed with an over-metering of the formulation, so each canister was filled with 40% overage of doses to satisfy the canister life with 120 actuations. Three canisters were discharged with H&T Presspart actuators with different orifice sizes e.g. 0.25 mm 0.35 mm, 0.40 mm and 0.46 mm. According to Section 2.8.2, uniformity of delivered dose was measured by metal DUSA. In the beginning (shot 5, 6, 7, and 8), middle (shot 58, 59, 60, and 61) and end (117, 118, 119, and 120) of canister life, each canister was discharged once in each stage of canister life, in total 3 actuations. The aerodynamic particle distribution was measured by a standard NGI (Section 2.8.3).

A further formulation was prepared at 1:5 (w/w) ratio of Tio: SV003 and suspended in HFA 227, and the ex-valve dose was set as 11.25 µg per actuation. The manufacture of this 60-canister batch was according to Section 2.4 to 2.7. Each canister contained 120 doses plus 40% overage. Five canisters were discharged with H&T Presspart® actuators with orifice size of 0.25 mm and 0.46 mm. The uniformity of delivered dose was measured according to Section 2.8.2, and aerodynamic particle distribution was according to Section 2.8.3.

4.4.5 Investigation of Canister Coating

The inner surface of pMDI canisters has direct and intimate contact with the formulation. Potential catalytic degradation of the drug and drug deposition on the canister inner surface therefore represents challenges in pMDI devices. Canister surface coating, e.g. fluorocarbon polymerisation (FCP) plasma treatment, has been widely used to reduce unintended drug loss. As discussed in Section 4.5.7, either lactose SV010 or leucine were used as the model secondary particle.

Two batches of formulations were prepared at 1:5 (w/w) ratio of Tio: SV010 and suspended in HFA 227 in either 14 mL plain aluminium (CAN Plain) or 14 mL FCP-coated aluminium (CAN FCP) canisters. The manufacture process was referred to Section 2.4 to 2.7. In section 2.5, Tio was de-aggregated by a 20-µm sieve and the particle size of leucine was further narrowed down to less than 20 µm by re-sieving the whole mixture with a 20-µm sieve. The target ex-valve dose was set as 11.25 µg, and a total of 120 doses plus 40% overage filled in each canister, crimped with 25 µL VARI KHFA valves. All the data was generated with 0.25 mm Presspart® actuators. Uniformity of delivered dose was measured according to Section 2.8.2, and the aerodynamic particle distribution was measured according to Section 2.8.3.

Two more batches of formulations were prepared at 1:5 (w/w) ratio of Tio: Leu and suspended in HFA 227 in 14 mL plain aluminium or FCP-coated aluminium canisters. The manufacture of these batches was according to Section 2.4 to 2.7. In section 2.5, Tio was de-aggregated and the whole mixture was then re-sieved by a 20-µm sieve. The ex-valve dose was designed as 9 µg per actuation as described in Section 4.4.4. Each canister was filled with 120 doses plus 40% overage. All the generated data was based in 0.25 mm H&T Presspart® actuators. Uniformity of delivered dose was measured according to Section 2.8.2, and the aerodynamic particle distribution was measured according to Section 2.8.3.

4.4.6 Investigation of Excipient Ratio

The effects of excipient ratio on the performance of the novel suspension formulation were investigated in this section. Formulations were prepared with different ratios of Tio: SV003 (w/w) including. 1: 2.5, 1: 5, 1: 10 and 1:25, coded as EXCIP 1:2.5/1:5/1:10/1:25, and suspended in HFA 134a in 19 mL plain aluminium canisters, crimped with 50 µL KHFA metering valves. All the generated aerosol data was based on 0.25 mm H&T Presspart® actuators. Four 25-canister batches were prepared according to Section 2.3 to 2.6, and a 38 µm sieve was used. Uniformity of delivered dose was measured according to Section 2.8.2, and the aerodynamic particle distribution was measured according to Section 2.8.3.

The influence of metering valve volume was discussed in Section 4.5.2, and 25 µL was proven to be more effective to deliver the formulation containing 1:5 (w/w) Tio: SV003. Therefore, a metering valve volume of 25 µL was also used for a single formulation containing 1:5 (w/w) Tio: SV003. This formulation was prepared and crimped with 25 µL metering valve, referred to Section 2.3 to 2.6. The bulk formulation powder was treated with 38 µm sieve, and the manufactured canisters were equipped with 0.25 mm H&T Presspart® actuators. Uniformity of delivered dose was measured according to Section 2.8.2, and the aerodynamic particle distribution was measured according to Section 2.8.3.

4.4.7 Investigation of Excipient Types

The patent of Dickinson and Warren (2004), listed various types of secondary particle including carbohydrates, amino acids, poly-peptides and proteins. However, commercial choices of excipients are limited in pulmonary application, and only lactose (Spiriva®), glucose (Bronchodual®) and mannitol (Exubera®) have been approved by the FDA. In this research, two types of commercialised inhalation grade lactose, Respitose® SV003 and SV010, and one type of amino acid, leucine, were investigated in novel Tio pMDI formulations. The particle sizes of these raw materials were analysed according to Section 2.5. The control canisters were filled with Tio only without addition of any excipient, and then suspended in HFA 227 in 14 mL plain aluminium canisters by two-stage filling (Section 2.6).The experimental formulations were prepared at 1:5 (w/w) ratio of Tio: excipients, e.g. SV003, SV010 and leucine according to Section 2.3 to 2.5. As described in Section 2.4, leucine was treated with mechanical vibrating and the particle fraction of 90-63 µm was used. During the dry mixing and blending (Section 2.5), the particle size of SV003 was sieved to <38 µm, and SV010 and leucine were sieved to <20 µm. Each formulation was weighed separately into 14 mL plain aluminium canisters and suspended in HFA 227 wherein Tio: SV010 and Tio: Leu were prepared by two-stage filling, and Tio: SV003 was prepared by cold transfer. Each canister contained 168 doses, and was crimped with 25 µL VARI KHFA metering valves. All the generated aerosol data was based on 0.25 mm H&T Presspart® actuators. Uniformity of delivered dose was measured using a metal DUSA, according to Section 2.8.2. The aerodynamic particle distribution was measured by a standard NGI according to Section 2.8.3.

4.4.8 Investigation of Excipient Particle Size

In the format of dry powder, inter-particulate interactions are influenced by the physico-chemical properties of the materials, particularly the particle size distribution (Louey et al, 2003). In suspension these interactions will occur amongst and between drug particles, carrier particles, and propellant molecules. The effect of particle size of the excipient was investigated. A Mastersizer 2000 laser diffraction technique was used to determine the particle size distribution based on the particle volume, with particle size calculated as an equivalent sphere diameter. According to Section 2.4, different portions of SV010 were prepared by sieve grinding including <90 µm (FRAC <90 µm), <63 µm (FRAC <63 µm) and <38 µm (FRAC <38 µm). Excipient size analysis was conducted by Mastersizer® 2000 particle size analyser as described in Section 2.5. Three batches of formulations were prepared at 1: 5 (w/w) ratio of Tio: SV010 and suspended in HFA 227 in 14 mL FCP canisters. Modification was made to the dry mixing and blending process in Section 2.4. Following the standardised trituration, the whole bulk formulation powder containing SV010 <90 µm was sieved through a 63 µm mesh, the powder containing SV010 <63 µm was through a 38 µm mesh, and the powder containing SV010 <38 µm was through a 20 µm mesh separately. The obtained SV010 particle fractions included <63 µm, <38 µm and <20 µm. Uniformity of delivered dose was measured by metal DUSA (Section 2.8.2), and the aerodynamic particle distribution was measured by a standard NGI (Section 2.8.3).

Leucine as a prospective secondary particle has been investigated in Section 4.5.7. Three batches of formulations were prepared at 1: 5 (w/w) ratio of Tio: leucine and suspended in HFA 227 in 14 mL FCP canisters. According to Section 2.3, the pre-treatment of leucine was required. Different sieve portions of leucine (e.g. 90-63 µm and 63-38 µm) were obtained by mechanical vibrating using a M200 minor sieve shaker, while the portion <20 µm was sieved manually by a laboratory test

sieve with 20 µm aperture. The manufacture process was according to Section 2.4 to 2.7. In Section 2.4, standardised trituration process was conducted and afterwards the whole formulation powder containing leucine 90-63 µm was sieved through a 63 µm mesh, the powder containing leucine 63-38 µm was through a 38 µm mesh, and the powder containing leucine <20 µm was through a 20 µm mesh separately. The obtained leucine particle fractions included <63 µm, <38 µm and <20 µm. Uniformity of delivered dose was measured according to Section 2.8.2, and the aerodynamic particle distribution was measured according to Section 2.8.3.

4.5 Results and Discussion

4.5.1 Canister Volume

The canister of the pMDI system plays a role as a container to store the filled formulation and maintain the physical and chemical stability. However, the electronegative property of HFA propellants can cause intense interaction between particle and device surfaces which leads to potential adhesion of the drug particles on the inner surface of the canister (Jinks, 2008). The size of the canister or canister volume is assumed to affect the drug concentration over the shelf life, and finally reduce the dose that patients receive. The canister volume is normally in the range of 10 to 22 mL, and in this section, 14 mL and 19 mL plain aluminium Presspart® canisters are utilized. Tio: SV003 (w/w) 1:5 is suspended in HFA 134a as the experimental groups, and control groups containing Tio only in HFA 134a are prepared to co-evaluate the influence of canister volume and secondary particles on the physical stability and aerosol performance of Tio suspension.

Table 4. 1 Aerosol Characteristics (ex-actuator) of Tio Controls (Tio Only) and Tio: SV003 with Different Canister Volumes (14 mL Control: n=5; mean ± SD, 14 mL/19 mL Control/19 mL: n=3; mean ± SD)

Canister Volume	14 mL Control (Tio Only) (a)	14 mL Tio: SV003 (b)	19 mL Control (Tio Only) (c)	19 mL Tio: SV003 (d)
Tio FPF (% < 5 µm)	20.70 ± 1.69	34.67 ± 3.32	18.10 ± 2.56	36.78 ± 3.07
Tio MMAD (µm)	5.43 ± 0.21	3.33 ± 0.05	4.57 ± 0.30	2.38 ± 0.20
Tio FPD (µg < 5 µm)	1.43 ± 0.17	2.77 ± 0.43	2.25 ± 0.63	3.39 ± 0.16
Tio GSD	2.05 ± 0.10	2.27 ± 0.13	2.13 ± 0.08	2.17 ± 0.21

((a):Tio Only/HFA 134a/VAL 25 µL/CAN 14 mL/CAN Plain/ACT 0.25 mm, (b) Tio:SV003/EXCIP 1:5/FRAC <38 µm/HFA 134a/VAL 25 µL/CAN 14 mL/CAN Plain/ACT 0.25 mm, (c) Tio Only/HFA 134a/VAL 25 µL/CAN 19 mL/CAN Plain/ACT 0.25 mm, (d) Tio:SV003/EXCIP 1:5/FRAC <38 µm/HFA 134a/VAL 25 µL/CAN 19 mL/CAN Plain/ACT 0.25 mm)

Table 4.1 illustrates parts of the aerodynamic distribution characteristics of Tio formulations using different volume canisters. In control groups (Tio Only), the ex-actuator emitted doses are 6.93 ± 0.66 µg (14 mL) and 12.31 ± 1.66 (19 mL) µg

respectively. As the volume of the canister increases, FPD increases (t-test, $P< 0.05$) and MMADs decreases (t-test, $P< 0.05$). No significant difference is found in the FPF (t-test, $P> 0.05$) and GSD (t-test, $P> 0.05$). The increase of FPD in 19 mL canisters may be caused by high ex-actuator emitted doses but a large fraction about 66% of the ex-actuator dose is captured in the IP stage in Figure 4.1. This suggests the formation of big agglomerates and the suspension is not well dispersed in 19 mL canisters. In experimental groups (Tio: SV003), the ex-actuator emitted doses are 8.03 ± 1.39 μg (14 mL) and 9.23 ± 0.35 μg (19 mL) respectively. The MMAD of formulation from the 19 mL canister was significantly smaller than 14 mL canister (t-test, $P< 0.05$), and other characteristics, e.g. FPF and FPD, are similar (t-test, $P> 0.05$). The MMAD is reduced in 19 mL canisters but the same as the control group, a large fraction about 56% of ex-actuator emitted dose deposits in the IP stage, which indicates worse particle uniformity in 19 mL canisters. It is suggested that increase of canister volume has more obvious effects on control group (Tio Only) than experimental groups (Tio: SV003). The FPF (t-test, $P< 0.05$) and FPD (t-test, $P< 0.05$) of formulations containing Tio and SV003 is increased significantly when compared against the control group, when either volume of canister is used. Furthermore, the MMAD of this binary formulation is reduced to that of Tio only. These findings suggest with addition of SV003, the in-vitro performance of the canister is more efficient regardless of canister volumes.

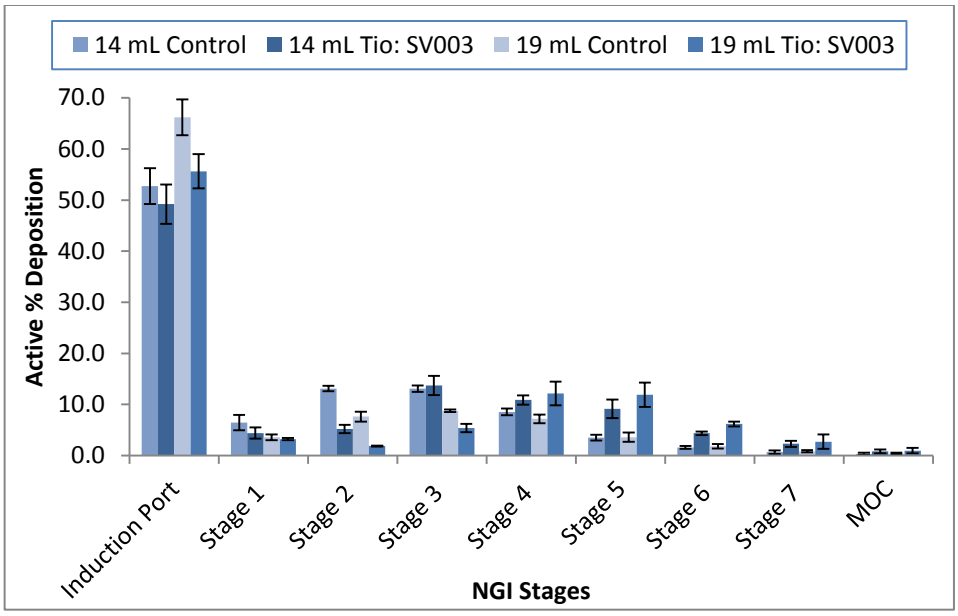

Figure 4. 1 NGI Distribution Pattern (%) of Tio Controls and Tio: SV003 with Different Canister Volumes (14 mL Control: n=5; mean ± SD, 14 mL/19 mL Control/19 mL: n=3; mean ± SD)

Particle size distributions of control (Tio Only) and experimental canisters (Tio: SV003) are illustrated in Figure 4.1. All the groups show a high IP deposition in the range of 49% and 66%, and this is significantly higher than the reference pMDI, the Tiova®, which is in the range of 34% and 41 % (Section 3.5.5). There is a trend of increased IP deposition with the increase of canister volume, when either in the formulation containing Tio only or Tio: SV003. When combining the IP, Stage 1 and 2 together as the non-respiratory fraction (particle diameter > 6.4 µm), and combining Stage 3 to MOC as the respiratory fraction (particle diameter < 3.99 µm), the data is shown in Table 4.2. There is no significant differences in non-respiratory and respiratory fraction in both control (t-test, P> 0.05) and experimental groups (t-test, P> 0.05), when either volume of canister is used. However, the addition of SV003 significantly decreases the non-respiratory fraction and accordingly increases the respiratory fraction when compared against Tio only in either 14 mL (t-test, P< 0.05) or 19 mL canisters (t-test, P< 0.05). The aerosol performance is improved with co-suspended SV003 and is independent of the canister volume.

Table 4. 2 Grouping of the Deposition Fraction of Different NGI Stages (14 mL Control: n=5; mean ± SD, 14 mL/19 mL Control/19 mL: n=3; mean ± SD)

Canister Volume	14 mL Control (a)	14 mL Tio: SV003 (b)	19 mL Control (c)	19 mL Tio: SV003 (d)
Non-respiratory fraction (> 6.4 µm) (%)	72.29 ± 1.97	58.81 ± 4.10	77.39 ± 2.55	60.72 ± 3.40
Respiratory fraction (< 3.99 µm) (%)	27.71 ± 1.97	41.19 ± 4.10	22.61 ± 2.55	39.28 ± 3.40

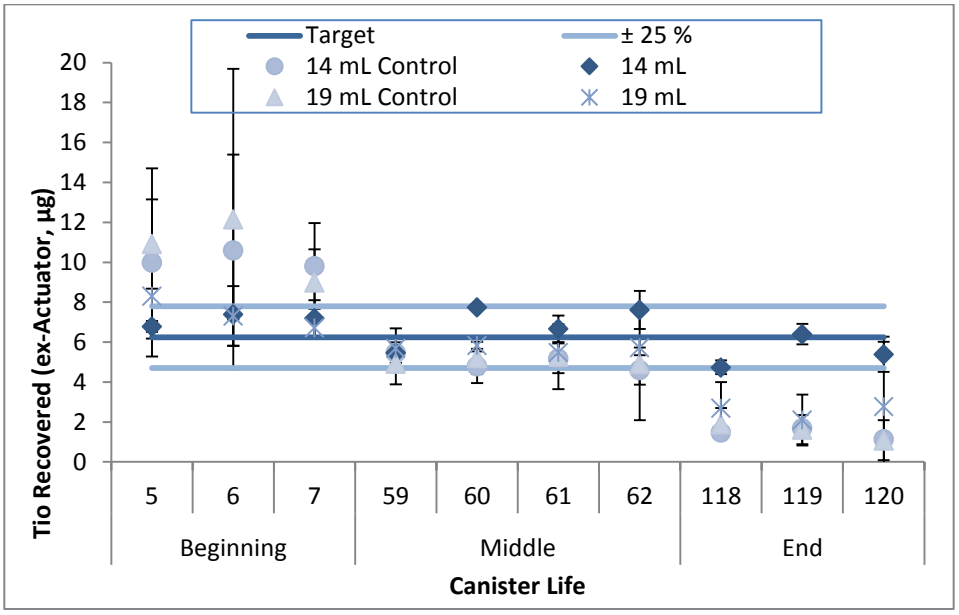

Figure 4. 2 Canister Life Content Uniformity of Tio Controls and Tio: SV003 with Different Canister Volumes (14 mL Control: n=5; mean ± SD, 14 mL/19 mL Control/19 mL: n=3; mean ± SD)

In the test of canister life content uniformity, 10 actuation per canister is collected by the DUSA over the canister life. The average ex-valve doses are 8.60 ± 5.34 µg (14 mL Control), 9.89 ± 1.19 µg (14 mL), 9.32 ± 6.04 µg (19 mL Control), 9.22 ± 3.64 µg (19 mL) respectively. A large standard deviation is observed especially with control groups, and accordingly, ex-actuator doses show the same variability as show in Figure 4.2. It is obvious that higher and variable doses are released in the beginning of canister life with both 14 and 19 mL control canisters, which leads to

lower doses forwards the end of canister life. It is assumed that drug particle-particle interaction induces the growth of particles size, and large agglomerated particles settle quicker than fine particles after shaking the canister. This increases the metered mass of Tio in the beginning but reduces the drug concentration in the bulk formulation. In experimental groups, 14 mL canisters show relatively stable ex-actuator doses in the range of ± 25% of the target over the canister life, whereas 19 mL canisters show a dramatic decrease in the end. The beginning and middle doses of both canisters illustrates a uniform formulation is obtained, but as the increase of the canister inner surface area, more drugs could be lost in canisters. When compared between 14 mL control and 14 mL experimental groups, a constant ex-actuator dose distribution is achieved with the addition of SV003 even in plain aluminium canisters, which suggests the secondary particle could prevent the drug loss over the canister life caused by drug particle-particle interaction and drug particle-surface interaction. Therefore, effect is seen with both formulations, perhaps significantly greater with Tio alone formulation in through can life data. Canister volume is mostly dependent on the volume of drug formulation. Large canister volume not only leads to inconsistent doses to be delivered but increases the air and moisture fractions inside the canisters. Due to the small dosing requirement, 14 mL canisters are recommended to store the novel formulation and maintain the physical stability.

4.5.2 Valve Volume

The performance of metering valves is a critical parameter during the development of a pMDI formulation. Under depression of the valve, the metered formulation is atomised into an aerosol plume at the actuator orifice. The characteristics of the aerosol plume are affected by the design of the valve, and the reproducibility of emitted dose can also be improved by reducing the variability from the valve (Williams et al, 1997). The dispersed volume of the formulation per actuation relies on the volume capacity of the valve metering chamber, which is normally in the range of 25 µL to 100 µL. VARI® KHFA valves are well established to use with HFA formulations including solution, suspension and hybrid (suspension/solution) in previous research (Monti et al, 2011). In this section, a variety of VARI® KHFA valves are used with chamber volumes of 25 µL, 35 µL and 50 µL. To keep the same amount of the drug delivered per actuation, the concentration of both drugs and secondary particles is varied. The influence of the valve volume on the aerosol emitted is investigated based on the formulation of Tio: SV003 (w/w) 1: 25.

Table 4. 3 Valve Performance of Tio: SV003 pMDI Formulation (n=3; mean ± SD)

Valve Volume	25 µL	35 µL	50 µL
Metered Weight (mg)	32.13 ± 1.35	49.24 ± 1.84	64.16 ± 0.41
Emitted Dose (ex-valve) (µg)	11.19 ± 0.69	10.42 ± 1.99	14.28 ± 2.46
Emitted Dose (ex-actuator) (µg)	9.23 ± 0.35	8.91 ± 2.02	11.51 ± 2.62

(Metering weight: canister weight difference between pre- and post-actuation, which includes the weight of the formulation and propellant; Ex-valve emitted dose: the dose emitted from the valve; Ex-actuator emitted dose: the dose emitted from the actuator)

The metered weight including the weight of the formulation and propellant, the ex-valve dose and ex-actuated dose are presented in Table 4.25. According to Section 4.4.2, target metered weights are 30.74 mg (25 µL), 42.92 mg (35 µL) and 61.19 mg (50 µL), while experimental weights were 4.52%, 14.73% and 4.85% above these targets respectively. The 25 µL and 50 µL valves show more accurate metering of the formulation than the 35 µL valve. The metering accuracy of the 35 µL valve is

slightly outside the acceptable range of ±10% of the target set by the manufacturer. Furthermore, the variability of metering weights over the canister life is least for the largest metering volume (50 µL), indicated by the present SD of 0.41, whist similar variability for the 25 µL (1.35) and 35 µL (1.84) valves. However, relative standard deviations (RSD) of all valves are less than 5% which indicates consistent valve measuring of the bulk formulation. As described in the introduction of this chapter, the target ex-valve dose is 11.25 µg. The difference between the mean of ex-valve doses and the target are 0.53% (25 µL), 7.38% (35 µL) and 26.93% (50 µL) respectively, which indicate the smallest metering volume (25 µL) gives the most accurate measuring of Tio. Therefore, it is concluded that 25 µL valve presents consistent and accurate metering of the drug among different metering volumes (25 µL – 50 µL). Furthermore, similar actuator performance is found independent of the valve volume, which is indicated by the actuator deposition of 18% (25 µL), 14% (35 µL) and 19% (50 µL). Therefore, the accurate metering of both the bulk formulation and the drug suggests the components of 25 µL valves are more compatible with the Tio: SV003 formulation.

Table 4. 4 Aerosol Characteristics (ex-actuator) of Tio: SV003 Formulation with Different Valve Volumes (n=3; mean ± SD)

Valve Chamber Volume	25 µL (a)	35 µL (b)	50 µL (c)
Tio FPF (% < 5 µm)	36.78 ± 3.07	27.65 ± 8.88	24.40 ± 2.32
Tio MMAD (µm)	2.38 ± 0.20	4.91 ± 2.23	3.08 ± 0.19
Tio FPD (µg < 5 µm)	3.39 ± 0.16	2.39 ± 0.66	2.79 ± 0.57
Tio GSD	2.17 ± 0.21	2.82 ± 0.35	2.85 ± 0.25

((a): Tio: SV003/EXCIP 1:25/FRAC <38 µm/HFA 134a/VAL 25 µL/CAN 19 mL/CAN Plain/ACT 0.25 mm, (b): Tio: SV003/EXCIP 1:25/FRAC <38 µm/HFA 134a/VAL 35 µL/CAN 19 mL/CAN Plain/ACT 0.25 mm, (c): Tio: SV003/EXCIP 1:25/FRAC <38 µm/HFA 134a/VAL 50 µL/CAN 19 mL/CAN Plain/ACT 0.25 mm)

The aerosol characteristics of the formulation equipped with different metering valves are illustrated in Table 4.4. Due to the limited sample number and significant outliers in FPF and FPD, and MMAD of the 35 µL valve, big in-group variance has a negative effect on one-way ANOVA. Large variability indicate incompatibility

between the 35 µL valve and the formulation. Therefore, FPF, FPD and MMAD of the 25 µL are compared against the 50 µL valve by unpaired t-tests. It is found that the FPF of the 25 µL valve is significant higher (t-test, P< 0.05), and MMAD significant lower (t-test, P< 0.05) than the 50 µL valve. This suggests more fine particles in the 25 µL valve are generated through the actuator, which might be relevant to the valve volume, or the drug and SV003 concentrations. Higher Tio and SV003 concentrations in 25 µL valves could more effectively prevent the particle aggregation and maintain the suspension uniformity. However, there is no significant difference in FPD (t-test, P< 0.05), which could be explained by the higher ex-actuator dose in the 50 µL valve as shown in Table 4.3. Variable ex-actuator doses of the 50 µL valve also cause inconsistence of the FPD as indicated by which the RSD of 20%. GSD of 25 µL, 35 µL and 50 µL valves are analysed by an ANOVA and Tukey HSD test, and the 25 µL valve also shows the least value with significant difference (P< 0.05), which suggests a less poly-dispersed particle size distribution in smaller size valves. Therefore, the valve with lower chamber volume generates more fine particles (< 5 µm) during the atomisation process.

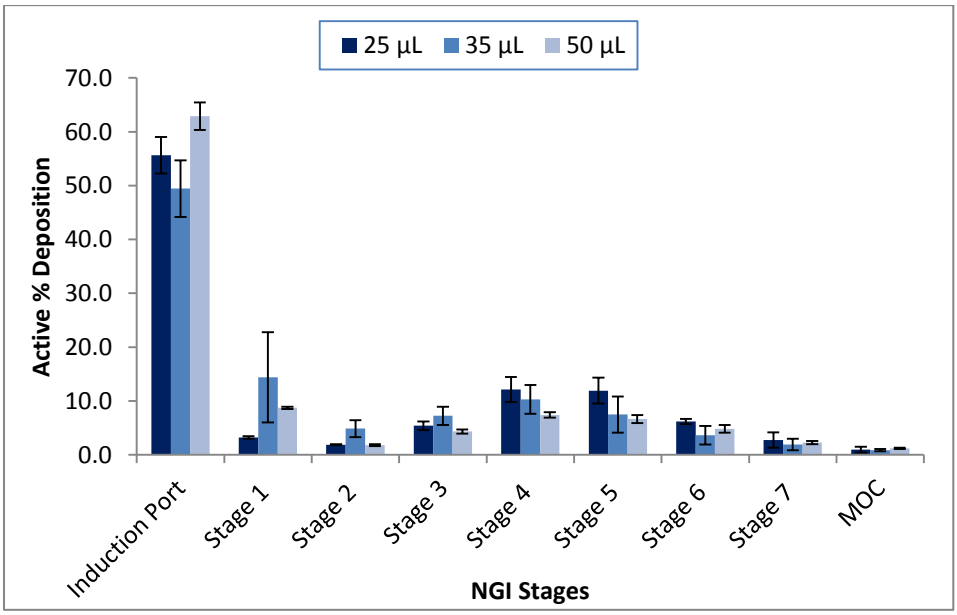

Figure 4. 3 NGI Distribution Pattern (%) of Tio Formulations with Different Valve Volumes (n=3; mean ± SD)

The percentage of particle deposition is plotted against NGI stages as shown in Figure 4.3. The 35-µL valve has great variability in most stages of the distribution pattern. The 25-µL valve shows relatively lower IP deposition than 50 µL but no significance is identified (t-test, $P > 0.05$). However, less drug particles are captured on Stage 1 (t-test, $P < 0.05$) and more in Stage 4 (t-test, $P < 0.05$) and 5 (t-test, $P < 0.05$) by 25 µL valves. According to Williams et al (1997), 130 mg of the model drug is suspended in HFA 134a containing ethanol and crimped with 50, 75 and 100 µL type DF10 RC valves respectively. No significant difference was found in respirable fraction and MMAD between the three metering chamber volumes investigated by an Anderson Cascade Impactor (ACI). Nevertheless, Berry et al (2003) found that a batch of suspension formulation containing micronised drug substances, oleic acid and ethanol is suspended in HFA 227 and crimped with 25 µL or 63 µL valves. As the volume of metering valve increased from 25 µL to 63 µL, there was a trend of decrease in FPF (< 4.7 µm) and reduction in droplet size, which was the same as the results we get. However, both experiments were performed by an ACI, and both formulations contained ethanol and without secondary excipients. It is assumed if actuated using the same actuator orifice, larger metering volumes will provide longer actuation periods. The inspiration volumes are the same for all the pMDIs, so smaller valves could lead to more efficient droplet evaporation and deposition. With the addition of SV003, the initial droplet size and number could be also affected during the atomisation. Furthermore, the emitted dose per actuation is the same for all the valves, so for valves with smaller volume, the drug and excipient are more concentrated in the propellant. Drug contents and concentrations were reported to be relevant to aerosol particle size, stability of the suspension and emitted dose uniformity (Tiwari et al, 1998). Therefore, it is assumed in higher concentrations of the formulation, decreased propellant fractions results in more-efficient evaporation of the propellant and accordingly more fine particles are observed.

Because a low excipient ratio is proven to advance actuator performance in Section 4.5.6, a further batch containing Tio: SV003 (w/w) 1:5 is formulated and crimped with 25 µL valves. According to Figure 4.4, when excipient ratio is reduced from 1: 25 to 1: 5, FPF decreases (t-test, P< 0.05) and MMAD increases (t-test, P< 0.05) significantly. FPD and GSD are comparable with no significant difference. The in-vitro performance of both Tio: SV003 1: 25 and 1:5 are shown in Figure 4.5. The peak deposition in the NGI body is shifted from Stage 4 (1: 25) to Stage 3 (1: 5). When combining the IP, Stage 1 and 2 together as the non-respiratory fraction (particle diameter > 6.4 µm), the ratio of 1: 25 is significant lower than 1: 5 (t-test, P< 0.05). When combining Stage 3 to MOC as the respiratory fraction (particle diameter < 3.99 µm), the ratio of 1: 25 is significant higher than 1: 5 (t-test, P< 0.05). A low ratio of SV003 is proven to prevent actuator clogging issues but sacrifices the generation of fine particles and delivery efficiency.

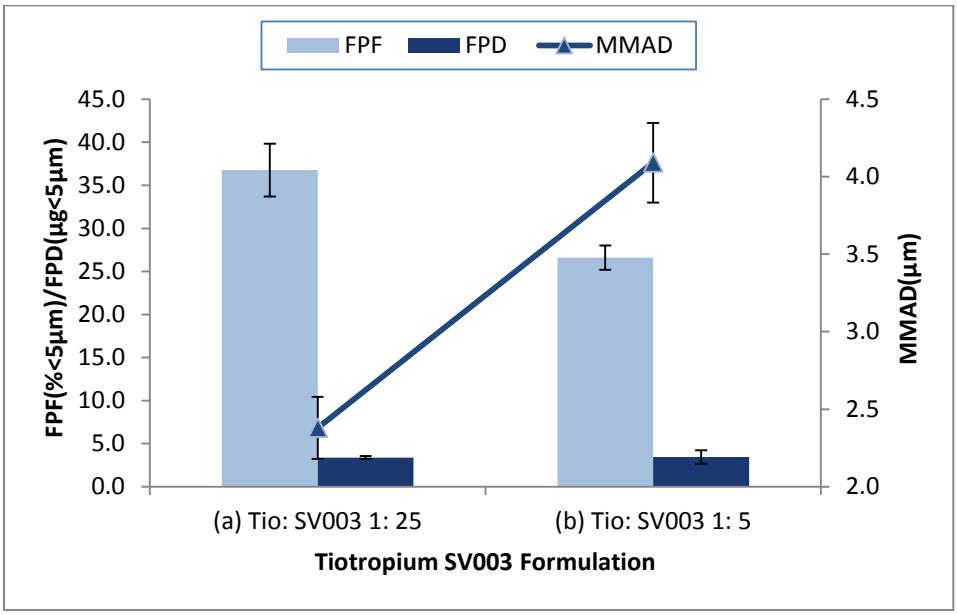

Figure 4. 4 Aerosol Characteristics (ex-actuator) of Tio Formulations with Ratios of 1: 25 and 1:5 with 25 µL VARI® KHFA Metering Valve (Tio: SV003 1: 25: n=3; mean ± SD, Tio: SV003 1: 5: n=5; mean ± SD) ((a)Tio: SV003/EXCIP 1:25/FRAC <38 µm/HFA 134a/VAL 25 µL/CAN 19 mL/CAN Plain/ACT 0.25 mm, (b) Tio: SV003/EXCIP 1:5/FRAC <38 µm/HFA 134a/VAL 25 µL/CAN 19 mL/CAN Plain/ACT 0.25 mm)

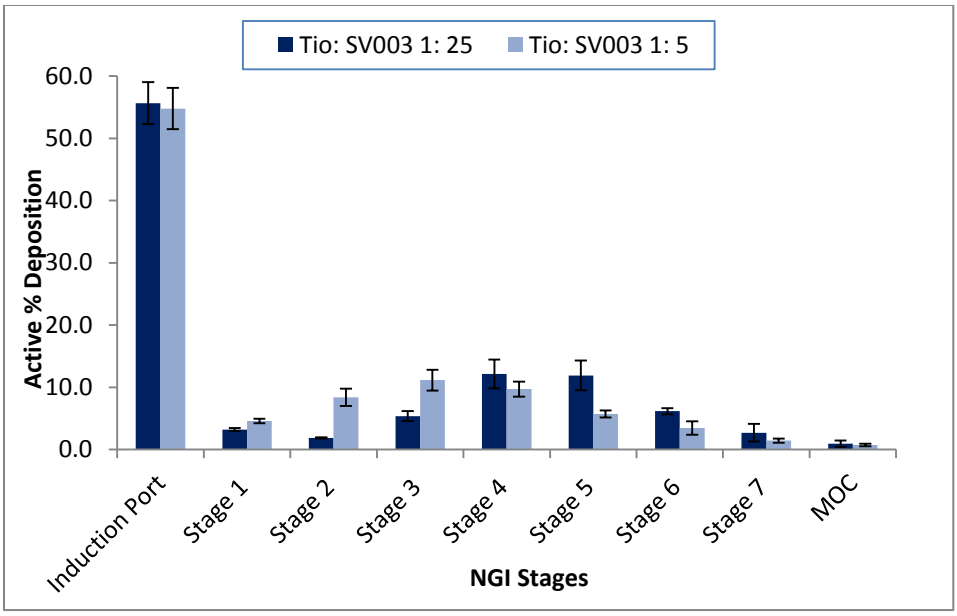

Figure 4. 5 NGI Distribution Pattern (%) of Tio Formulations with Ratios of 1: 25 and 1: 5, Crimped with 25 µL VARI® KHFA metering Valves (Tio: SV003 1: 25: n=3; mean ± SD, Tio: SV003 1: 5: n=5; mean ± SD)

4.5.3 Propellant Type

Current pMDIs use liquefied and pressurised gases as the dispersion phase and propellants to form a solution or suspension. In a sealed environment, a dynamic equilibrium is reached between the liquefied propellant and the vapor phase. The saturated vapor pressure, relevant to the physical properties of the propellant, mixture of propellants, ambient temperature or the incorporated excipients, is maintained consistently during the product life, which assures the identical delivery pattern by the same product (Newman, 2005). In the current EU and US pharmaceutical market, two types of HFA propellants are available including HFA 134a (1,1,1,2-tetrafluoroethane) and HFA 227 (1,1,1,2,3,3,3-heptafluoropropane). Several physico-chemical properties of HFA 134a and HFA 227 are shown in Table 4.5. HFA 134a has a relatively lower density and viscosity, but higher vapor pressure. The density of propellants and viscosity are relevant to the sedimentation properties of suspended drug particles, and consequently influence the physical stability of the suspension (Steckel and Wehle, 2004). The vapor pressure determines the evaporation process e.g. plume speed and evaporation rate. Boiling points of both propellants are in the range of -15°C and -30°C, which could provide sufficient atomisation of the formulation to provide aerosol droplets in ambient temperature (Noakes, 2002).

Table 4. 5 Physiochemical Properties of HFA propellants (HFA 134a and 227) (McDonald and Martin, 2000)

Propellant	Density (g/mL)	Viscosity (mPas)	Boiling Point (°C)	Vapor Pressure (bar)
HFA 134a	1.21	0.22	-26.5	5.72 at 20 °C
HFA 227	1.41	0.26	-17.3	3.9 at 20 °C

The performance of a pMDI suspension is highly dependent on the uniformity of particles and the particle size distribution (Myrdal et al, 2014). In an ideal formulation, suspended particles are expected to remain separate, without aggregating, and uniformly distribute throughout the dispersion phase. However, due to the solvency of the drug, particle interaction or quick sedimentation by

gravity, agglomeration and caking are commonly observed, which are dependent on the physico-chemical properties of both the propellant and the drug. Even when drug particles are maintained stable in propellants, the distribution of aerosolised particles can also be affected by the plume speed and evaporation rate. Consequently, selection of suitable propellants is necessary to avoid or reduce these influences.

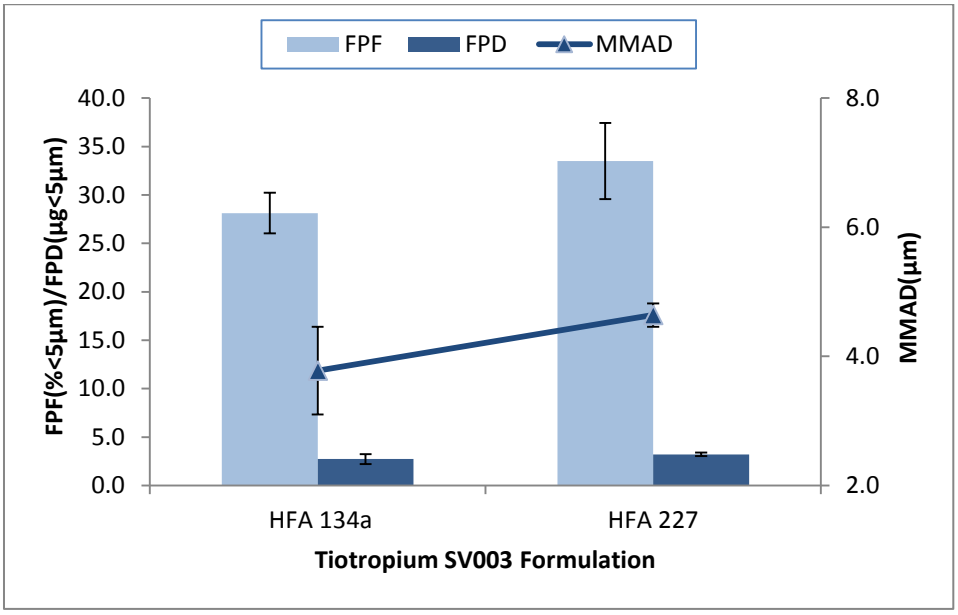

Figure 4. 6 Aerosol Characteristics (ex-actuator) of Tio: SV003 Formulations with Different Propellants, e.g.HFA134a and HFA 227 (n=8; mean ± SD)

Table 4. 6 Aerosol Characteristics (ex-actuator) of Tio: SV003 Formulations with Different Propellants, e.g.HFA134a and HFA 227(n=8; mean ± SD)

Propellant Type	HFA 134a	HFA 227
Tio FPF (% < 5 µm)	28.13 ± 2.09	33.51 ± 3.93
Tio MMAD (µm)	3.78 ± 0.68	4.64 ± 0.18
Tio FPD (µg < 5 µm)	2.73 ± 0.52	3.22 ± 0.19
Tio GSD	2.43 ± 0.11	2.29 ± 0.08

((a): Tio: SV003/EXCIP 1:5/FRAC <38 µm/HFA 134a/VAL 25 µL/CAN 14 mL/CAN Plain/ACT 0.25 mm, (b): Tio: SV003/EXCIP 1:5/FRAC <38 µm/HFA 134a/VAL 25 µL/CAN 14 mL/CAN Plain/ACT 0.25 mm)

In this research, Respitose® SV003 was used as the model secondary particle to co-suspend Tio in HFA 134a and HFA 227. Both propellants solvency characteristics were described in Chapter 2. The in-vitro aerosol performance of both propellants is shown in Figure 4.6 and Table 4.6. FPF of HFA 227 is 33.51 ± 3.93 %, which is significantly higher than 28.13 ± 2.09 % of HFA 134a (t-test, P< 0.05). FPD of HFA 227 is 3.22 ± 0.19 μg, which is also significantly higher than 2.73 ± 0.52 μg of HFA 134a (t-test, P< 0.05). Due to the relative high vapor pressure of HFA 134a (Table 4.5), a high speed of aerosol plume is expected to generate from the actuator orifice. However, there is an incomplete understanding of the droplet formation process during atomisation. It is considered that the pressure gradient between the saturated vapor and the atmosphere induces the evaporation of the propellant, and forces the atomisation and emission of the initial droplets (Smyth and Hickey, 2011). The velocity of the aerosol is proportional to the pressure gradient, and high velocity causes high particle inertia, which are supported by the particle size distribution (Figure 4.7). Higher IP deposition was observed for HFA 134a, 54.32 ± 4.38% than HFA 227, 38.69 ± 4.05% (t-test, P< 0.05). More drug particles are trapped in the IP stage rather than pass into the lower stages. The MMAD of HFA 227 is 4.64 ± 0.18 μm, which is significant higher than HFA 134a, 3.78 ± 0.68 μg (t-test, P< 0.05). The high vapor pressure of HFA 134a contributes to rapid evaporation of initial droplets, which is defined as the droplets generated immediately after atomisation (Sheth et al, 2013). In solution formulation, it is better understood that the size distribution of initial particles is related to that of the residual droplets, which is defined as the droplets without volatile propellants and semi-volatile cosolvents (Stein and Myrdal, 2004). Smaller residual particle size of HFA 134a indicates that more fine initial droplets could be generated during the atomisation, or rapid evaporation of the initial droplets produces more fine residual particles, which are supported by less deposition on Stage 1 to 5 (particle diameter >0.83 μm), and greater deposition on Stage 6 to MOC (particle diameter <0.83 μm) (t-test, P< 0.05). This is also supported by previous research and that an

increase in vapor pressure by using HFA 134a in solution formulations or incorporating HFA 134a into HFA 227 in suspension formulations leads to a smaller MMAD (Williams et al, 1998; Brambilla et al, 1999; Stein and Myrdal, 2004). The GSD values of HFA 134a was also found to be significantly higher than HFA 227 (t-test, P< 0.05), but both are greater than 2, predicting the formation of poly-dispersions.

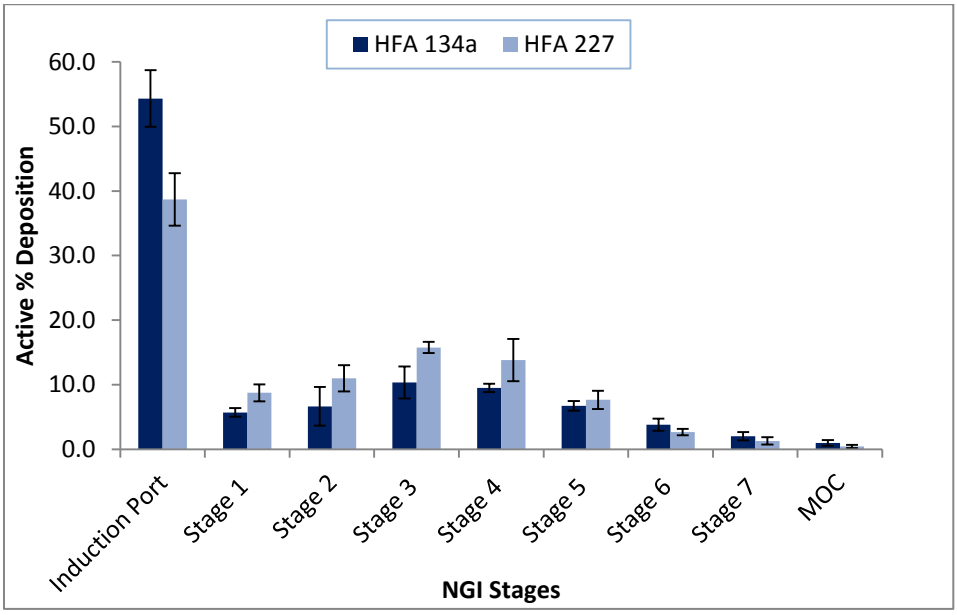

Figure 4. 7 NGI Distribution Pattern (%) of Tio: SV003 Formulations using Different Propellants (HFA134a and HFA 227) (n=8; mean ± SD)

For suspension formulations, the density of propellants can affect particle settling or creaming behaviour (Myrdal et al, 2014). To accurately meter the suspension, an appropriate sedimentation velocity is required. The sedimentation rate is expressed by Stokes's Law (10):

$$v = \frac{d^2(\rho_{s-}\ _{o})g}{18\eta_o} \quad (10)$$

(v: the velocity of sedimentation, d: the diameter of the particle, ρ_s: the density of the dispersed phase, ρ_o: the density of dispersion phase, g: gravity acceleration, η_o: the viscosity of the dispersion medium)

Depending on the density of the drug and the propellant, drug particles tend to either 'cream' (float on the top) or clay (sink to the bottom and compact) (Noakes, 2002). It has been reported that formulations show higher physical stability and more consistent dose delivery as the propellant density approaches the drug density (Williams et al, 1998). HFA 227 presents higher density and viscosity than HFA 134. Based on Stokes' law, both Tio and SV003 will have slower sedimentation rates in HFA 227 than in HFA 134a. The metering doses or the ex-valve emitted doses are 10.37 ± 3.02 μg (HFA 134a) and 10.08 ± 1.25 μg (HFA 227). There is no significant difference in metering doses with HFA 134a and HFA 227 (t-test, $P > 0.05$), so it is considered that both propellants can provide adequate suspending of Tio and SV003. The mass balance of both HFA 134a and HFA 227 is satisfactory as all the emitted doses are in the acceptable range of 8.43 - 14.06. However, there is trend of a decrease in the emitted dose at the end of canister life, which will be discussed in the following paragraph.

Accordingly, the ex-actuator dose of HFA 227 (6.72 ± 1.15 μg) is comparable to HFA 134a (7.00 ± 2.16 μg) (t-test, $P > 0.05$). As shown in Figure 4.8, more drug in HFA 134a (34.02 ± 5.44%) is lost in the actuator than HFA 227 (30.02 ± 6.35%) (t-test, $P < 0.05$). Previous research also showed that HFA 134a indicated a higher actuator deposition than CFC in a salbutamol suspension formulation (Cheng et al, 2001). It is considered that the fluid dynamics of the propellants could affect the geometry of generated plume. It is well-known that the aerosol velocity decrease with the travelling distances. For example, HFA 134a (Airomir®) start from 14 m/sec to 3 m/sec in 10 milliseconds, and CFC (Ventolin®) start 17 m/sec to 7 m/sec in 10 milliseconds (Barry and O'Callaghan, 1997). Therefore, the different physiochemical properties of HFA 134a and HFA 227 could be also relevant to the spray pattern and actuator deposition, which will need to be further investigated. The canister

life content uniformity is illustrated in Figure 4.9. It is noticed that both formulations show high ex-actuator doses at the beginning of canister life and a drop of the doses at the end. As there is no indication of chemical degradation of Tio, which will be discussed in Chapter 5, the decrease in ex-actuator doses could be due to poor uniformity of the formulation, and the decreased ex-actuator doses could be caused by decreased Tio concentration during the use of pMDIs. The influence of the canister surface is further investigated in Section 3.57.

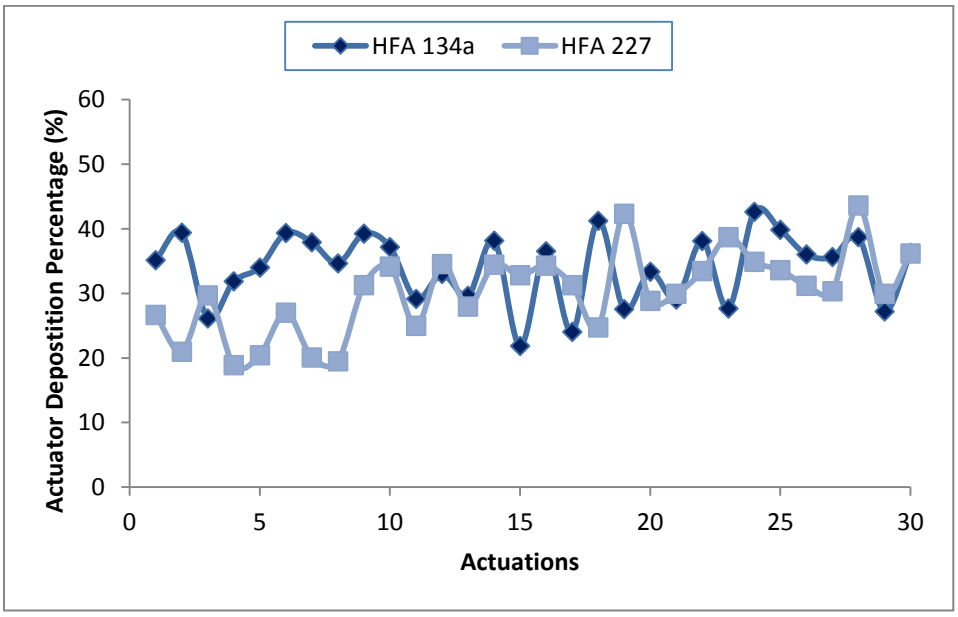

Figure 4. 8 Actuator Deposition of pMDI Formulation with Different Propellants over 30 Actuations (HFA134a and HFA 227)

The different physicochemical properties of HFA propellants, especially the vapor pressure, are proven to affect the atomisation process and the fluid dynamic properties of the formulation. Though it is unclear about the factors influencing the formation of the initial droplets of the suspension formulation, it is found the droplet diameter is inversely proportional to the pressure gradient. Both propellants can provide sufficient sedimentation rate for Tio and SV003, and HFA 227 also shows higher FPF and FPD, along with lower IP stage and actuator deposition. In the case of Tio and SV003 suspension, HFA 227 indicates a better

aerosol performance than HFA 134a. However, decreased emitted doses are noticed for both propellants in the end of canister life. Further investigations are required to improve the dosing consistency. Modification of the vapour pressure by using mixture of propellants, and evaluation of the spray pattern and plume geometry might be useful in the future for better understanding the atomisation process of the suspension formulation.

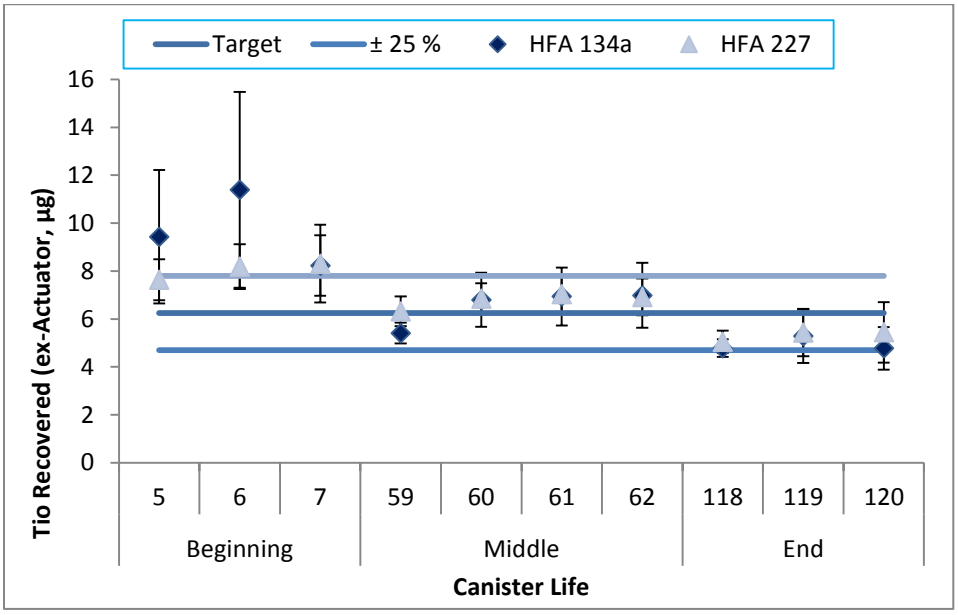

Figure 4. 9 Canister Life Content Uniformity of Tio: SV003 Formulations using Different Propellants (HFA134a and HFA 227) (n=8; mean ± SD)

4.5.4 Actuator Orifice

A typical actuator contains the actuator body, the stem block, the expansion chamber, the actuator orifice and the mouthpiece. The metered formulation is released through the canister stem into the expansion chamber where it initially boils, and then atomises at the actuator orifice. As the formation of aerosols is a complex dynamic process, multiple factors should be investigated during actuator design. It has been shown that the design of the orifice, especially the geometry of the orifice, volume of expansion chamber and dimension of the orifice, is relevant to the atomisation process and can affect particle deposition (Chen et al, 2015; Ju et al, 2012). Smyth et al (2006) found the spray pattern of the actuator was affected by orifice size, with the geometry of the generated plume correlating to the performance of the actuator and the formulation. In a budesonide solution formulation, higher FPFs were identified by smaller actuator orifice (Ganderton et al, 2003). In a suspension system, smaller orifice could also cause smaller droplets and de-aggregation of the drug particles (Vervaet and Byron, 1999). In this section, the influence of orifice size of the actuator is the main research emphasis on the in-vitro aerosol performance. During the atomisation, the valve stem is required to fit tightly with the stem block to prevent leakage of the expansion chamber (Myrdal et al, 2014). Suitable actuators should accompany the crimped VARI KHFA valve, so all the data in this research were generated based on H&T Presspart actuators.

The diameter of the actuator orifice is normally in the range of 0.22 mm to 0.6 mm, while in this section four types of orifices are used in the range of 0.25 to 0.46 mm including 0.25 mm, 0.35 mm, 0.40 mm and 0.46 mm. All the actuators were tested with the same batch of canisters. As seen from Table 4.7, the metered weight of the valve equipped with 0.25 mm actuators was significantly less than the rest of actuators tested e.g. 0.35 mm, 0.40 mm and 0.46 mm (ANOVA & Tukey HSD test, $P< 0.05$), which could be caused by valve metering variability. Even with fast-fill and

fast-drain advantages, KHFA valves still need to be properly primed before using for the first time. The lower metering weight can also explain the significant lower ex-valve doses (ANOVA & Tukey HSD test, $P< 0.05$) and ex-actuator doses (ANOVA & Tukey HSD test, $P< 0.05$) with 0.25 mm actuators. The target ex-valve dose of Tio: Leu (20 µm) formulation is 9 µg and ex-actuator dose is 6.25 µm (Section 4.5.8), where most of emitted doses of all actuators locate in the range of ± 25% of the target. Table 4.7 suggests 0.35 mm actuators give the greatest actuator deposition (ANOVA & Tukey HSD test, $P< 0.05$) while similar deposition patterns are found between 0.25, 0.40 and 0.46 mm actuators.

Table 4. 7 Valve and Actuator Performance Tio: Leu Formulations with Different Actuator Orifices (n=9; mean ± SD)

Actuator Orifice	Metered Weight (mg)	Emitted Dose (Ex-valve) (µg)	Emitted Dose (ex-actuator) (µg)	Actuator Deposition (%)
0.25 mm	39.28 ± 1.07	8.01 ± 0.31	5.85 ± 0.53	27.06 ± 4.02
0.35 mm	40.24 ± 0.82	9.35 ± 0.72	6.33 ± 0.71	32.38 ± 3.77
0.40 mm	41.08 ± 1.16	9.56 ± 0.52	6.91 ± 0.62	27.85 ± 3.66
0.46 mm	41.23 ± 1.14	9.28 ± 0.60	6.68 ± 0.60	28.07 ± 3.03

(Metered weight: canister weight difference between pre- and post-actuation, which includes the weight of the formulation and propellant; Ex-valve emitted dose: the dose emitted from the valve; Ex-actuator emitted dose: the dose emitted from the actuator; Actuator deposition: the percentage of the dose deposited on the actuator)

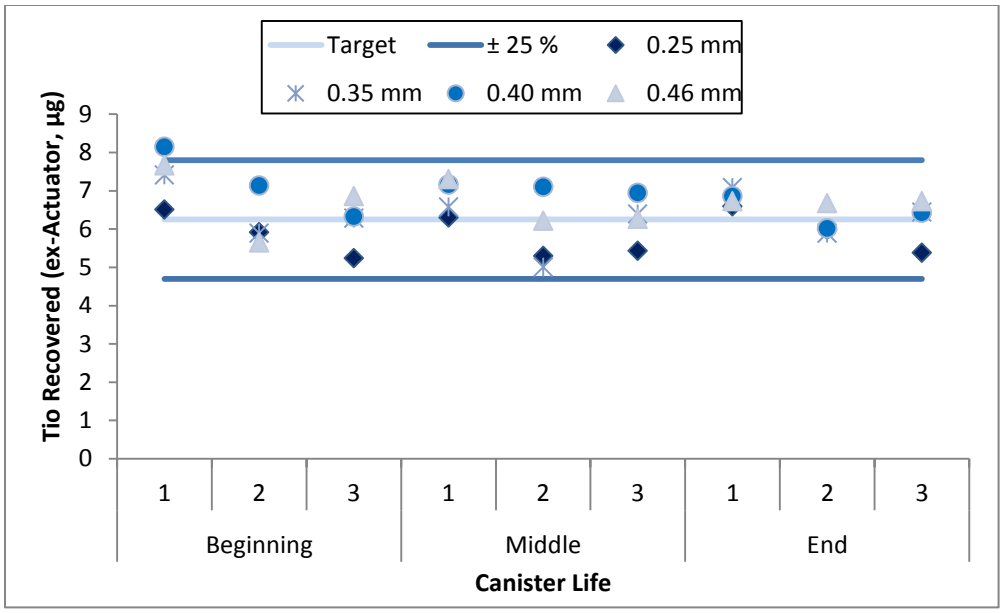

Figure 4. 10 Canister Life Content Uniformity of Tio: Leu Formulations with Different Actuator Orifices (3 single actuations per actuator are recorded in the beginning, middle and end of canister life respectively)

As shown in Figure 4.10, the distribution of 9 single actuations at the beginning, middle and end of the canister life shows consistent delivery of tiotropium regardless of actuator orifice size. 97% of the ex-actuator doses were in the range of ± 25% of the target, 6.25 µg along with an outlier shot at the beginning of the canister life. Although the actuation number is limited to 9 per actuator, the result gives us a better understanding that the formulation at ratio of Tio: Leu (w/w) 1: 5 is compatible with 25 µL valve and plain aluminium canisters.

Table 4. 8 Aerosol Characteristics (ex-actuator) of Tio: Leu Formulations with Different Actuator Orifices (n=3; mean ± SD)

Nozzle Diameter	0.25 mm (a)	0.35 mm (b)	0.40 mm (c)	0.46 mm (d)
Tio FPF (% < 5 µm)	54.76 ± 0.87	47.46 ± 2.70	42.99 ± 1.43	45.33 ± 2.34
Tio MMAD (µm)	2.95 ± 0.15	2.96 ± 0.16	3.04 ± 0.13	3.04 ± 0.14
Tio FPD (µg < 5 µm)	3.89 ± 0.20	3.28 ± 0.18	3.06 ± 0.25	3.06 ± 0.21
Tio GSD	2.25 ± 0.09	2.12 ± 0.06	2.14 ± 0.08	2.11 ± 0.11

((a) Tio:Leu/EXCIP 1:5/FRAC <20 µm/HFA 227/VAL 25 µL/CAN 14 mL/CAN Plain/ACT 0.25 mm, (b) Tio:Leu/EXCIP 1:5/FRAC <20 µm/HFA 227/VAL 25 µL/CAN 14 mL/CAN Plain/ACT 0.35 mm, (c) Tio:Leu/EXCIP 1:5/FRAC <20 µm/HFA 227/VAL 25 µL/CAN 14 mL/CAN Plain/ACT 0.40 mm, (d) Tio:Leu/EXCIP 1:5/FRAC <20 µm/HFA 227/VAL 25 µL/CAN 14 mL/CAN Plain/ACT 0.46 mm)

Table 4.8 illustrates the aerosol characteristics with different actuators. 0.25 mm actuators show the greatest (ANOVA & Tukey HSD test, P< 0.05) and most consistent FPF values compared with other actuators. The 0.35, 0.40 and 0.46 mm actuators are similar. 0.25 mm actuators also present the highest FPD values (ANOVA & Tukey HSD test, P< 0.05), while 0.35, 0.40 and 0.46 mm actuators are comparable. No significant difference is identified in MMAD (ANOVA, P> 0.05) and GSD (ANOVA, P> 0.05). In pMDIs equipped with smaller orifice-size actuators, it is assumed that the formulation is atomised into a finer mist by increased shear force, and drug particles are effectively de-aggregated from leucine particles. The quick evaporation of the smaller initial droplets contributes to a decrease in the plume momentum, and therefore a greater fraction of fine particles, and more drugs are found to penetrate into the lower stages of the NGI (Stein et al, 2014).

As shown in Figure 4.11, 0.25 mm actuators show the lowest IP deposition (ANOVA & Tukey HSD test, P< 0.05), and higher deposition in lower stages e.g. Stage 4, 6, 7 and MOC. Gabrio et al (1999) also found IP deposition of a pMDI solution system containing beclomethasone dipropionate was sensitive to the orifice diameter of the actuator, and increased with the orifice size in the range of 0.29 mm to 0.40 mm. It is assumed that a slow aerosol speed and smaller initial aerosol particles

are generated by actuators with smaller orifice size. The slow plume or smaller aerosol particles could lead to effective evaporation of the aerosol droplets. Fewer particles were deposited on the IP stages by gravitational force and inertial impaction, and more small particles go through the NGI and deposit into lower stages. The influence of high plume velocity generated by actuators with orifice size> 0.35 mm, or larger aerosol particles could overwhelm the effect of shear force, so no significance difference was found in the performance of the formulation by these actuators.

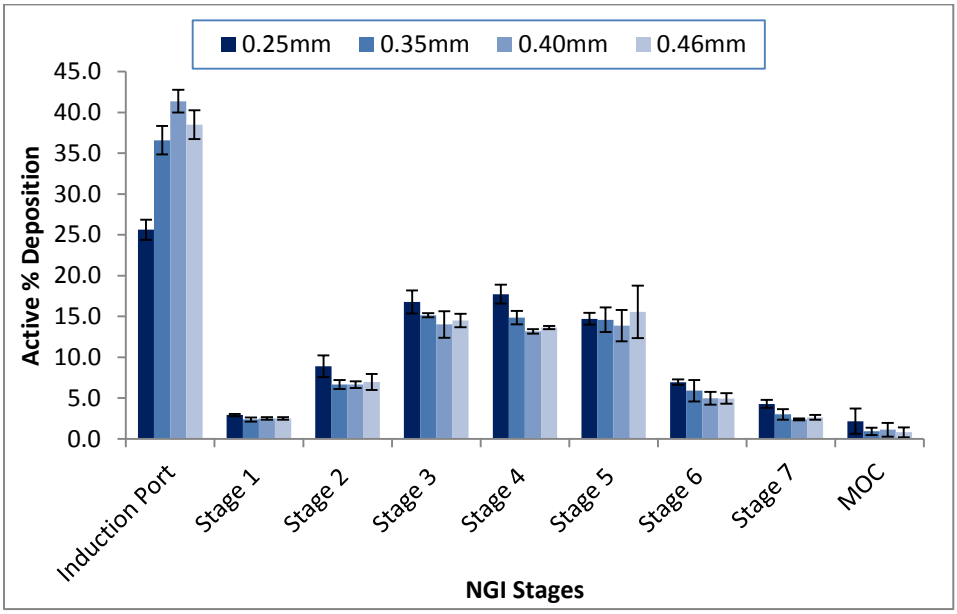

Figure 4. 11 NGI Distribution Pattern (%) of Tio: Leu Formulations with Different Actuator Orifices (n=3; mean ± SD)

A dyed propellant can be used as an indicator of spray pattern of actuators with different orifice sizes, when sprayed against a piece of paper. 0.25 mm actuators present the smallest area, with the spray coverage increasing with the increase of actuator orifice size. The larger spray coverage represents a broader spray angle by bigger orifices, which increase the chance of collision between drug particles and the IP inner wall (Figure 4.12). Because atomisation is a dynamic process, dynamic

spray patterns, e.g. the spray cone geometry, velocity and cone angle, need to be measured by integrated instruments using laser and high-speed digital camera.

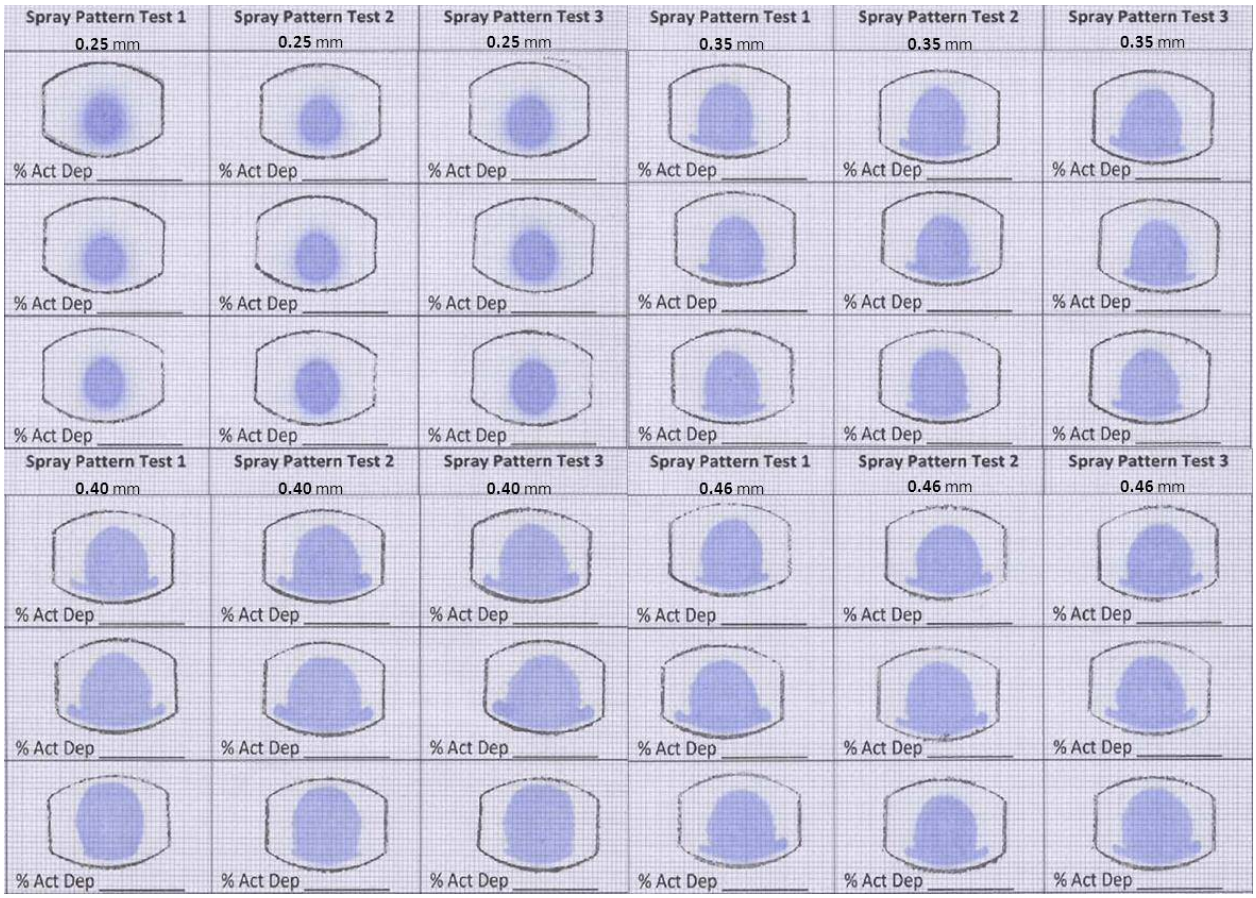

Figure 4. 12 Spray Pattern of H&T Presspart® Actuators with Orifice Size of 0.25 mm, 0.35 mm, 0.40 mm and 0.46 mm (Brilliant blue was dissolved in canisters containing HFA 227 and used as the indicator of spray patterns. The canister containing brilliant blue and HFA 227 was fired through the tested actuators against a piece of paper.)

In further studies leucine was substituted by the commercial inhalable excipient, Respitose® SV003, which has been approved for usage in pulmonary delivery by regulatory agents. The aerosol properties of Tio: SV003 (w/w) 1: 5 and Tio: Leu (w/w) 1: 5 are illustrated in Table 4.9. According to two-way ANOVA test, there is no statistically significant interaction in FPF, FPD, MMAD and GSD respectively between excipient type and orifice size ($P > 0.05$). Tio: SV003 and Tio: Leu show significant higher FPF by actuators with smaller orifice size of 0.25 mm ($P < 0.05$).

Nevertheless, FPFs of Tio: Leu are significantly higher than Tio: SV003 ($P< 0.05$), when either actuator is used. However, the ex-actuator dose of Tio: SV003 with 0.46 mm actuators (12.78 ± 4.01 µg) is higher than 0.25 mm actuators (10.54 ± 0.38 µg), which leads to similar FPDs ($P> 0.05$). Tio: SV003 shows the same pattern as Tio: Leu in that higher FPF is achieved by actuators with smaller orifice size (t-test, $P< 0.05$), and MMAD and GSD values are comparable between 0.25 mm and 0.46 mm actuators (t-test, $P> 0.05$). It is also apparent that a larger standard deviation of FPD is observed within Tio: SV003 formulation with 0.46 mm actuators. There are no statistic difference in GSD which is independent of excipient type and orifice size. However, when compared with Tio: Leu formulations, there is significant decrease of FPF and significant increase of MMAD in Tio: SV003 formulations when either actuator is used. The excipient type suggests a different aerosol performance, and the same results is also found in Section 4.5.7.

Table 4. 9 Aerosol Characteristics (ex-actuator) of Tio: SV003 Formulations and Tio: Leu Formulations with 0.25 mm and 0.46 mm Actuators (Tio: SV003: n=5; mean ± SD, Tio: Leu: n=3; mean ± SD)

Actuator Orifice	0.25 mm SV003 (a)	0.25 mm Leu (b)	0.46 mm SV003 (c)	0.46 mm Leu (d)
Tio FPF (% < 5 µm)	30.75 ± 1.04	54.76 ± 0.87	21.78 ± 2.09	45.33 ± 2.34
Tio MMAD (µm)	4.73 ± 0.12	2.95 ± 0.15	5.09 ± 0.32	3.04 ± 0.14
Tio FPD (µg < 5 µm)	3.24 ± 0.20	3.89 ± 0.20	2.82 ± 1.05	3.06 ± 0.21
Tio GSD	2.27 ± 0.08	2.25 ± 0.09	2.27 ± 0.14	2.11 ± 0.11

((a)&(c) Tio:SV003/EXCIP 1:5/FRAC <38 µm/HFA 227/VAL 25 µL/CAN 14 mL/CAN Plain/ACT 0.25 mm, (b)&(d) Tio:Leu/EXCIP 1:5/FRAC <20 µm/HFA 227/VAL 25 µL/CAN 14 mL/CAN Plain/ACT 0.25 mm)

The distribution pattern of Tio: SV003 and the leucine counterpart are illustrated in Figure 4.13. In Tio: SV003, more particles are captured on the IP stage and less particles deposits on lower stages e.g. Stage 4, 6, 7 and MOC with 0.46 mm actuators than 0.25 mm actuators, which shows the same pattern as the Tio: Leu. However, leucine can more effectively decrease the number of large aerosol particles and prevent the large deposition on IP and upper stages. The effect of

excipient types is further discussed in Section 4.5.6. Overall, in this section, it is found at the ratio of 1:5 Tio: Excipient, the novel formation is compatible with H&T Presspart® actuators in the range of 0.25 mm to 0.46 mm, and actuators with smaller orifice size presented better aerosol performance and delivery efficacy.

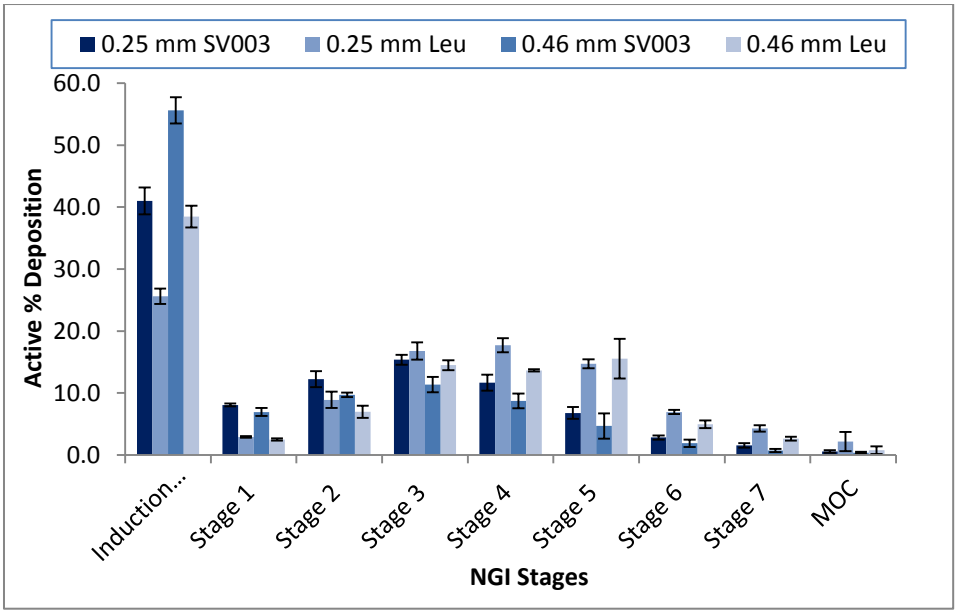

Figure 4. 13 NGI Distribution Pattern (%) of Tio: SV003 Formulations with 0.25 mm and 0.46 mm Actuator Orifices (Tio: SV003: n=5; mean ± SD, Tio: Leu: n=3; mean ± SD)

4.5.5 Canister Coating

Irreversible adhesion of the drug on the surface of the canister results in a decrease of metered dose, which will lead to a reduction in dose delivered to the patients. In the case of low dose medications the influence of adhesion of the drug can be critical (Traini et al, 2006). Aluminium is the most commonly used material in the manufacture of pMDI canisters. Although aluminium is almost inert, the adsorption of specific drugs can cause physical instability of the formulation and even chemical degradation (Newman, 2005; Young et al, 2003). An inner coating, as either a protective barrier or low surface-energy coating, is employed to increase the stability of the formulation, maintain the performance of the canister and extend the shelf life of the product. The coating treatments include barrier coatings, e.g. canister anodisation, and low surface energy coating such as fluorocarbon polymer, e.g. perfluorocarbon, hydrofluorocarbon and chlorofluorocarbon (Ashurst et al, 2003; (Turner, 2010). Canisters are coated with one or more polymers, and with one or more layers. According to Section 4.5.7, SV010 and leucine are found with advantages in improving aerosol performance of Tio against SV003 and Tio Only. Therefore in this section, novel formulations including Tio: SV010 and Tio: Leu, are investigated using the plain and fluorocarbon polymerisation (FCP) treated H&T Presspart® aluminium canisters.

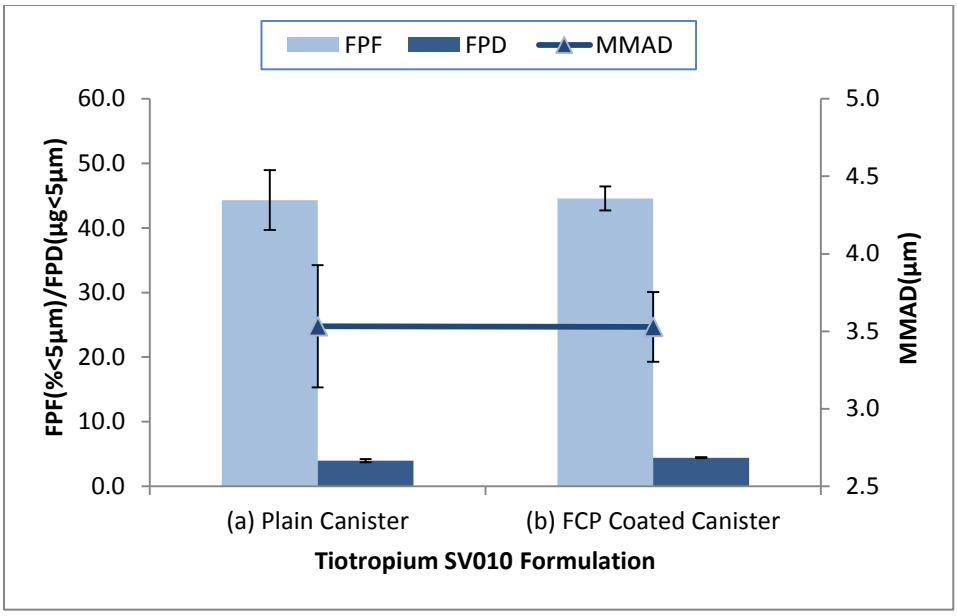

Figure 4. 14 Aerosol Characteristics (ex-actuator) of Tio: SV010 Formulations with Different Canister Coatings (n=3; mean ± SD)

Table 4. 10 Aerosol Characteristics (ex-actuator) of Tio: SV010 Formulations with Different Canister Coatings (n=3; mean ± SD)

Canister Coating	Plain Canister	FCP Coated Canister
Tio FPF (% < 5 µm)	44.32 ± 4.63	44.57 ± 1.84
Tio MMAD (µm)	3.53 ± 0.39	3.53 ± 0.22
Tio FPD (µg < 5 µm)	3.96 ± 0.26	4.42 ± 0.08
Tio GSD	2.22 ± 0.01	2.35 ± 0.17

((a) Tio:SV010/EXCIP 1:5/FRAC <38 µm/HFA 134a/VAL 25 µL/CAN 14 mL/CAN Plain/ACT 0.25 mm, (b) Tio:SV010/EXCIP 1:5/FRAC <38 µm/HFA 134a/VAL 25 µL/CAN 19 mL/CAN Plain/ACT 0.25 mm)

In the group of 1:5 Tio: SV010, ex-valve doses of plain canisters (10.29 ± 0.47 µg) are comparable to FCP canisters (10.77 ± 0.44 µg) (t-test, P> 0.05). As shown in Figure 4.14 and Table 4.10, both types of canisters present similar aerosol performance, and there was no significant difference in FPF (t-test, P> 0.05) and FPD (t-test, P> 0.05), and MMAD (t-test, P>0.05). However, standard deviations of FPF, FPD and MMAD are doubled when using plain canisters, which suggests surface coating could improve dose uniformity and valve performance, which is

also supported in Figure 4.15. However, these aerosol performance tests are performed at the beginning of canister life, which avoids the influence of potential drug absorption over the canister life and storage period. Therefore whilst canister surface coating may appear to result in more consistent delivery, further tests after a certain period of storage are required and discussed in Chapter 5.

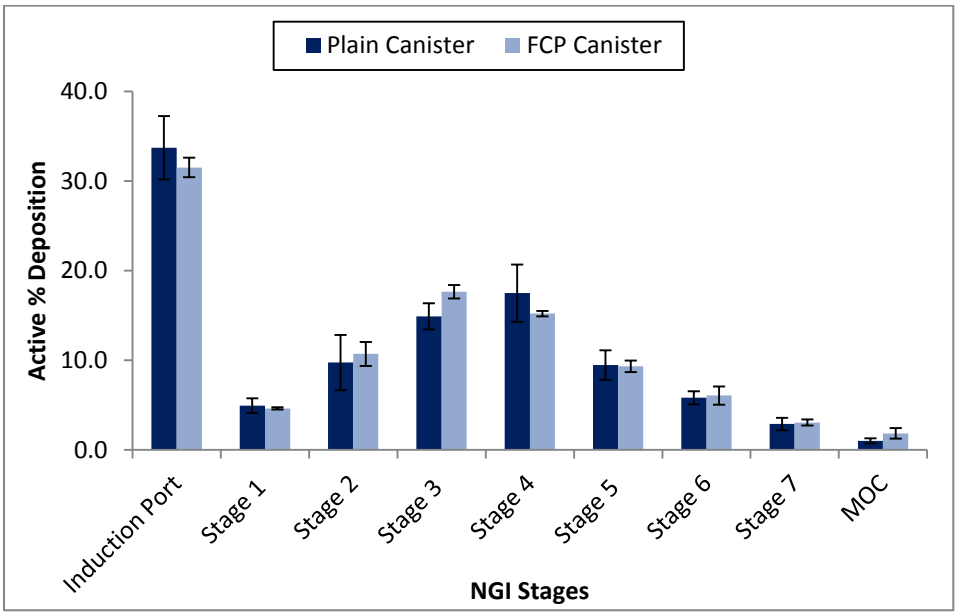

Figure 4. 15 NGI Distribution Pattern (%) of Tio: SV010 Formulations Using Different Canister Coatings (n=3; mean ± SD)

The particle size distribution is shown in Figure 4.15. Both types of canisters show similar distribution patterns, however increased variability of stage deposition is apparent in plain canisters. Over the canister life, the average ex-actuator doses are 6.48 ± 1.45 µg for plain canisters and 7.18 ± 1.19 µg for FCP canisters. Canister surface coating ensures delivery of higher and less variable ex-actuator doses than plain aluminium surface with statistical significance (t-test, $P< 0.05$). As shown in Figure 4.16, FCP canisters maintain consistent emitted doses over the canister life, but plain canisters shows high doses at the beginning and an obvious decrease at the end. The dropping in ex-actuator doses suggests decreased drug concentration

in the bulk formulation. As a low surface energy coating material, FCP can effectively reduce the potential drug absorption on the canister inner surface.

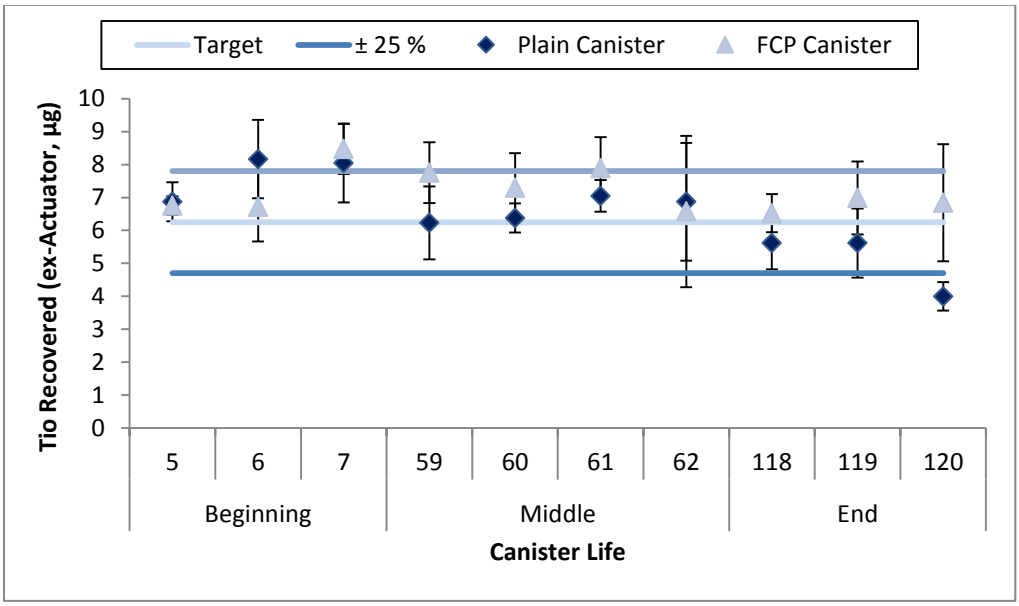

Figure 4. 16 Canister Life Content Uniformity of Tio: SV010 Formulations Using Different Canister Coatings (n=3; mean ± SD)

In the group of Tio: Leu 1:5, no significant difference in ex-valve doses was identified between plain (8.02 ± 1.59 µg) and FCP (7.82 ± 0.56 µg) canisters (t-test, P> 0.05). The aerosol performance is shown in Figure 4.17 and Table 4.11. The FPF (t-test, P< 0.05) and MMAD (t-test, P< 0.05) of FCP canisters are significantly higher than plain canisters, while FPD (t-test, P> 0.05) and GSD (t-test, P> 0.05) are similar. As the ex-actuator dose of plain canisters is 7.76 ± 0.92 µg, which is greater than FCP canisters of 6.53 ± 0.26 µg, therefore the increase in ex-actuator dose compensates for the difference in FPD. Compared with Tio: SV010 (Table 4.11), Tio: Leu shows an increase FPF, and a decrease in MMAD and GSD with statistic difference (ANOVA, P< 0.05), when either canister is used. This suggests Tio: Leu has a better aerosol performance than Tio: SV010 by producing more fine Tio particles and less polydispersity of the particle distribution, and the same result is described in Section 4.5.7. Leucine and SV010 also show different aerodynamic

behaviours within HFA 227. At the beginning of canister life, SV010 provides equivalent aerosol performance regardless of canister coating, whilst leucine appears to be more susceptible to fine particle deposition on the plain aluminium surface.

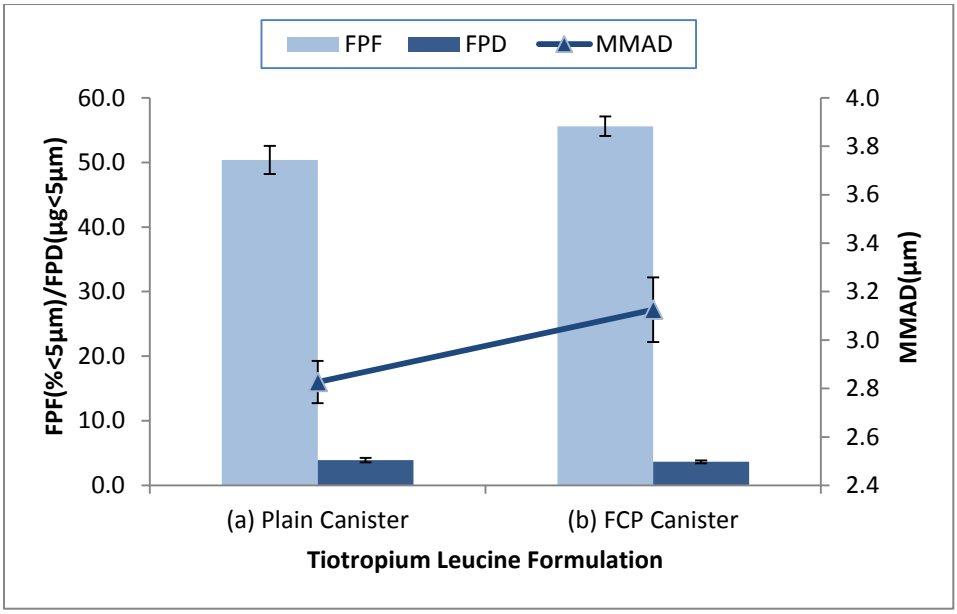

Figure 4. 17 Aerosol Characteristics (ex-actuator) of Tio: Leu Formulations with Different Canister Coatings (n=3; mean ± SD)

Table 4. 11 Aerosol Characteristics (ex-actuator) of Tio: Leu Formulations with Different Canister Coatings (n=3; mean ± SD)

Canister Coating	(a) Plain Canister	(b) FCP Coated Canister
Tio FPF (% < 5 µm)	50.38 ± 2.18	55.61 ± 1.50
Tio MMAD (µm)	2.83 ± 0.09	3.13 ± 0.13
Tio FPD (µg < 5 µm)	3.90 ± 0.34	3.64 ± 0.23
Tio GSD	1.96 ± 0.06	1.90 ± 0.09

((a) Tio:Leu/EXCIP 1:5/FRAC <38 µm/HFA 134a/VAL 25 µL/CAN 14 mL/CAN Plain/ACT 0.25 mm, (b) Tio:Leu/EXCIP 1:5/FRAC <20 µm/HFA 134a/VAL 25 µL/CAN 19 mL/CAN Plain/ACT 0.25 mm)

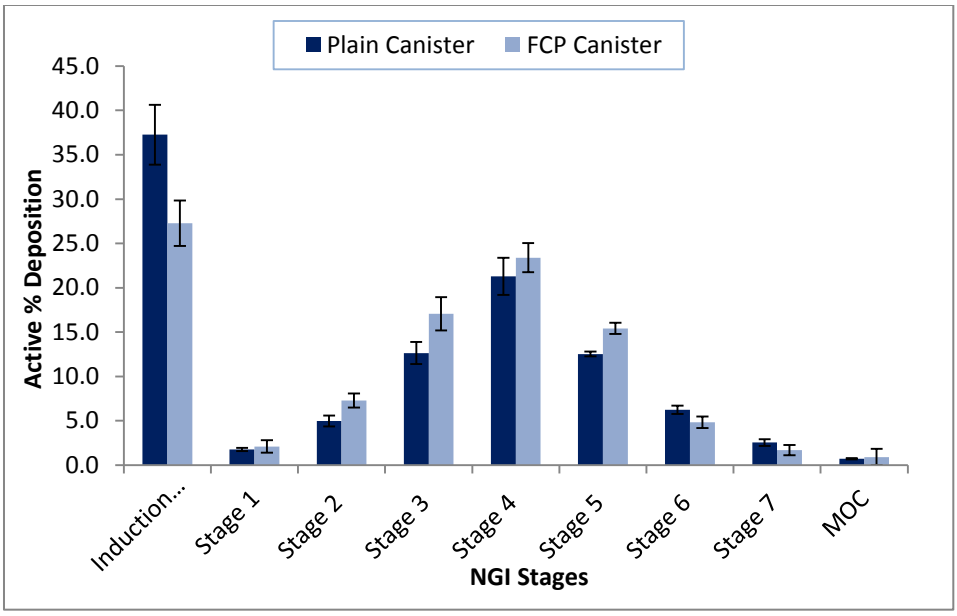

Figure 4. 18 NGI Distribution Pattern (%) of Tio: Leu Formulations Using Different Canister Coatings (n=3; mean ± SD)

The particle size distribution is presented in Figure 4.18. Distribution patterns of the two types of canisters are similar with the peak deposition on Stage 4. However, a significant increase of deposition on IP (t-test, $P< 0.05$), and accordingly decrease on lower stages, e.g. Stage 3 (t-test, $P< 0.05$) and 5 (t-test, $P< 0.05$) are identified in plain canisters. As shown in Figure 4.19, the ex-actuator dose uniformity of FCP canisters remains constant over the canister life, whilst the performance of plain canisters diminish towards the end of canister life. Ex-actuator doses of FCP canisters (5.47 ± 0.44 µg) are also significantly higher than plain canisters (4.76 ± 1.18 µg) (t-test, $P< 0.05$). Consequently, FCP coated surfaces can prevent particle-particle and particle device interaction, improve the aerosol performance of the formulation Tio: Leu, and maintain constant dose delivery over the canister life.

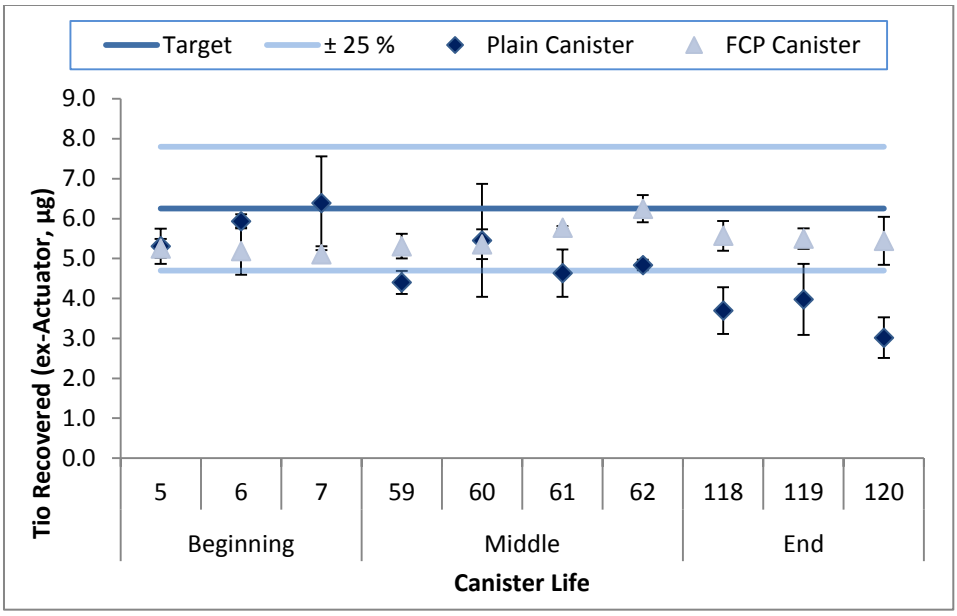

Figure 4. 19 Canister Life Content Uniformity of Tio: Leu Formulations Using Different Canister Coatings (n=3; mean ± SD)

4.5.6 Excipient Ratio

In a dry powder inhaler (DPI), excipients or carriers normally account for more than 80% of the formulation weight. Low doses of micronised drug particles are mixed with the carriers to improve the powder flowability and facilitate the metering and handling processes. The ratio of carriers is assumed to affect adhesion of drug particles, content uniformity or even particle distribution (Le et al, 2012). Similarly, the addition of secondary particles in pMDIs is used to facilitate the dispensing and manufacturing process of the low-dose therapeutics, but a lower amount of secondary particles are required compared to the DPI products. It is also assumed that the secondary particle could improve the dispersibility of drugs in propellants and further affect the aerodynamic behaviour of drug particles. Though there is no full understanding of the atomisation process of the suspension formulation, fewer droplets containing drugs are observed than the solution formulation since many of the generated droplets contain no drugs (Stein, 2008). Consequently, this research will focus on the influence of the ratio of large excipient particles on formulation stability and aerodynamic behaviour in a suspension formulation.

According to the patent of Dickinson and Warren (2004), the weight ratio of the drug and the secondary particles was defined to cover the range of 1:0.1 to 1:500 and more preferable between 1: 5 and 1:50 in pMDI systems. To investigate the influence of the mass of the secondary particles, Respitose® SV003 is selected as the model excipient, and formulations containing different Tio: SV003 ratios, e.g. 1:2.5, 1:5, 1:10 and 1:25, were produced. The blend uniformity of the four formulations is 1.60% (1: 2.5), 1.51% (1: 5), 0.86% (1: 10) and 0.57% (1: 25) respectively, which is expressed as the relative standard deviation (RSD) of recovery percentage from 5 separated measurements (Section 2.4). Blend uniformity is within the acceptable level (<5%) for a uniform mixture for all of the formulations, decreasing the ratio of SV003 led to an increase in uniformity.

Table 4. 12 Valve and Actuator Performance of pMDI Formulation with Different SV003 Ratios (1:2.5, 1:5, 1:10 and 1:25) (n=30; mean ± SD)

Tio: Excipient Ratio	(a) 1: 2.5	(b) 1: 5	(c) 1: 10	(d) 1: 25
Metered Weight (mg)	63.46 ± 3.19 (a1)	64.58 ± 1.96 (b1)	62.45 ± 2.20 (c1)	62.26 ± 2.56 (d1)
Ex-valve Dose (µg)	11.73 ± 2.65	11.40 ± 3.40	10.85 ± 3.10	10.33 ± 2.65
Actuator Deposition (%)	39.10 ± 4.47 (a2)	35.02 ± 4.95 (b2)	32.98 ± 3.44 (c2)	28.18 ± 5.19 (d2)

(Metered weight: canister weight difference between pre- and post-actuation, which includes the weight of the formulation and propellant; Ex-valve emitted dose: the dose emitted from the valve; Actuator deposition: the percentage of the dose deposited on the actuator) (b1 is significant higher than c1 and d1 ($P<0.05$). a2 is the greatest ($P< 0.05$), d2 is the least of all the ratios ($P< 0.05$), and b2 is comparable to c2 ($P> 0.05$)

The valve and actuator performance is shown in Table 4.12. According to Section 4.4.3, the target metered weights per actuation of the four formulations are 60.94 mg (1: 2.5), 60.97 mg (1: 5), 61.02 mg (1: 10), and 61.19 mg (1: 25), and the experimental weights are 4%, 6%, 2% and 2% higher than targets respectively. According to ANOVA and Tukey HSD test, the metered weight of 1: 5 Ratios is slightly higher than the 1: 10 ($P< 0.05$) and 1: 25 ($P< 0.05$), but weights are consistent over the 30 actuations, indicated by low standard deviations (SD) of all the ratios tested. This result suggests that the VARI® metering valve is compatible with formulations containing Tio: SV003 within the ratio range of 1:2.5 and 1: 25. However, ex-valve doses of all the four formulations shows variable results with relative standard deviations (RSD) higher than 20%. The actuator deposition decreases as the ratio of excipient decreases (ANOVA, $P< 0.05$). According to Tukey HSD test, SV003 (1:2.5) is significantly higher ($P< 0.05$), and SV003 (1:25) is significantly lower than the rest ($P< 0.05$), whereas SV003 (1:5) is comparable to SV003 (1:10) ($P> 0.05$). The increased chance of collision between the airborne particles and the deposited particles on actuator surface may accelerate the detachment. However, the increase in excipient ratio also causes the blockage of actuators with repetitive use. The regular cleaning or change to larger orifice

actuators are recommended for formulations containing high SV003 ratio (Tio: SV003⩾ 10).

Table 4 13 Aerosol Characteristics (ex-actuator) of Formulations with Different SV003 Ratios (1:2.5, 1:5, 1:10 and 1:25) (n=3; mean ± SD)

Tio: Excipient Ratio	(a) 1:2.5	(b) 1:5	(c) 1:10	(d) 1:25
Tio FPF (% < 5 μm)	25.15 ± 3.88	22.33 ± 3.05	25.57 ± 2.84	26.24 ± 2.24
Tio MMAD (μm)	3.80 ± 0.08	4.02 ± 0.14	2.76 ± 0.08	3.26 ± 0.09
Tio FPD (μg < 5 μm)	2.57 ± 0.42	2.46 ± 0.38	2.18 ± 0.19	2.59 ± 0.29
Tio GSD	2.30 ± 0.17	2.31 ± 0.12	2.46 ± 0.06	2.59 ± 0.08

((a) Tio:SV003/EXCIP 1:2.5/FRAC <38 μm/HFA 134a/VAL 50 μL/CAN 19 mL/CAN Plain/ACT 0.25 mm, (b) Tio:SV003/EXCIP 1:5/FRAC <38 μm/HFA 134a/VAL 50 μL/CAN 19 mL/CAN Plain/ACT 0.25 mm, (c) Tio:SV003/EXCIP 1:10/FRAC <38 μm/HFA 134a/VAL 50 μL/CAN 19 mL/CAN Plain/ACT 0.25 mm, (d) Tio:SV003/EXCIP 1:25/FRAC <38 μm/HFA 134a/VAL 50 μL/CAN 19 mL/CAN Plain/ACT 0.25 mm)

Table 4.13 illustrates parts of the aerodynamic distribution characteristics of Tio formulations with different SV003 ratios. There is no significant difference in FPFs (ANOVA, P> 0.05). However, FPFs are much lower than the values presented in Table 4.1 (Section 4.5.1) and Table 4.6 (Section 4.5.3), which is caused by equipping with 50 μL metering valves, and similar result is gained with Tio: SV003 and 50 μL metering valve in Table 4.4 (Section 4.5.2). In DPIs where carriers are more often used, some research illustrated fine particle fraction of APIs increased with the increase of the API concentration (Harjunen et al, 2003; Le et al, 2012). This could be explained by increased proportion of active in the blend which contributes to the adhesion of drug particles to the low energy sites of the carriers, which accelerates the de-aggregation and increases the number of fine particles. However, the relationship between the active: carrier ratio and FPF is not straightforward and highly dependent on the physicochemical properties of both drugs and carriers, and a balance between the adhesion and cohesion forces (Harjunen et al, 2003). However, in the suspension formulation, previous research conducted by Jones (2004) illustrated there was no defined interaction identified between excipient ratio and FPF using salbutamol sulphate and leucine. The same results were

concluded by Vehring et al (2012) in that engineered porous phospholipid was co-suspended with a variety of strengths of glycopyrrolate in HFA 134a to prepare a pMDI suspension. The ratio of phospholipid microparticles and glycopyrrolate did not impact on the aerosol performance, e.g. FPF and MMAD, of the co-suspension pMDI. As the excipient concentration is much higher than the drug concentration, the saturated binding of drug particles on high-energy binding points of the carriers make no difference on particle interaction. Similarly, FPDs are between 2.18 μg and 2.59 μg, and no significant difference is identified (ANOVA, $P > 0.05$). However, distinctive patterns between excipient ratios and aerodynamic diameters are noticed (ANOVA, $P< 0.05$). According to Tukey HSD test, MMAD values are comparable in high drug ratios (1:2.5 and 1:5) ($P> 0.05$), while significant decrease is found in low drug ratios (1: 10 and 1: 25), with the increase of the mass of SV003, the aggregation of drug particles may be prevented more effectively. There are no significant differences of GSD values between SV003 (1:2.5), SV003 (1:5) and SV003 (1:10) ($P> 0.05$), but SV003 (1: 25) is found more hetero-dispersed.

The addition of excipients has been assumed to affect the formulation uniformity during storage and formation of initial droplets during atomisation, and the formation of initial droplets would further influence the particle distribution of the aerosols (Dickinson and Warren, 2004; Sheth et al, 2015). The increase in the number of larger excipients was considered to increase the binding points where the drug particles were attached (Hersey, 1975). However, Figure 4.20 shows the aerosol particle size distribution pattern of formulations, which are similar with different SV003 ratios. It is obvious that all the formulations had very high IP deposition (in excess of 55%), which is relevant to the high vapor pressure of HFA 134a and the volume of metering valves. The similar IP deposition is gained Tio: SV003 in HFA 134a as shown in Figure 4.7 and the influence of propellant type has been discussed in Section 4.5.3. The large valve volume of 50 μL affects the IP

deposition as discussed later in this section. The ANOVA test illustrates there is no significant difference between IP stage deposition for different SV003 ratios (P> 0.05). However, the low efficiency of the four formulations may overcome the influence of the excipient ratio. It can be concluded that Tio: SV003 (in the range of 1:2.5 to 1:25) has no significant effect on de-aggregation of Tio particles from larger SV003 carrier particles during the atomisation but a further investigation on formulations with an improved aerosol performance is required.

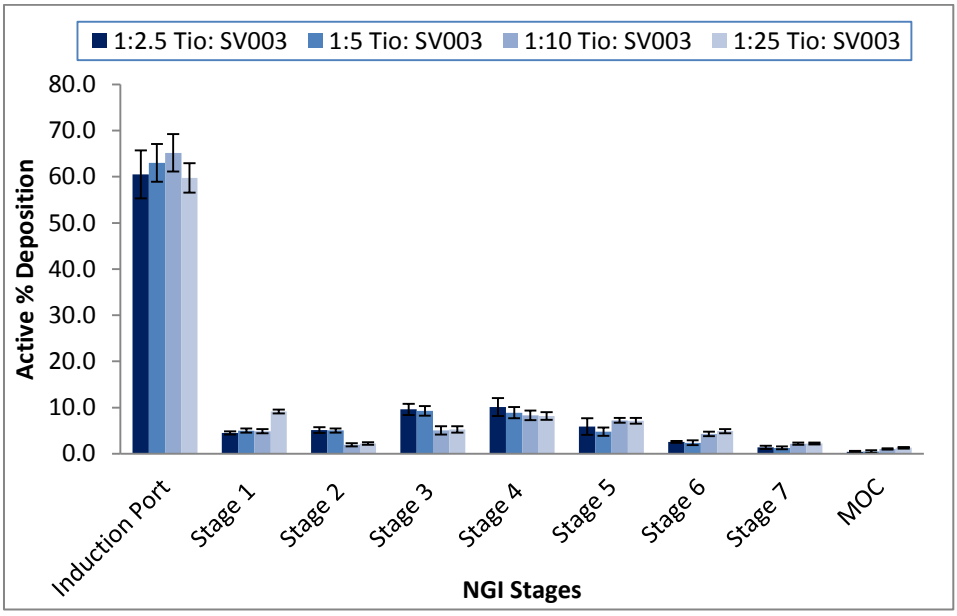

Figure 4. 20 NGI Distribution Pattern (%) of Tio Formulations with Different SV003 Ratios (1:2.5, 1:5, 1:10 and 1:25) (n=3; mean ± SD)

The ex-actuator emitted doses are 7.18 ± 1.69 µg (1:2.5), 7.39 ± 2.00 µg (1:5), 7.27 ± 1.52 µg (1:10) and 7.39 ± 1.27 µg (1:25) respectively. There is no significant difference between different SV003 ratios (ANOVA, P> 0.05). As shown in Figure 4.21, high and variable emitted doses are noticed at the beginning of canister life, which could be due to the compatibility of the 50 µL valves, or decreased Tio concentration in the bulk at the end of canister life. In conclusion, no evidence of a direct relation was identified between Tio: secondary particle ratio (in the range of 1:2.5 to 1:25) and the performance of pMDIs. The addition of higher ratios of

secondary particles prevents the aggregation of drug particles in suspension as found in Figure 4.4 and facilitates the measuring of potent drugs but also increases the risk of blocking the actuator orifice.

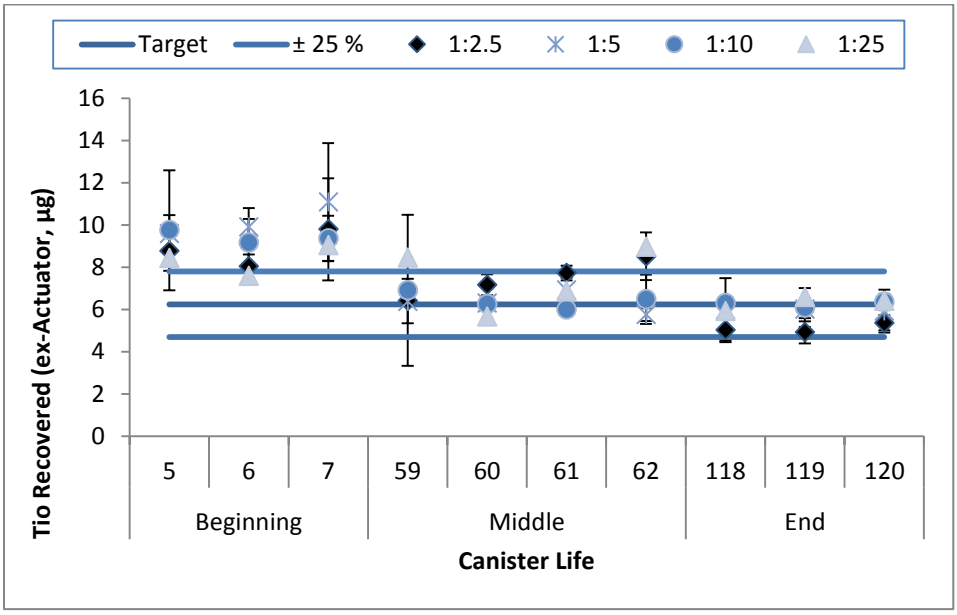

Figure 4. 21 Canister Life Content Uniformity of Tio Formulations with Different SV003 Ratios (1:2.5, 1:5, 1:10 and 1:25) (n=3, mean ± SD)

According to Section 4.5.2, smaller valve metering volume gives a higher concentration of the drug and secondary particle. Increased chance of particle interaction and more efficient evaporation after atomisation indicates a better aerosol performance. A new batch is prepared with 25 µL VARI KHFA metering valve and compared with 50 µL valves at the ratio of 1:5. The aerosol characteristics are illustrated in Figure 4.22. The FPF and dose of 25 µL valve is higher than 50 µL but with no significant difference (t-test, P> 0.05). The MMAD of both are in respiratory range (< 5 µm) but the value of 25 µL valve (4.38 ± 0.16 µm) is significant higher than 50 µL (4.02 ± 0.14 µm). These are different with the findings in Section 4.5.2, where the ratio of Tio: SV003 was 1: 25 and canisters were crimped with 50 µL valves. FPF was significantly higher and MMAD was

significantly lower with 25 µL valves. This indicates the increase the ratio of SV003 has a different effect on valves with different volumes.

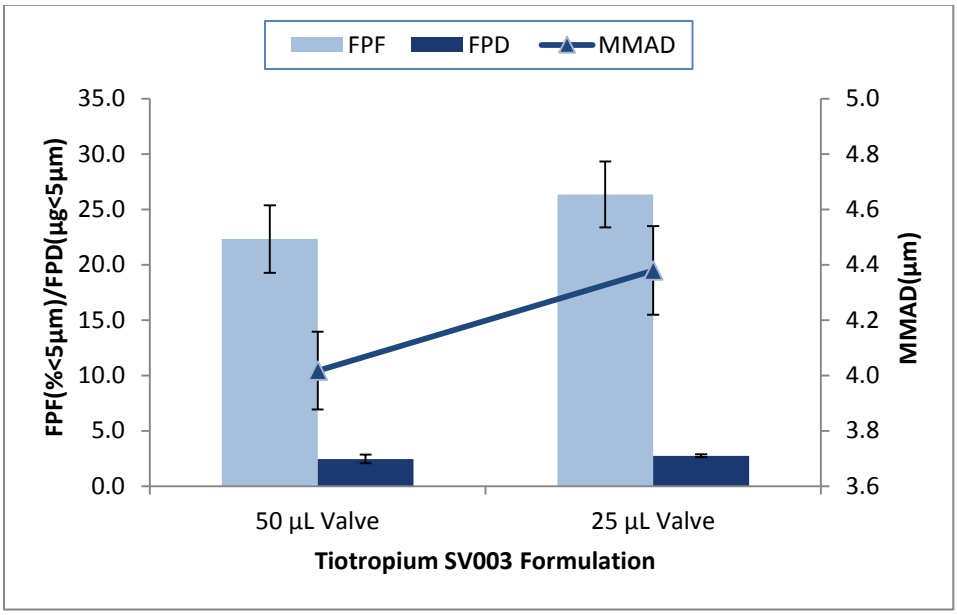

Figure 4. 22 Aerosol Characteristics (ex-actuator) of Tio Formulations at the Ratio of 1:5 with 50 µL and 25 µL VARI® Metering Valve (50 µL: n=3; mean ± SD, 25 µL: n=5; mean ± SD)

The aerodynamic distribution is shown in Figure 4.23. The 25 µL valve gives a lower IP deposition (t-test, $P< 0.05$) and higher Stage 2 deposition (t-test, $P< 0.05$) than the 50 µL valve. Similar IP depositions were obtained with 25 µL and 50 µL valves in Section 4.5.2, but increase of deposition percentage in lower stages, e.g. Stage 4, 5 and 6, was also found with 25 µL valves in Figure 4.3. Therefore, a high ratio of SV003 has a more beneficial effect on low-volume valves such as 25 µL. When combining the IP deposition, Stage 1 and Stage 2, where the aerodynamic particle size is over than 6.4 µm, both values are comparable (t-test, $P> 0.05$). Furthermore, more drug particles are captured in Stage 3 of 25 µL valve (t-test, $P< 0.05$), but this has limited impact on overall aerodynamic performance. Consequently, it is considered that distribution patterns of two different types of valves are similar.

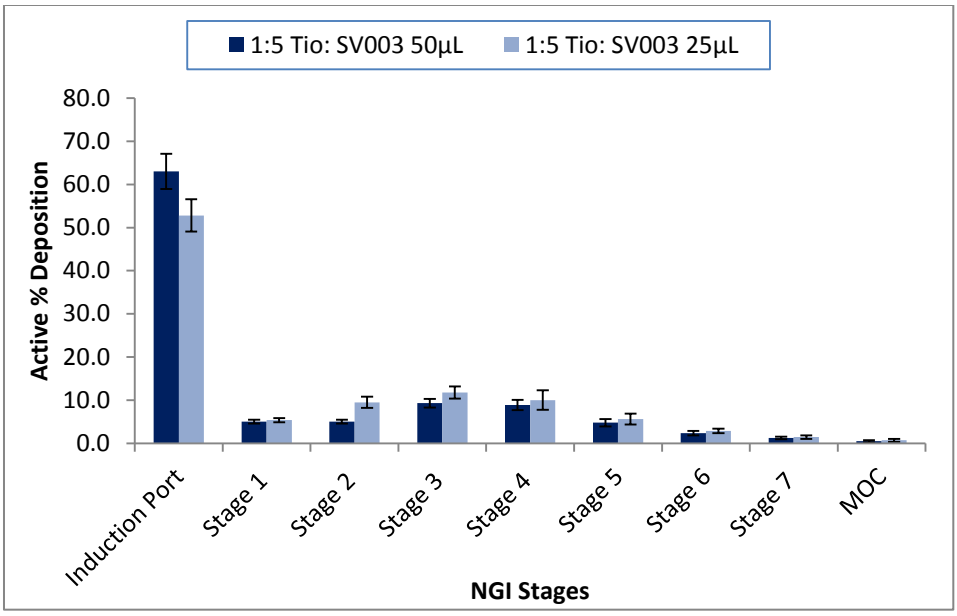

Figure 4. 23 NGI Distribution Pattern (%) of Tio Formulations at the Ratio of 1:5 with 50 µL and 25 µL VARI® Metering Valve (50 µL: n=3; mean ± SD, 25 µL: n=5; mean ± SD)

The content uniformity over the canister life is plotted in Figure 4.24. Similarly, ex-actuator doses are found higher than the target at the beginning of canister life, and lower at the end. Although metered weights of both valves are consistent, the drug concentration in the bulk formulation decreases over time. The drug loss could be due to drug particle agglomeration, degradation or adhesion to the contactable surface of the device, e.g. canister inner surface. The previous research in Section 4.5.1 and 4.5.5 has demonstrated that large canister volume and uncoated inner surface could decrease the dose uniformity over the canister life but the potential agglomeration could also contribute to reproducible emitted dose. Therefore, further researches on factors of excipient type and excipient particle size are investigated in Section 4.5.7 and 4.5.8.

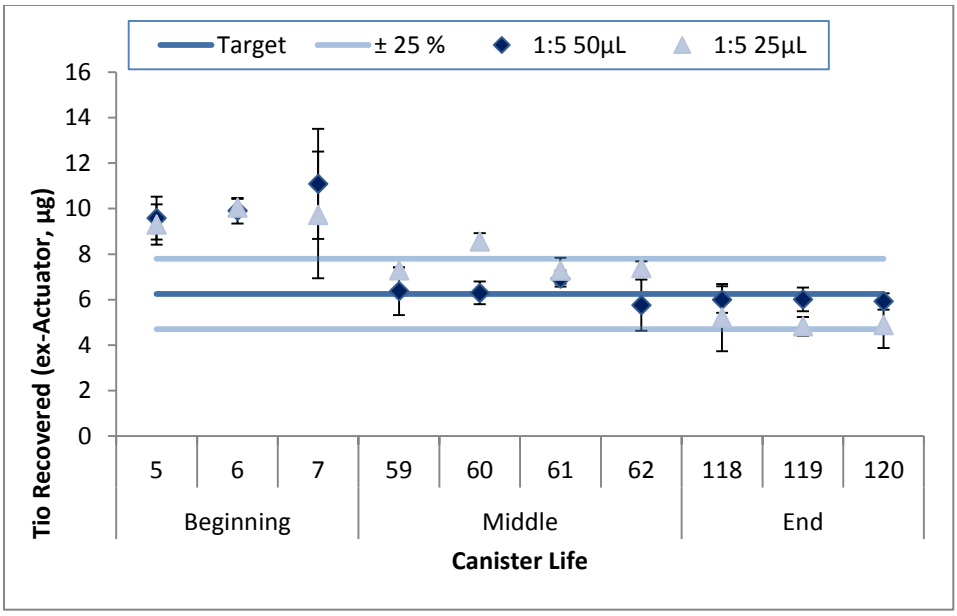

Figure 4. 24 Canister Life Content Uniformity of Tio Formulations at the Ratio of 1:5 with 50 µL and 25 µL VARI® Metering Valve (50 µL: n=3; mean ± SD, 25 µL: n=5; mean ± SD)

4.5.7 Excipient Type

The mechanisms whereby the secondary particles stabilise and optimise aerosol characteristics were investigated using different types of excipients in Tio formulations. Our preference was to choose substances with regulatory approval as the secondary particles. FDA approved excipients, used for pulmonary delivery, include lactose and other sugars, e.g. mannitol and glucose. Lactose is the most widely used excipient in inhalation formulation and Leucine, as an alternative excipient, has also been used as a ternary component to the formulation containing API and lactose to increase the performance of aerosols (Labiris and Dolovich, 2003b). In this section, coarse lactose including Respitose® SV003 and SV010, and leucine are selected to assess the feasibility to use in the novel pMDI formulations.

Table 4. 14 Particle Distribution of Different Raw Secondary Particles

Excipient	d(0.1) (µm)	d(0.5) (µm)	d(0.9) (µm)	Span
Respitose® SV003	15.39 ± 0.15	48.37 ± 0.04	90.64 ± 0.01	1.55
Respitose® SV010	29.89 ± 0.58	94.72 ± 0.29	183.33 ± 0.48	1.62
Leucine (90-106µm)	17.93 ± 2.46	78.57 ± 2.45	166.25 ± 1.60	1.88

(Leucine 90-106µm: sieve fractions received through mechanical vibrating; d(0.1): particle size under which 10% volume of particles lie, d(0.5): particle size under which 50% volume of particles lie, d(0.9): particle size under which 90% volume of particles lie)

Particle size determinations for three types of secondary particles are illustrated in Table 4.14. SV010 indicates the largest mass median diameter with a d(0.5) of 94.72 ± 0.29 µm and SV003 the smallest with a d(0.5) of 48.37 ± 0.04 µg. According to the product specification, d(0.5) of SV003 is 53 - 66 µm, and d(0.5) of SV010 is 95 - 126 µm, which are slightly lower than the results received from Mastersizer 2000. Leucine shows the widest particle distribution with the span of 1.88 while SV003 shows the narrowest with the span of 1.55. As the fraction of leucine (90 – 106 µm) is received through mechanical vibrating, it is noticed that d(0.5) of leucine (90 - 106 µm) is lower than the mesh size of the top sieve, 90 µm, and d(0.9) is

higher than mesh size of the bottom sieve, 106 μm, which also indicates a wider particle size distribution.

The blend uniformity of the formulations containing SV003, SV010 and leucine is 2.39%, 3.19% and 0.30% respectively, which is expressed as the relative standard deviation (RSD) of recovery percentage from 5 separated measurements (Section 2.4). All the mixtures are uniformly dispersed with a RSD less than 5%. Therefore, the addition of the secondary particles can facilitate accurate dose dispensing for manufacturing purposes. The total metered weight, defined as the metering mass including the formulation and propellant, and the ex-valve emitted dose per actuation of the four batches are illustrated in Table 4.15 and Figure 4.25. According to ANOVA and Tukey HSD test, the metered weights of Tio control and Tio: SV010 are comparable with Tio: Leu ($P > 0.05$), and Tio: SV003 is significantly less than the rest ($P < 0.05$). However, Tio control shows the biggest weight variability indicated by a large standard deviation of 2.46. Furthermore, the target metered weight based on the formulation calculation, is 35.06 mg and all the formulations are over the target value. However, as there was no trend of decrease of metering weights over the canister life as shown in Figure 4.25, so the 25 μL metering valves is considered compatible with HFA 227, which is independent of the formulation used.

The targeting ex-valve emitted dose is 11.25 μg per actuation. According to Table 4.15, with the addition of the secondary excipients, less ex-valve emitted doses are measured. There is no significant difference of the ex-valve doses between Tio: SV003, Tio: SV010 and Tio: Leu (ANOVA, $P > 0.05$). Higher emitted doses are found in Tio control than Tio with secondary particles, and large dose variability is also indicated by a large standard deviation of 3.55. The large variability in metering weights and emitted doses are observed with Tio Control which can be due to

aggregation of Tio particles, and similar result is illustrated by Figure 5.4 in Chapter 5. The other reason is the potential adsorption of Tio on the surface of plain aluminium canisters and this is also proven in Figure 4.16 (Section 4.5.5) Nevertheless, it is necessary to conduct post-canister-life tests to calculate the mass balance of the testing canisters. It seems all these secondary particles are found to prevent interaction between Tio particles and the pMDI device, and maintain the suspension uniformity.

Table 4. 15 Valve and Actuator Performance of pMDI Formulations with Different Excipients (n=28; mean ± SD)

Excipient Type	Tio	SV003	SV010	Leucine
Metered Weight (mg)	41.22 ± 2.46	37.92 ± 1.28	40.64 ± 1.05	41.38 ± 1.33
Emitted Dose (ex-valve) (μg)	12.40 ± 3.55	9.91 ± 1.44	10.29 ± 1.93	10.02 ± 1.98
Actuator Deposition (%)	23.20 ± 2.42	30.23 ± 0.68	35.64 ± 1.91	41.02 ± 0.58

(Metered weight: canister weight difference between pre- and post-actuation, which includes the weight of the formulation and propellant; Ex-valve emitted dose: the dose emitted from the valve; Actuator deposition: the percentage of the dose deposited on the actuator)

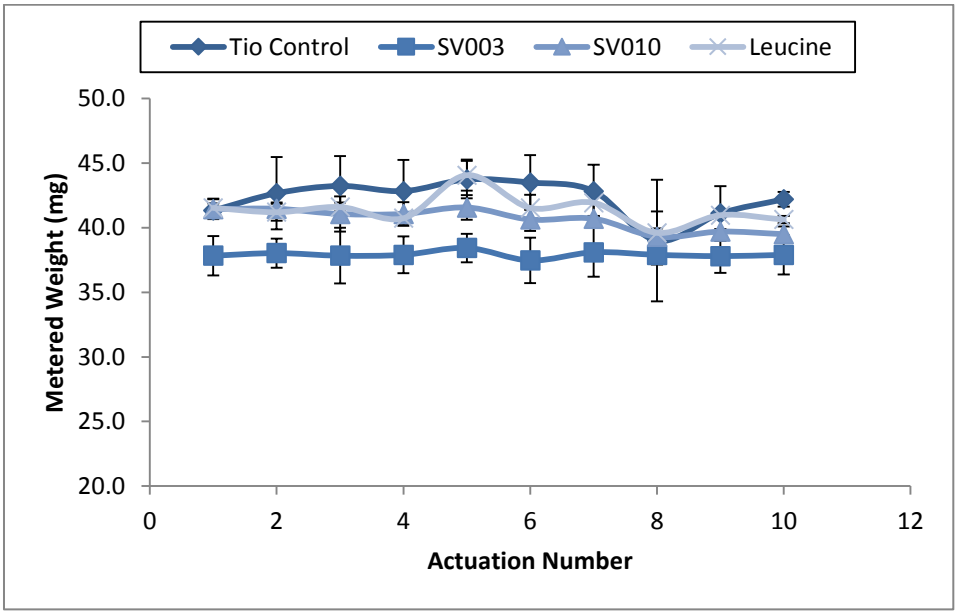

Figure 4. 25 Metered Weight of Tio Formulations with Different Excipients (SV003, SV010 and Leucine) (n=3; mean ± SD)

The four batches of pMDIs are equipped with H&T Presspart actuators with the same orifice size, 0.25 mm. The percentage of actuator deposition of each batch is illustrated in Table 4.15, there are significant differences (ANOVA, P< 0.05). According to Tukey HSD test, the lowest actuator deposition is found with Tio control while with the greatest variability (P< 0.05). Tio: Leu has the greatest deposition (P< 0.05), followed by Tio: SV010 (P< 0.05), and Tio: SV003 (P< 0.05). However, the deposition percentage of all formulations tested is much higher than some commercialised products, e.g. 10% (Tiova® tiotropium bromide/ HFA 227), 17% (Ventolin® salbutamol sulphate/ HFA 134a) and 13% (Pulmicort® budesonide /HFA 134a), which were tested by our in-house methods. It is assumed that the addition of the secondary particles influences the atomisation process as more particles presenting in the aerosol plume, which increase the chance of particle collision and impaction on the surface of actuators. Furthermore, though ionizing bar is applied during the sieving, the electrostatic charges could be generated. The induced electrostatic charges during atomisation may attract more drug particles or drug-secondary particle aggregates.

Table 4. 16 Aerosol Characteristics (ex-actuator) of Formulations with Different Excipients (SV003, SV010 and Leucine) (n=3; mean ± SD)

Excipient	(a) Tio Control	(b) SV003	(c) SV010	(d) Leucine
Tio FPF (% < 5 µm)	40.08 ± 3.55	38.11 ± 1.03	44.32 ± 4.63	50.38 ± 2.18
Tio MMAD (µm)	3.00 ± 0.25	4.49 ± 0.17	3.53 ± 0.39	2.83 ± 0.09
Tio FPD (µg < 5 µm)	3.59 ± 0.89	3.18 ± 0.20	3.96 ± 0.26	3.90 ± 0.34
Tio GSD	2.46 ± 0.12	2.31 ± 0.09	2.22 ± 0.01	1.96 ± 0.06

((a) Tio Only /HFA 227/VAL 25 µL/CAN 14 mL/CAN Plain/ACT 0.25 mm, (b) Tio:SV003/EXCIP 1:5/FRAC <38 µm/HFA 227/VAL 25 µL/CAN 14 mL/CAN Plain/ACT 0.25 mm, (c) Tio:SV010/EXCIP 1:5/FRAC <20 µm/HFA 227/VAL 25 µL/CAN 14 mL/CAN Plain/ACT 0.25 mm, (d) Tio:Leu/EXCIP 1:5/FRAC <20 µm/HFA 227/VAL 25 µL/CAN 14 mL/CAN Plain/ACT 0.25 mm)

The in-vitro aerosol performance was tested at the beginning of canister life and illustrated in Table 4.16. According to ANOVA and Tukey HSD test, FPF of Tio: SV003 is comparable to Tio SV010 (P> 0.01). The Tio: Leu shows the greatest FPF (P< 0.01),

followed by Tio: SV010 (P< 0.01). Tio control (Tio only) is found much higher than data illustrated in Table 4.1 (Chapter 4), and Table 5.5 (Chapter 5). The data presented here is the original data tested as the Tio was received but Table 4.1 and Table is re-tested data a couple of years after the purchase of the Tio source. Co-suspended secondary particles, e.g. SV010 and leucine, are believed to form adequate bonding with the micronised Tio particles, which is strong enough to retain drug particles in suspension, and weak enough to break up during the atomisation. In previous research, it was also found that co-suspension of one kind of porous phospholipid microparticles with micronised therapeutic agents, e.g. glycopyrrolate processed great efficient dose delivery with high FPFs of 68%, and low IP deposition of less than 33% (Tarara et al, 2004; Vehring et al, 2012). The difference in physicochemical characteristics of the secondary particles, e.g. roughness, influences the interaction with the drug particles and aerodynamic behaviour during the atomisation (Steckel and Bolzen, 2004). It was recognised that the increased rugosity of microparticles could prevent the close contact and influence the agglomeration properties of the dispersed particles (Tarara et al, 2004). Consequently, the effects of particle surface characteristics regarding secondary particles are needed for future investigation.

The FPDs of the four groups are shown to be similar (ANOVA, P> 0.05). According to Table 4.37, Tio: SV003 has the greatest aerodynamic diameter (P< 0.05) while Tio control is comparable to Tio: SV010 and Tio: Leu (P> 0.05). During the atomisation process, the initial droplets, which is defined as the droplets generated immediately after atomisation is relevant to the residual droplets, which is defined as the final droplets that deposit in the NGI. Sheth et al (2013) found the size of the initial droplets depend on the dispersed particle size and number, and determined the size of the residual droplets size. Therefore, the added secondary particles are assumed to affect the formation of aerosol mist or the initial droplets. Furthermore,

the interaction between the drug particle and secondary particles exists in the suspension, and effective detachment between these particles is required during the atomisation. Tio: SV003 shows the least fine particles and the greatest aerodynamic diameter, which indicate a strong interaction between drug and SV003 particles may influence the de-aggregation.

The in-vitro particle size distribution is illustrated in Figure 4.26, Tio control shows the highest deposition on induction port (IP) (44.42 ± 6.44%) and the micro-orifice collector (MOC) (2.63 ± 1.29%), so both large aggregates and extra-fine particles are found, which are not expected in an ideal pMDI. The drug or aggregates in the case of corticosteroids in a DPI formulation could deposit in the oropharyngeal region and caused local side effects if they are not detached from the excipients after aerosolisation (Labiris and Dolovich, 2003b). The decreased IP deposition indicates the effective de-aggregation of Tio particles from Tio aggregates and the secondary particles. The extra-fine particles (<0.45 μm) may be exhaled without lung deposition, which represent a dosing problem and may compromise the effectiveness of therapy (Shekunov et al, 2007; Labiris and Dolovich, 2003b). As shown in Table 4.15, Tio control shows the mostly variable metering dose and the greatest GSD of the particle size distribution. With the addition of larger secondary particles, the collision between Tio particles could be reduced and the suspension is more physically stabilised. The uniformity of both the bulk and metered dose are achieved, and effective de-aggregation also contributes to the improvement of aerosol performance.

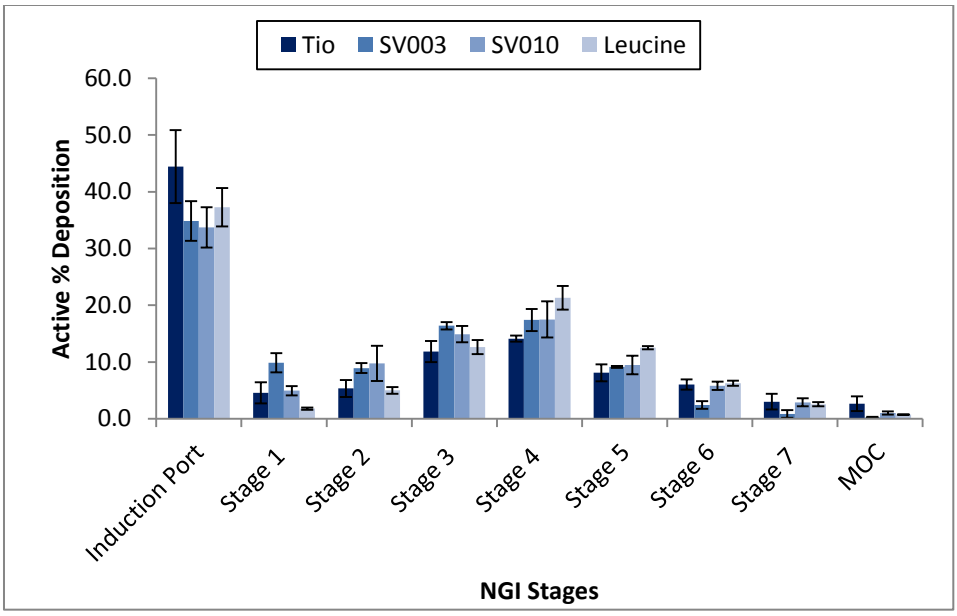

Figure 4. 26 NGI Distribution Pattern (%) of Tio Control and Formulations with Different Excipients (SV003, SV010 and Leucine) (n=3; mean ± SD)

The canister life content uniformity of all the batches is shown in Figure 4.27. The target ex-actuator dose is set as 6.25 µg. Tio control shows the highest ex-actuator dose, 9.44 ± 1.79 µg, around 50% greater than the target, which can be explained by the greatest ex-valve emitted dose and the lowest actuator deposition. At the beginning and the end of canister life, the emitted doses are found to be more variable than the other testing formulations. The ex-actuator doses of Tio: SV003, Tio: SV010 and Tio: Leu are 6.94 ± 1.23 µg, 6.48 ± 1.23 µg and 5.95 ± 1.30 µg respectively, most of which are in the range of 4.7-7.8 µg, ± 25% of the targeting dose. Tio with excipient also shows more consistent ex-actuator dose. In previous research, it has been proven that pMDI formulations containing salbutamol sulphate, and a secondary particle showed improved canister life performance (Jones, 2004). The inclusion of a larger and coarse excipient particle can effectively maintain the suspension uniformity and contribute to consistent delivery of therapeutic agents. However, there is a trend for all the formulations for the emitted doses to decrease at the end of canister life. This could be due to the

decreased drug concentration in the bulk caused by the loss of drugs on the inner surface of the plain canister, which was investigated and proven in Section 4.5.5.

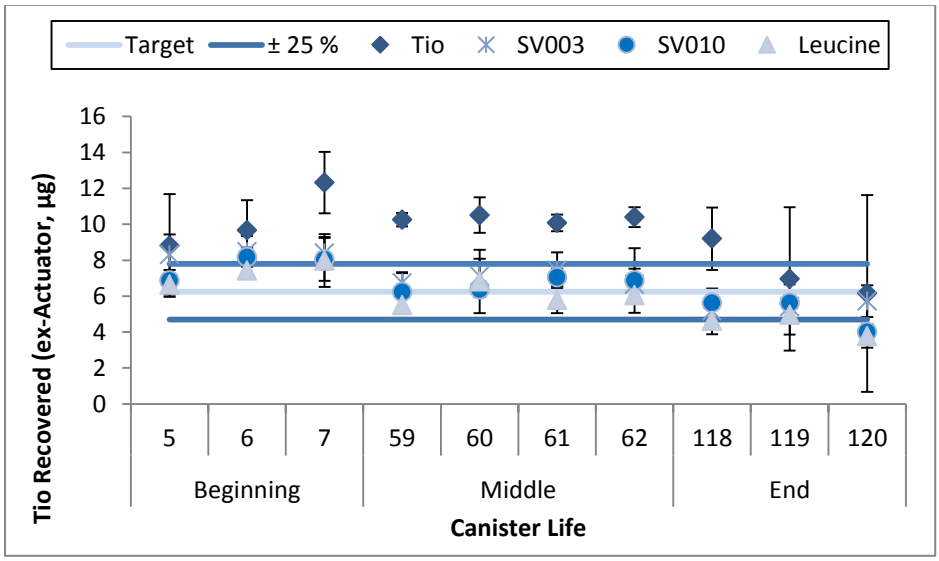

Figure 4. 27 Canister Life Content Uniformity of Tio Formulations with Different Excipients (SV003, SV010 and Leucine) (n=3; mean ± SD)

Large carrier particles, such as α-lactose monohydrate, are generally used to reduce the cohesion and increase the flowability of drug particles in a DPI formulation (Schiavone et al, 2004). Therefore, during the powder preparation, good dry blend uniformity is achieved for all the formulations containing SV003, SV010 and leucine. Compared with Tio control, the addition of the secondary particle can prevent the particle interaction in the suspension to increase the physical stability of micronised Tio, and maintain or enhance the aerosol performance. Lactose is most widely used in inhalation formulations but not preferable where interaction with drugs is observed, e.g. formoterol or peptide and protein drugs (Steckel and Bolzen, 2004). Lactose is also unsuitable for patients with lactose intolerance and a potential issue for diabetic patients. It is found the performance of leucine is comparable and even better than lactose, so leucine could be used as an alternative excipient in pMDI suspension formulations.

4.5.8 Excipient Particle Size

As a critical physiochemical parameter in pharmaceutical products, particle size of both active ingredients and excipients may have significant effects on bioavailability, dissolution and release rate, and safety of active ingredients. For pulmonary delivery, particle size is essential for the content and dose uniformity, and aerosolisation behaviour of respiratory formulations (Shekunov et al, 2007). However, most of the researches regarding the influence of excipient particle size were relevant to DPI products. The addition of lactose fine particles (<5 µm) was found with increased DPI performance in some papers (Islam et al, 2004; Jones and Price, 2006), but few works have been done about influence of excipient size on a pMDI suspension. Jones (2004) reported in the formulation containing salbutamol sulphate and leucine in HFA 134a, there was no well-defined pattern regarding the effect of leucine fractions, e.g. 38-63 µm, 63-90 µm and 90-125 µm, on the aerosol performance. Therefore, in this section, fine excipient particles (<20 µm) is also included. Based on Section 4.5.7, Respitose® SV010 and L-leucine are used as the model secondary particles, and different sieve fractions, e.g. <20 µm, <38 µm and <63 µm, are investigated based on the in-vitro aerosol performance.

4.5.8.1 SV010

Different portions of SV010 samples were prepared according to Section 2.3. The coarse SV010 was sieved through Endecott® sieves with mesh sizes of 38 µm, 63 µm and 90 µm respectively. Particle size data of these different portions of SV010, gathered from Mastersizer 2000 are shown in Table 4.17. As the residual% is less than 1% which indicates the default refractive index and absorption values is fitted with all the samples. There is significant difference in the span between the four groups investigated (ANOVA, $P < 0.05$). According to Tukey HSD test, SV010 (<38 µm) is comparable to SV010 (<63 µm) ($P > 0.05$), and significantly different from SV010 (< 90 µm) ($P < 0.05$) and coarse SV010 ($P < 0.05$). There is significant

difference between SV010 (< 90 µm) and the other groups and the same result is found with coarse SV010. The width of particle size distribution become wider as the increase of the mesh size and slight change was found with sieves less than 38 µm.

Table 4. 17 Characteristics of Laser Diffraction of Different SV010 Fractions (SV010 (<38 µm), SV010 (<63 µm), SV010 (<90 µm), coarse SV010)

Excipient	Residual%	Obscuration%	Span
SV010 (<38µm)	0.28 ± 0.00	2.99 ± 0.10	2.50 ± 0.09
SV010 (<63µm)	0.44 ± 0.02	0.77 ± 0.02	2.45 ± 0.04
SV010 (<90 µm)	0.58 ± 0.15	1.19 ± 0.10	1.94 ± 0.02
Coarse SV010	0.88 ± 0.22	1.14 ± 0.08	1.62 ± 0.02

Particle size distributions of SV010 analysed by Mastersizer 2000 are illustrated Figure 4.28. Particle fractions sieved with mesh sizes of 38 µm, 63 µm and 90 µm are compared with the coarse lactose. The criteria included d(0.1), d(0.5) and d(0.9) that refer to the volume diameter under which 10%, 50% and 90% of the particles lied. As shown in Figure 4.29, the mass median diameter of the coarse SV010 decreases from 94.72 µm to 54.89 µm (SV010 < 90 µm), 25.03 µm (SV010 < 63 µm) and 13.97 µm (SV010 < 38 µm). After the sieving process, more fine particles are observed with smaller mesh size, and there are more than 10% of coarse material less than 10 µm with 90 µm sieve, and even 5 µm with 63 µm and 38 µm sieves. d(0.9) of coarse SV010, SV010 (< 90 µm), SV010 (< 63 µm) and SV010 (< 38 µm) are 183.33 µm, 113.94 µm, 64.47 µm and 37.76 µm respectively, which are found similar to the corresponding mesh size.

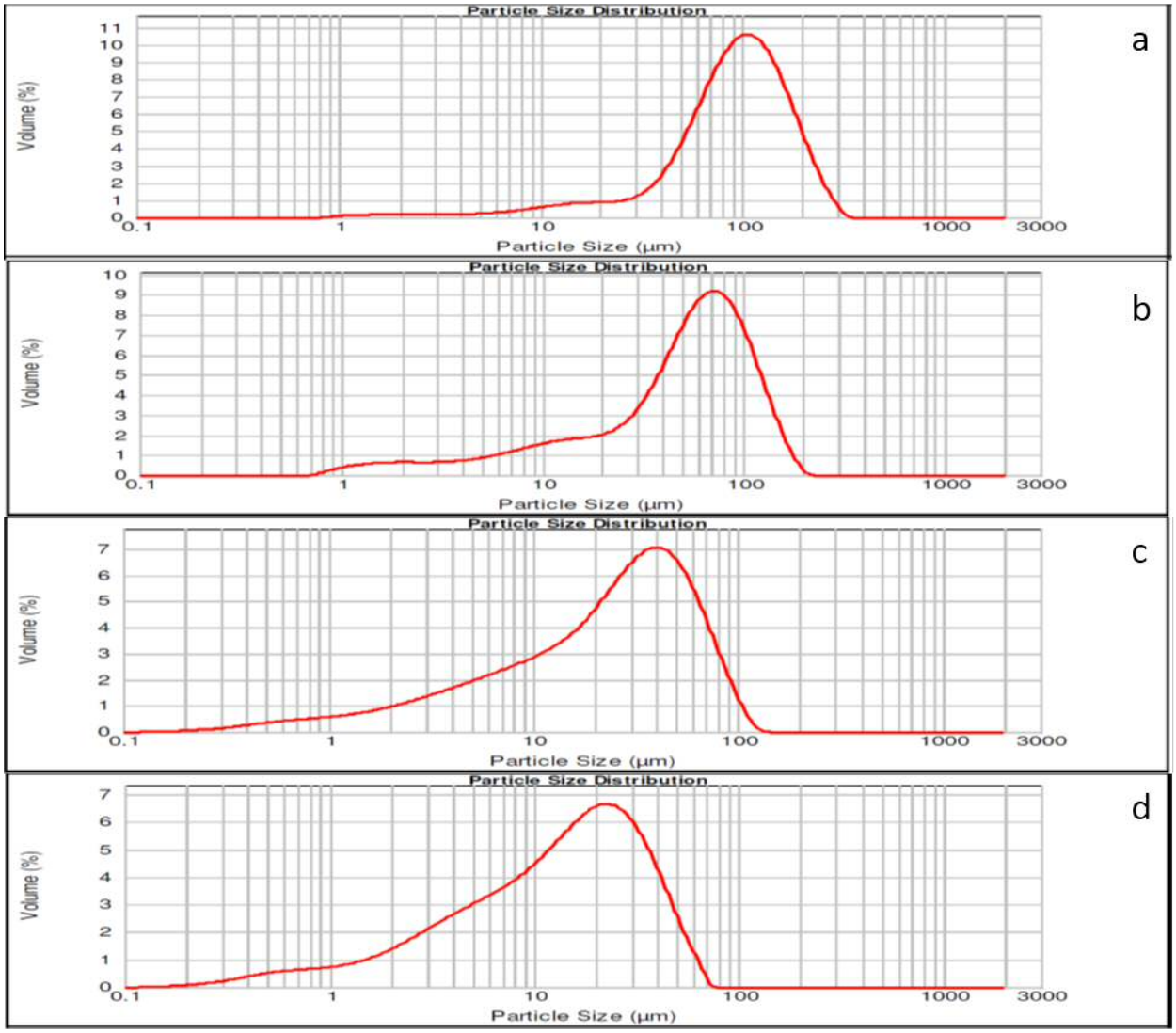

Figure 4. 28 Frequency Size Distribution of SV010 Treated with Different Sieve Size (a: Coarse SV010; b: SV010 by a 90 µm sieve; c: SV010 by a 63 µm sieve; d: SV010 by a 38 µm sieve)

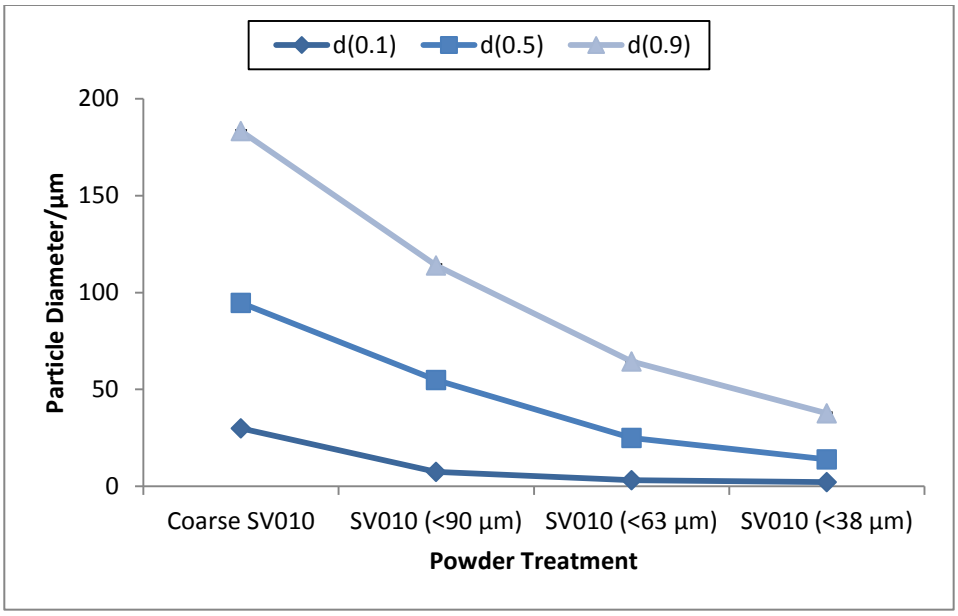

Figure 4. 29 Particle Size Distribution for SV010 (coarse material, <90 µm, <63 µm and <38 µm) Treated by De-aggregation Process (n=3; mean ± SD; d (0.1): particle size under which 10% volume of particles lie, d(0.5): particle size under which 50% volume of particles lie, d(0.9): particle size under which 90% volume of particles lie.)

It is found that the Endecotts® sieves could effectively decrease the particle size of crystal lactose. With the decrease in mesh size, more fine particles less than 10 µm are observed in the bulk, which lead to a wider particle size distribution. However, the produced fine particles may not be removed by the compressed air during the particle size analysis, which could further affect the determination of particle size distribution (Steckel et al, 2006). These undetected fine materials might be determined by visual evaluation using a scanning electron microscope (SEM) in the future. As crystal lactose has a characteristic tomahawk shape, it is obvious that diameter of most particles was grinded close to the mesh size. The sieving process changes physical properties of the powders, which could further affect the mixing process of the micronised Tio particles and the in-vitro performance of the formulation.

To evaluate the effect of particle sizes on the bulk properties and the delivery efficiency of the novel formulation, Tio is blended with different portions of SV010 including <20 µm, <38 µm and <63 µm, and the blend uniformity is 3.46%, 2.55% and 2.12% respectively. With the decrease of particle size of SV010, the uniformity of the formulation decreases. The aerodynamic assessment of the blends is further investigated by in-vitro performance tests. The average ex-valve doses of these three blends are determined through canister-life uniformity test (n=30, mean ± SD). The target ex-valve dose of these formulations is 11.25 µg, and the acceptable range is is 8.4 - 14.1 µg, ± 25% of the target. The values are 10.77 ± 0.66 µg (<20 µm), 10.16 ± 1.14 µg (<38 µm) and 10.76 ± 0.77 µg (<63 µm), which are in the range of ± 5% of the target, with no significant differences (ANOVA, P> 0.05). The addition of SV010 can maintain accurate metering of the formulation, and no interaction between the particle size and valve metering properties is identified. However, high relative standard deviations (RSD) are found with all three formulations, 6.13% (< 20 µm), 11.22% (<38 µm) and 7.12% (<63 µm).

Table 4. 18 Aerosol Characteristics (ex-actuator) of Tio Formulations with Different SV010 Sieve Fractions (<20 µm, <38 µm and <63 µm) (n=3; mean ± SD)

Excipient Size	SV010 (< 20 µm) (a)	SV010 (< 38 µm) (b)	SV010 (< 63 µm) (c)
Tio FPF (% < 5 µm)	44.57 ± 1.84 (a1)	38.19 ± 4.79 (b1)	35.35 ± 3.15 (c1)
Tio MMAD (µm)	3.53 ± 0.22	3.34 ± 0.08	3.64 ± 0.15
Tio FPD (µg < 5 µm)	4.42 ± 0.08 (a2)	3.08 ± 0.66 (b2)	2.67 ± 0.29 (c2)
Tio GSD	2.35 ± 0.17	2.22 ± 0.11	2.07 ± 0.10

((a) Tio:SV010/EXCIP 1:5/FRAC <20 µm/HFA 227/VAL 25 µL/CAN 14 mL/CAN FCP/ACT 0.25 mm, (a) Tio:SV010/EXCIP 1:5/FRAC <38 µm/HFA 227/VAL 25 µL/CAN 14 mL/CAN FCP/ACT 0.25 mm, (c) Tio:SV010/EXCIP 1:5/FRAC <63 µm/HFA 227/VAL 25 µL/CAN 14 mL/CAN FCP/ACT 0.25 mm) (a1 is the greatest (P<0.05) and c1 is the lowest FPF (P< 0.05). No difference is found between a1 and b1 (P> 0.05). a2 is the greatest FPD (P< 0.05) and b2 is similar to c2 (P> 0.05).)

The aerodynamic distribution characteristics of Tio formulations with different SV010 sieve fractions are summarised in Table 4.18. There is significant difference in FPF (ANOVA, P< 0.05). According to Tukey HSD test, the formulation with <63 µm

SV010 shows the lowest FPF (P< 0.05), and the formulation with <20 μm SV010 obtain the greatest and less variable FPF but no significant difference was found with <38 μm SV010 (P> 0.05). There is also significant difference in FPD (ANOVA, P< 0.05). According to Tukey HSD test, the formulation with <20 μm SV010 also obtains the greatest and less variable FPD, compared with <38 μm (P< 0.05) and <63 μm (P< 0.05), and <38 μm is found comparable to <63 μm (P> 0.05). There are no distinctive patterns of these three groups in MMAD (ANOVA, P > 0.05) and GSD (ANOVA, P > 0.05). In a dry powder format, drug particles predominantly attach to larger carrier particles as single entities, and the rest form multiple-particle agglomerates with fine materials (Podczeck, 1999). During inhalation, the drug particles are required to de-aggregate from carrier particles. With the decrease in particle size, the increased cohesive properties of the formulation results in the increase in aerodynamic drag force and particle-particle collision, which could accelerate the de-aggregation process (Shur et al, 2008). However, in the suspension format, it is hard to predict their dynamic properties and particle interaction in high-vapour-pressure HFA propellants. Through in-vitro evaluation, it is found that the SV010 fraction with a smaller particle size increases the aerosol performance as more fine drug particles (<5 μm) are generated. This indicates the particle size of lactose affects the atomisation process to generate aerosols in pMDIs. James et al (2008) found sub-micro lactose exhibited a lower surface free energy and adhesion values between sub-micro lactose and APIs than micronised lactose in a pMDI suspension, where used 2H, 3H decafluoropentane as the model propellant. Consequently, the shear force inducted by evaporation of the propellant may be more likely to de-aggregate drug particles from lactose with smaller particle size.

The NGI distribution patterns of the three formulations are illustrated in Figure 4.30. SV010 (< 20 μm) shows the lowest IP deposition (ANOVA, P< 0.05), suggesting

that more drug particles are captured in IP with the increase of the particle size of SV010. One explanation is that finer SV010 could prevent Tio particle aggregation more effectively, or the fine materials prevent the formation of strong adhesive bond between drug and lactose particles. However, the greatest deposition in Stage 2 (ECD=6.4 µm) (ANOVA, P< 0.05) implies the aggregates of both particles may still exist but due to the decreased particles size, they by-pass the IP and deposit in lower stages. Consequently, the decrease in particle size could avoid the IP deposition which contributes to prevention of loss of the drug on the mouth and throat, and relief of the potential local adverse effects. As Coarse SV010 was ground into smaller particles using a sieve, the excipient number is also increased with the decrease of particle size. The increased number of SV010 particles are assumed to increase the chance of Tio - SV010 interactions, and further blind the adsorption of Tio particles on SV010 particles. Alternatively, the decreased excipient particle sizes are assumed to decrease the initial droplet size and prevent inertial deposition. These assumptions need to be further investigated.

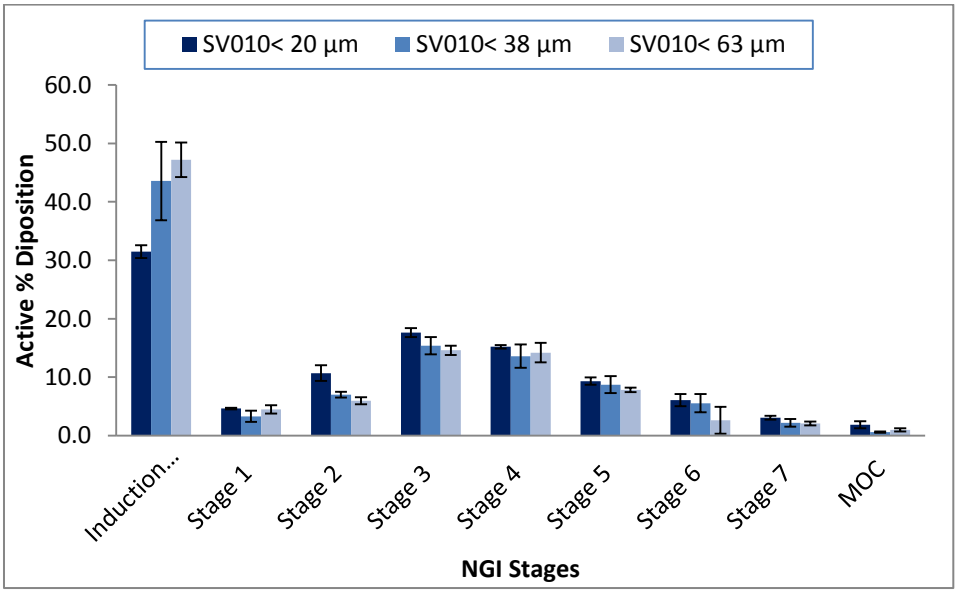

Figure 4. 30 NGI Distribution Pattern (%) of Tio Formulations with Different SV010 Sieve Fractions (<20 µm, <38 µm and <63 µm) (n=3; mean ± SD)

Table 4. 19 Valve and Actuator Performance of Tio pMDI Formulation with Different SV010 Sieve Fractions (<20 µm, <38 µm and <63 µm) (n=30; mean ± SD)

Fraction	SV010 < 20 µm	SV010 < 38 µm	SV010 < 63 µm
Actuator Deposition%	33.45 ± 8.49	37.68 ± 6.80	31.80 ± 3.83
Ex-valve Emitted Dose (µg)	10.77 ± 0.96	10.16 ± 1.30	10.76 ± 1.02
Ex-actuator Emitted Dose (µg)	7.18 ± 1.19	6.34 ± 1.10	7.34 ± 0.75

(Actuator Deposition%: percentage of ex-valve emitted dose on the actuator; Ex-valve Emitted Dose: emitted dose from the valve; Ex-actuator Emitted Dose: emitted dose from the actuator)

During the canister life test, high and variable actuator depositions are observed within all the formulations (Table 4.19), which is similar to the results in Table 4.12 in previous section. There is no interactions between SV010 particle size and actuator deposition, and this is also supported by Jones (2004) that similar actuator deposition was observed in a suspension formulation containing salbutamol sulphate with different size fractions of leucine. Although in the research of a DPI formulation, Zeng et al (2000) found more drug particles tended to deposit on the inner surface of the inhaler device with incorporation of the fine lactose particles, pMDI seems a different performance. However great variations in actuator deposition patterns indicate the poor efficiency of the device in all the formulations, which could be relevant to broad distribution of SV010 fractions, which are indicated by Figure 4.28. The heterogeneity of the particle size distribution of SV010 may attribute to changes in vaporisation characteristics and plume geometry (Young et al, 2009). All the ex-valve emitted doses are found in the range of ±25% of the target dose, 11.25 µg, but a large variation in ex-actuator doses is observed with all the formulations tested, indicated by relative standard deviations of 17% (<20 µm), 17% (<38 µm)and 10% (<63 µm)respectively.

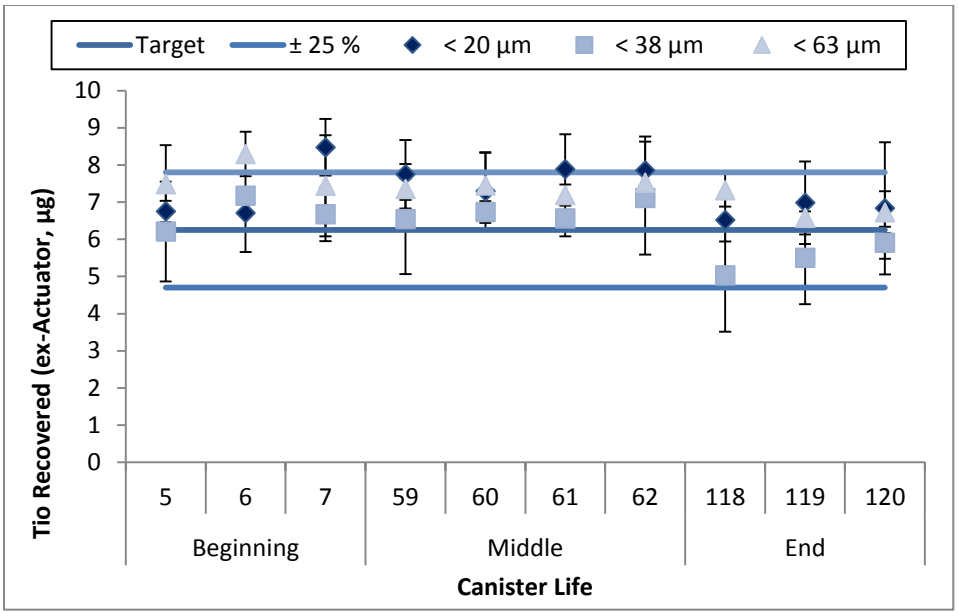

Figure 4. 31 Canister Life Content Uniformity of Tio Formulations with Different SV010 Sieve Fractions (n=3; mean ± SD) (Different sieve fractions e.g. <20 µm, <38 µm and <63 µm of SV010 is co-blended with Tio)

The canister life content uniformity is shown in Figure 4.31. All the canisters with different sieve fractions showed variations in ex-actuator emitted doses. Though no trends of decreased doses are identified in the end of canister life, there are 10 out of 30 points in SV010 (<20 µm) outside the range of ± 25% of the target dose, 6.25 µg, and 3 and 4 out of 30 points for SV010 (< 38 µm) and SV010 (< 63 µm) respectively. Previous studies have suggested that sub-micron lactose increased the valve performance with an indication of reduction in "loss of dose", and benefited dosing consistency of a high potency beta-2 agonist (Jinks, 2008), but there is no indication from our results. As the raw material of sieve fractions of SV010 are prepared simple sieving process, other size reduction techniques are required to prepare a narrower size distribution of secondary particles.

In this section, it is found that with the addition of a secondary particle, lactose, the physical stability of the formulation is maintained regardless of the particle size.

The formulation prepared with smaller particle size of lactose, showed significantly improved aerosol performance. In the formulation containing SV010 (<20 μm), more fine particles are generated for deep penetration into the NGI apparatus, along with decreased deposition on the IP stage. Several previous findings support the function of carrier particles with decreased particle size in the improvement of the aerosol performance but these are limited to the dry powder format (Kaialy, 2012; Islam and Cleary, 2012). In general, smaller lactose particles had higher amorphous content, smaller angularity, higher surface smoothness, higher specific surface area, higher porosity and smaller bulk density. The fine excipient particles could decrease the adhesive bond with drug particles and potentially cover the high-energy binding point in the coarse excipient particles, which increase the likelihood of de-aggregation induced by shearing forces. Furthermore, increased number of lactose particles due to the sieving process could also affect the physical stability and aerosol performance. Other physical properties of the secondary particle, e.g. surface smoothness and rugosity, also need to be further investigated.

4.5.8.2 L-leucine

As a prospective secondary excipient, the addition of leucine as a secondary particle has been proven to increase the aerosol performance of Tio suspension in previous sections, a novel formulation is prepared at the ratio of Tio: Leu (w/w) 1: 5 to investigate the influence of the size of excipient particles. Different sieve fractions of leucine including <20 µm, <38 µm and <63 µm are co-blended with Tio, and the blend uniformity is 5.73%, 3.92% and 1.19% respectively, which is reduced with the reduction of the Leu particle size. The aerosol performance of the three formulations is summarised in Table 4.20. The FPF is increased with the decease of leucine particle size. According to ANOVA and Tukey HSD test, the formulation with <63 µm Leu shows the lowest FPF ($P< 0.05$), but no significant difference is found between <20 µm and <38 µm Leu ($P> 0.05$). MMAD (ANOVA, $P> 0.05$) and GSD (ANOVA, $P> 0.05$) are also comparable regardless of the Leu particle size. The particle dose of <20 µm is the lowest between all these groups (ANOVA and Tukey HSD test, $P< 0.05$), and <38 µm and <63 µm are similar ($P> 0.05$). The low particle dose obtained by Leu< 20 µm is relevant to low ex-actuator doses, which is shown in Table 4.21., Therefore, Leu <20 µm and <38 µm suggest better aerosol performance than Leu <63µm.

Table 4. 20 Aerosol Characteristics (ex-actuator) of Tio Formulations with Different Leu Sieve Fractions (<20 µm, <38 µm and <63 µm) (<20 µm: n=3; mean ± SD, <38 and <63 µm: n=5; mean ± SD)

Excipient Size	Leu (< 20 µm) (a)	Leu (< 38 µm) (b)	Leu (< 63 µm) (c)
Tio FPF (% < 5 µm)	55.61 ± 1.50	53.69 ± 1.67	50.97 ± 2.54
Tio MMAD (µm)	3.13 ± 0.13	3.19 ± 0.10	3.39 ± 0.22
Tio FPD (µg < 5 µm)	3.64 ± 0.23	4.84 ± 0.26	5.33 ± 0.42
Tio GSD	1.90 ± 0.09	1.97 ± 0.06	2.01 ± 0.11

((a) Tio:Leu/EXCIP 1:5/FRAC <20 µm/HFA 227/VAL 25 µL/CAN 14 mL/CAN FCP/ACT 0.25 mm, (b) Tio:Leu/EXCIP 1:5/FRAC <38 µm/HFA 227/VAL 25 µL/CAN 14 mL/CAN FCP/ACT 0.25 mm, (c) Tio:Leu/EXCIP 1:5/FRAC <63 µm/HFA 227/VAL 25 µL/CAN 14 mL/CAN FCP/ACT 0.25 mm)

According to Figure 4.32, the distribution patterns of the three formulations are generally similar. There is no difference in IP deposition, and the peak deposition in the NGI appears on Stage 4 for each formulation. There was a trend of decreasing deposition on Stage 4 and 5, and increasing deposition on Stage 7 and MOC with increasing particle size. However, when combining IP, Stage 1 and 2 together as the non-respiratory fraction (particle diameter > 6.4 µm) (ANOVA, P > 0.05) and combining Stage 3 to MOC as the respiratory fraction (particle diameter < 3.99 µm) (ANOVA, P> 0.05), there is no significant different between these groups.

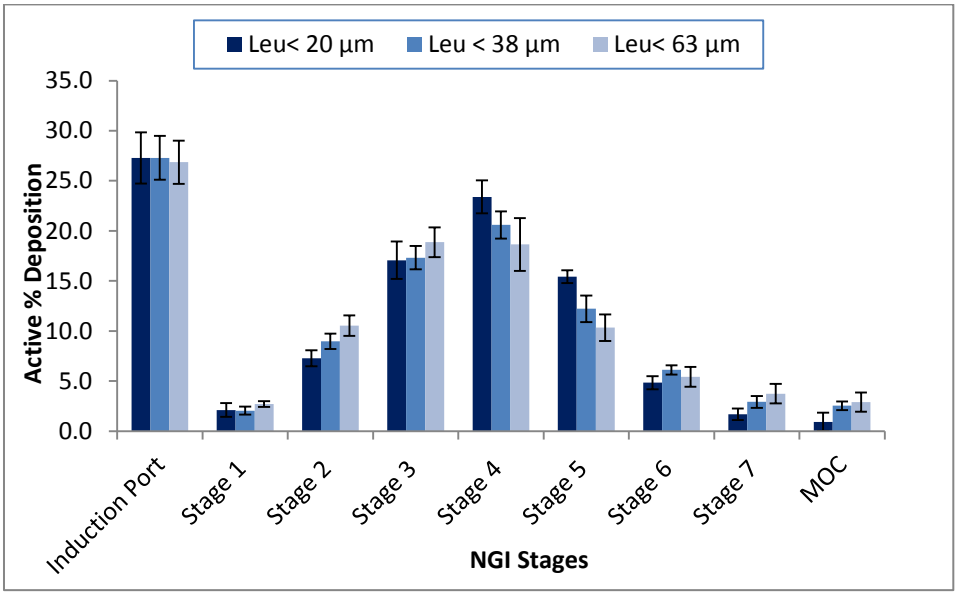

Figure 4. 32 NGI Distribution Pattern (%) of Tio Formulations with Different Leu Sieve Fractions (<20 µm, <38 µm and <63 µm) (<20 µm: n=3; mean ± SD, <38 and <63 µm: n=5; mean ± SD)

The valve and actuator performance is shown in Table 4.21. It appears from these findings that actuator deposition is constant and independent of the influence of the leucine particle size (ANOVA, P> 0.05). The ex-valve dose is increased with the increase of leucine particle size (ANOVA and Tukey HSD test, P< 0.05), and the same trend is found with the ex-actuator dose (ANOVA and Tukey HSD test, P< 0.05). However, the target ex-valve dose for Tio: Leu is 9 µg, and the corresponding ex-actuator dose is 6.25 µg. The ex-valve and ex-actuator doses of Leu <20 µm are

13% and 12% lower than the target respectively, Leu <38 µm are 15% and 16% higher, and Leu <63 µm are 26% and 32% higher. Furthermore, leucine with smaller particles size suggests better reproducibility of both ex-valve and ex-actuator emitted doses.

The content uniformity is assessed over the canister life. As shown in Figure 4.33, all the data points of Leu <20 µm lie in the range of ± 25% of the target. In the group of Leu <38 µm, there are 17 out of 50 points (Leu <38 µm) and 30 out of 50 points (Leu <63 µm) outside ± 25% of the target. Doses are quite consistent over the canister life, but large variability is seen with Leu <63 µm. All the formulations tested appear compatible with this pMDI system, and dose dropping is also prevented at the end of canister life. However, Leu <20 µm suggests the most reproducible doses with less dose variability.

Table 4. 21 Valve and Actuator Performance of Tio pMDI Formulation with Different Leu Sieve Fractions (<20 µm, <38 µm and <63 µm) (<20 µm: n=50; mean ± SD, <38 and <63 µm: n=50; mean ± SD)

Fraction	Leu < 20 µm	Leu < 38 µm	Leu < 63 µm
Actuator Deposition%	29.90 ± 3.84	29.83 ± 5.34	28.50 ± 5.01
Ex-valve Emitted Dose (µg)	7.82 ± 0.56	10.33 ± 1.32	11.32 ± 1.99
Ex-actuator Emitted Dose (µg)	5.47 ± 0.44	7.26 ± 1.11	8.27 ± 1.24

(Actuator Deposition%: percentage of ex-valve emitted dose on the actuator; Ex-valve Emitted Dose: emitted dose from the valve; Ex-actuator Emitted Dose: emitted dose from the actuator)

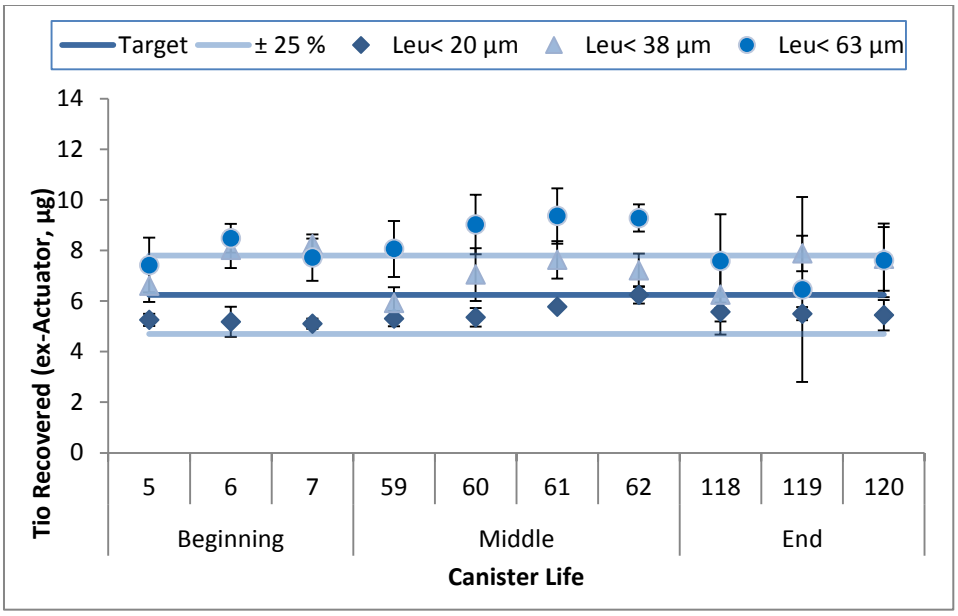

Figure 4. 33 Canister Life Content Uniformity of Tio Formulations with Different Leu Sieve Fractions (<20 µm: n=3; mean ± SD, <38 and <63 µm: n=5; mean ± SD) (Different sieve fractions e.g. <20 µm, <38 µm and <63 µm of Leu is co-blended with Tio)

In this section, it is found that leucine of smaller particle size can improve aerosol performance indicated by increased FPFs. Similar result is gained by SV010 but Tio: Leu demonstrates greater FPF, GSD and smaller MMAD than Tio: SV010. With the reduction in particle size, the number of leucine particles increases accordingly, which could increase the chance of particles collision and prevent the aggregation of drug particles. Efficient de-aggregation of the drug from leucine during atomisation may lead to a low IP deposition regardless of the particle size of leucine, while in Tio: SV010, the least IP deposition is observed with SV010 <20 µm. Furthermore, with the decrease of leucine particle size, the number of fine drug particles is also increased. In the content uniformity test, consistent ex-actuator doses indicate Leu <20 µm can maintain the physical stability of the novel formulation over the canister life, but the dose uniformity of Tio: SV010 seems independent of excipient particle size. Therefore, smaller particle size (<20 µm) of SV010 and leucine both suggest an improvement in FPF and a good maintenance of

physical stability of Tio particles, which are recommended for optimised Tio formulations described in Chapter 5.

4.6 Conclusion

The pMDI is a complex propellant-driven system, which needs coordination between the formulation, the hardware and the patient. Since its invention in 1956, the pMDI has changed in many aspects, e.g. propellant type, valve and canister design, added excipients and add-on devices. Since the phase out of CFC propellants, the re-formulation of pMDIs in new propellants has been a challenging task. As an innovative approach, secondary particles are co-suspended with drug particles aiming at improving the suspension stability and aerosol delivery efficiency. The aerosol performance of a pMDI depends on a multiple of factors, so both formulation and device factors are systematically evaluated in this chapter.

Choosing the appropriate canisters depends on the number of drug doses and the stability of the formulation. Features of canisters, e.g. canister volume (14 mL/19 mL canisters) and coating materials (plain/FCP canisters) were initially investigated in this research. At the beginning of canister life, it was proposed that the performance of the formulation was not affected by the canister volume as fewer drug particles were deposited and no chemical degradation was identified relevant to the contact with the canister surface (Table 4.1). However, over the shelf life of pMDIs, the larger inner surface of canisters was proven to increase the adhesion of the drug particles in both Tio control (Tio only) and Tio: SV003, which resulted in lower drug concentration and decreased ex-actuator doses at the end of canister life (Figure 4.2). Therefore, the canister volume of 14 mL is recommended for the novel Tio formulation. Regarding the coating materials, Tio: Leu was found with an increase of FPF in FCP canisters against plain canisters (Table 4.11) but not in Tio: SV010 (Table 4.10). Furthermore, over the canister life, a trend of decrease of ex-valve dose was identified in plain canisters containing both formulations of Tio: SV010 (Figure 4.15) and Tio: Leu (Figure 4.18). The coating polymers were proven to maintain the physical stability of the formulation without changing the aerosol

performance. Consequently, due to loss of the drug inside the plain canister, FCP coated canisters are recommended for the novel Tio formulation.

The metering valve is the core component of a pMDI system, which is responsible for accurate metering of doses, and the influence of design of the valve is critical to pMDI performance. Different volumes of the valve metering chamber, e.g. 25 µL, 35 µL and 50 µL, were investigated with formulations containing Tio: SV003 (w/w) 1:25 and Tio: SV003 (w/w) 1:5. In the formulation of Tio: SV003 (w/w) 1:25, as the metering volume reduced from 50 µL to 25 µL, more fine particles were generated during atomisation as shown in Table 2.4. In the experimental design, the ex-valve emitted dose and the ratio of Tio: SV003 was kept constant regardless of valve volumes, so there were two variables including valve volume and formulation concentration that could affect the aerosol performance. Therefore, the increase of FPF in 25 µL valves (Table 2.4) could be caused by more efficient evaporation of generated droplets as a small volume propellant is involved. The other reason could be that due to the increased concentration of SV003, the Tio-SV003 interactions were more intense and improved the de-aggregation process of smaller drug particles from larger secondary particles during the atomisation. This phenomenon was also observed in the formulation Tio: SV003 (w/w) 1:5, where a decrease in SV003 concentration showed a worse aerosol performance (Figure 4.4). Therefore, the valve volume and secondary particle concentration altered the performance of the novel formulation, and the 25-µL valve was more compatible and recommended to use with this novel suspension formulation.

As the dispersion phase and the propellant of the pMDI, HFAs are directly relevant to the physical stability and atomization of the suspension. Though HFA 134a and HFA 227 have different physico-chemical properties, e.g. viscosity and density, both show an adequate sedimentation rate for Tio and SV003 particles. It was found a

significant increase in FPF and FPD in Tio: SV003 with HFA 227 (Table 4.6). Although there was not fully understanding of the atomisation process of a suspension formulation, an appropriate shear force and aerosol velocity was generated by HFA 227. A future aerodynamic analysis of the spray pattern of a suspension is necessary. A high IP deposition (Figure 4.7) was observed in HFA 134a, which could be explained by the high vapor pressure of HFA 134a (Table 4.5) that leads to fast velocity of the aerosols. Furthermore, a high actuator deposition was also found in HFA 134a (Figure 4.8), and the relevance between actuator deposition and plume geometry study may be useful in the future to give a better understanding of the atomisation process of the suspension formulation. Based on these findings, HFA 227 is recommended for use with this novel formulation.

As a key component of the pMDI system, actuators play a vital role in the atomising process, which decides the final formation of the aerosol spray. Different designs have been utilised to improve the actuator performance, e.g. orifice geometry, size and sump volume. In this section, the influence of the actuator orifice on these novel formulations including Tio: Leu (w/w) 1:5 and Tio: SV003 (w/w) 1:5, was our priority. For the formulation of Tio: Leu (w/w) 1:5, it was found that smaller orifices, e.g. 0.25 mm, tended to generate smaller aerosol particles (Table 4.8) with a low velocity and narrow spray coverage (Figure 4.12). Slow velocity and effective evaporation of propellant after atomisation also contribute to a low IP deposition (Figure 4.11). The same results were also obtained for the formulation of Tio: SV003 (w/w) 1:5. Actuators with smaller orifice size, e.g. 0.25 mm, were advantageous to avoid drug loss inside the actuator and IP stage, and deliver the maximum fine particles to the target area regardless of the type of secondary particles (Table 4.9 and Figure 4.13). However, FPF was increased and MMAD was decreased with significant difference in Tio: Leu than Tio: SV003, when either actuator, e.g. 0.25 mm and 0.46 mm, was used. The same results was also obtained

in Section 4.5.7. Although orifice clogging of 0.25 mm actuators was observed at a high excipient ratio, e.g. Tio: SV003 1: 25, in Section 4.5.2 and Section 4.5.6, no blockage cases were found with a 1:5 ratio. Therefore, actuators with smaller orifice size, e.g. 0.25 mm actuators, is recommended for use with the novel formulation at a low excipient ratio.

The use of secondary particles to stabilise a suspension system is an innovative approach, which has been previously researched by Jones (2004) where L-leucine was initially investigated with low potent drugs including salbutamol sulphate and fluticasone propionate. As a follow up research, single physico-chemical factors of secondary particles were investigated with a high potent drug, e.g. Tio. The influence of the ratio of secondary particles, e.g. SV003, to drug is a key research question. In this section, an increase in blend uniformity is observed with an increase of the SV003 ratio. There is however no significant improvement on the dose uniformity (Figure 4.21) and the aerosolisation performance (Table 4.13) of the pMDI, which could be due to the relative high ratio of drug particles saturating the binding points on the secondary particles. Though a decrease of MMAD was observed in SV003 (1: 10) and SV003 (1:25), blockage issues of the actuators were noticed at high excipient ratios. Therefore, with consideration of easy handling, a 1:5 (w/w) ratio of Tio: excipient is recommended for further research.

The effects of modifications to secondary particles, e.g. lactose, L-arginine, L-glutamic acid and taurine, were examined by Jones (2004), where the aerosol performance of lactose was found more consistent than the equivalent leucine formulation with salbutamol sulphate. Therefore, in this section, two types of inhalable lactose including SV003 and SV010, were compared against the equivalent leucine formulation with Tio. Through the screening of the potential secondary particles used to stabilise the micronised Tio, SV003, SV010 and leucine

were proven to maintain good blend uniformity. Improvements in the suspension uniformity and dosing consistency (Table 4.15) against Tio control (Tio only) were observed over the canister life, and the same results was found in optimised formulations containing SV010 and leucine, described in Chapter 5. Although increased actuator deposition than Tio control was observed (Table 4.15), all the selected secondary particles can maintain (SV003) or improve (SV010 and leucine) delivery efficiency, e.g. FPF, as shown in Table 4.16. The decrease in IP deposition (Figure 4.26) and comparable FPDs (Table 4.16) could benefit the patients with acceptable therapeutic doses and less side effects on the oropharyngeal region. The addition of the secondary particles might affect atomisation process of the suspension and induced more electrostatic charges to the generated aerosols, which could affect the aerodynamic behaviour of drug particles. The influence of the surface characteristics on physical stability and atomisation of the suspension is necessary for future studies. The decrease of metering dose at the end of the canister life were observed with all the formulation tested, and this could be caused by the loss of Tio in the plain canisters which has been discussed in Section 4.5.5. So far, SV010 and leucine are identified as the prospective excipients to stabilise and improve the performance of the novel Tio formulations.

Several sieve fractions of SV010 and leucine were prepared by manual sieving. In the formulation of Tio: SV010, as more fine particles are generated during the sieving process, the particle size distribution got boarder with the decrease of the mesh size (Table 4.17). The induced stronger cohesion force of the smaller SV010 could decrease the bulk flowability and therefore the blend uniformity. Though the MMAD of Tio was similar with different size fractions of SV010 (<63 µm, <38 µm and <20 µm), the Tio formulation with SV010 (<20 µm) shows the greatest FPF and FPD (Table 4.18). The generated high ratio of fine lactose might decrease the adhesive bond of drug particles to larger SV010 particles by covering the high-

energy binding points and facilitate the de-aggregation during the atomisation, which was also approved by decreased IP deposition (Figure 4.30). However, poor efficiency of the actuators and the broad size distribution of SV010 fractions caused variable actuator deposition and ex-actuator emitted doses, which need to be further investigated. For the formulation with leucine, smaller particle size of leucine also showed improved aerosol performance, e.g. FPF, as shown in Table 4.20. In the case of leucine, similar IP deposition was found regardless of particle size. The decreased size of leucine e.g. <20 μm, and increased number of leucine particles suggested good physical stability over the canister life (Figure 4.33). Overall, the delivery efficiency is significantly improved with a decrease in the size of the secondary excipients. Therefore, particle size <20 μm of the secondary particle including SV010 and leucine is recommended for the optimized novel formulation described in Chapter 5.

5 Stability of Novel Optimised Tiotropium Formulation

5.1 Introduction

The development of a good pMDI product relies on rational selection of appropriate formulation and device components. Since the first introduction of Medihaler Epi™ by Riker Laboratories in 1956, processes such as formulation development and manufacturing of device components, and filling and assembly of the pMDI have continuously evolved to meet new clinical, industrial and regulatory requirements and standards. As a result of the replacement of CFC propellant by more environment friendly HFA propellants, differences in physico-chemical characteristics challenged the re-formulation of existing products and the development of new products. In this research, as an alternative route to stabilise a suspension formulation, a secondary particle is co-suspended to replace the use of surfactants and co-solvents without changing the delivery efficacy.

In previous chapters, research was focused on the evaluation of the influence of various formulation and hardware factors on aerosol performance. 14 mL FCP coated canisters were found to prevent the loss of Tio inside the canisters. The 25 µL metering valve and 0.25 mm actuator were able to improve the aerosol performance of the novel formulation containing 1:5 ratio of Tio: secondary particle in HFA 227. Furthermore, SV010 and leucine with particle size of <20 µm suggested a good maintenance of physical stability of the novel formulation over the canister life, and a significant increase of FPFs against drug alone and other secondary particle, i.e. SV003. Therefore, advantageous formulation and hardware parameters in optimised novel formulations were Tio: SV010/EXCIP 1:5/FRAC <20 µm/HFA 227/VAL 25 µL/CAN 14 mL/CAN FCP/ACT 0.25 mm and Tio: Leu/EXCIP 1:5/FRAC <20 µm/HFA 227/VAL 25 µL/CAN 14 mL/CAN FCP/ACT 0.25 mm. The formulations pre-blended at a 1:5 (w/w) ratio of Tio: Secondary Excipient (e.g. SV010 and leucine) were co-suspended in HFA 227 without addition of any

surfactants and co-solvents. The suspension was contained in 14 mL FCP coated aluminium containers, crimped by 25-µL VARI® valve, and accompanied with H&T Presspart® 0.25 mm actuators. A new batch of canisters containing Tio only were also manufactured as the control group and the tested commercialised products, e.g. Tiova®, Spiriva Handihaler® and Spiriva Respimat®, discussed in Chapter 3 were also used for reference.

All these experiments described in Chapter 4 were performed after a 3-day room temperature storage post-production. Due to the heterogeneous nature of the suspension formulation, formulation stability during the pMDI shelf-life is crucial for product performance and safety. In a suspension pMDI, the drug is considered to be practically insoluble in propellants, and drug particles are dispersed evenly with the aid of a surfactant and co-solvent (Pilcer and Amighi, 2010). However, a suspension is thermodynamically unstable, and particle agglomeration and size-specific particle growth, e.g. Ostwald ripening, are often observed upon storage. Ostwald ripening could accelerate the dissolution of small particles and increase the growth of larger particles, which further leads to physical stability issues, i.e. flocculation and sediment compaction (Berry et al, 2004). Furthermore, the ingress of water through the valve may also impact on solubility and chemical stability of the drug. Another phenomenon called the Brownian motion of sub-micro particles could overcome the effect of gravity, and the corresponding particle-particle collision and particle-device interaction could cause particle size growth and drug loss due to deposition on the device surface.

The potential dissolution of drug particles, particle size growth, and particle interactions including drug-drug and drug-device interactions could cause corresponding poor physical stability, loss of drug content, and finally lead to a failed product. In the approach of surfactant-free system, the addition of secondary

particles is assumed to interfere with particle interactions by attracting drugs to the surface of secondary particles and therefore increase the physical stability of the suspension. The reproducibility of emitted dose and aerodynamic behaviour are the prior concerns with a pMDI suspension formulation, which rely on the physical stability of the formulation. Therefore, in this chapter, the long term stability of the optimised formulations under accelerated conditions (40°C/75%) is investigated. The potential advantages of the secondary particle on pMDI suspension systems are evaluated against the control group in the storage stability study and the performance of the optimised novel formulation was compared against the commercial reference products.

5.2 Chapter Aims and Objectives

To perform formulation stability tests and evaluate the performance of the optimised formulation and hardware identified in Chapter 4 (Tio: SV010 (<20 µm) and Tio: Leu (<20 µm)) in comparison with Tio only as the control and marketed Tio products including Tiova®, Spiriva Handihaler® and Spiriva Respimat®.

The objectives of this chapter are as follows:

1) To prepare a range of formulations including Tio only as the control group, optimised Tio: SV010 (<20 µm) and Tio: Leu (<20 µm) formulations.

2) To determine leakage of propellant from pMDI canisters following 6 month storage under accelerated conditions of 40°C/75% RH.

3) To perform canister content test following 6 month storage under accelerated conditions of 40°C/75% RH.

4) To Investigate the ingress of moisture into the formulation over 6 months storage under accelerated conditions of 40°C/75% RH

5) To perform stability test with respect to aerosol performance and dose uniformity following 6 month storage under accelerated conditions of 40°C/75% RH.

6) To compare the aerosol performance and dose uniformity of optimised Tio formulations with Tio only and commercialised products, e.g. Tiova®, Spiriva Handihaler® and Spiriva Respimat®

5.3 Materials and Equipment

5.3.1 Materials

Tiotropium Bromide Monohydrate (Tecoland); Respitose® SV010 Monohydrate Lactose (DFE Pharma); L-Leucine (Sigma-Aldrich Co); HFA227 (Mexichem); Benzyltriethyl Ammonium Chloride (Sigma); 14 mL Plain & FCP Aluminum MDIs Canisters (H&T Presspart Manufacturing); 25 µL KHFA Metering Valve (Valvole Aerosol Research Italiana Spa, VARI); 0.25 mm Actuator (H&T Presspart Manufacturing); Spiriva Handihaler® (Boehringer Ingelheim) which was described as Handihaler® in the context; Spiriva Respimat® (Boehringer Ingelheim), which was described as Respimat® in the context; Tiova® (Cipla)

5.3.2 Equipment

The HPLC system used in this Chapter was described in Chapter 2; New Generation Impactor (Copley Scientific); Dosage Unit Sampling Apparatus (Metal & Copley Scientific); Dry Powder Controller Model TPK (Copley Scientific);NGI Gentle Rocker (Copley); Minor M200 Sieve Shaker (Endecotts) with Laboratory Test Sieve (Aperture: 20 µm); Turbula Mixer (Willy A. Bachofen AG Maschinenfabrik); Two Stage Filling Equipment (Pamasol) with laboratory scale propellant pump (X2008-00) and crimping head (X2002-0043/013); HPC SM12 Rotary Screw Air Compressor (Straightset); 5799-S Stability Storage Cabinets (Vindon Scientific) (40°C/75%); In-house Bespoke Piercing Tool and Adjustable Pipe Cutter (Wickes); 831 KF Coulometer (Metrohm)

5.4 Methods

Methods including preparation of canisters, canister content test, moisture test, dose uniformity test and aerodynamic particle size distribution test were described in Chapter 2. Modifications of any method are specified in this chapter.

5.4.1 Control Group

As shown in Table 5.1, the Tio Control group is prepared by suspending Tio only without addition of any excipient in HFA 227 in 14 mL plain aluminium canisters. All the canisters are crimped with 25 µL VARI® valves and accompanied with 0.25 mm H&T Presspart® actuators. Each canister contains 120 doses plus 30% overage and a total batch of 25 canisters is prepared using a two-stage filling process according to Section 2.6. The percentages of filled propellant were between 98.8% and 102.1% of the target of 5.46 g. All manufactured canisters were well shaken and kept inverted in the rack at room temperature. A minimum of 3-day storage was required before any further test.

Table 5. 1 pMDI Formulation of Tio Control Group

	Per actuation		Per Canister		% w/w
	µL	mg	mL	g	
HFA 227	25.00	35.00	3.90	5.46	99.97
Tiotropium		0.0113		0.0018	0.03
Totals	25.00	35.0113	3.90	5.4618	100.00
Total Solids				0.0018	

5.4.2 Novel Formulation

As shown in Table 5.2, the SV010 formulation is prepared at Tio: SV010 (w/w) 1: 5, and suspended in HFA 227 in 14 mL FCP coated aluminium canisters. Canisters are crimped with 25 µL VARI® valves and all the generated data is based on 0.25 mm H&T Presspart® actuators. A 60-canister batch is prepared according to Section 2.4 - 2.7, and each canister contains 120 doses plus 40% overage. In Section 2.5, the whole blend containing SV010 and Tio was re-sieved through a 20-µm mesh to decrease the size of SV010 to < 20 µm. All the canisters were filled by pressure filling, labelled and kept in inverted positions. All the generated data is based on 0.25 mm H&T Presspart® actuators. Uniformity of delivered dose was measured according to Section 2.8.2. The aerodynamic particle distribution was measured according to Section 2.8.3.

Table 5. 2 Novel pMDI Formulation of Tio: SV010

	Per actuation		Per Canister		% w/w	60 Can Batch	Batch+7%
	µL	mg	mL	g		g	g
HFA 227	25.00	35.00	4.20	5.88	99.8075	352.80	352.80
SV010		0.0563		0.0095	0.1604	0.5670	0.5670
Tiotropium		0.0113		0.0019	0.0321	0.1134	0.1213
Totals	25.00	35.0675	4.20	5.8913	100.0	353.4804	353.4883
Total Solids				0.0113		0.6804	0.6883

As shown in Table 5.3, the leucine formulation was prepared at Tio: Leu (w/w) 1: 5, and suspended in HFA 227 in 14 mL FCP coated canisters. The manufacture of this batch was according to Section 2.4 to 2.7. In section 2.5, Tio was de-aggregated by 20-µm sieve and the particle size of leucine was further narrowed down to <20 µm by re-sieving the whole mixture with a 20 µm sieve. The average ex-actuator dose was over 25% of the target 6.25 µg. The metering dose of Tio was set as 9 µg, rather than 11.25 µg, to target 6.25 µg of the ex-actuator dose. All the canisters were filled by pressure filling, crimped with 25 µL VARI® valves, labelled, weighed and kept in inverted positions. All the generated data is based on 0.25 mm H&T

Presspart® actuators. Uniformity of delivered dose was measured according to Section 2.8.2. The aerodynamic particle distribution was measured according to Section 2.8.3.

Table 5. 3 Novel pMDI Formulation of Tio: Leu

	Per actuation		Per Canister		% w/w	60 Can Batch	Batch+7 %
	µL	mg	mL	g		g	g
HFA 227	25.00	35.00	4.20	5.88	99.8460	352.80	352.80
Leucine (106-90 µm)		0.0450		0.0076	0.1284	0.4536	0.4536
Tiotropium		0.0090		0.0015	0.0257	0.0907	0.0971
Totals	25.00	35.0540	4.20	5.8891	100.0	353.3443	353.3507
Total Solids				0.0091		0.5443	0.5507

5.4.3 Leakage and Content Test

As the pMDI is an enclosed system, the quality of sealing is essential to the stability and consistency of the formulation. Leakage, or degradation, of the formulation could affect the drug concentration or total doses in an individual canister (FDA, 1998). Therefore, leakage of the canisters was monitored by the weight difference following a period of storage. Canister weights were recorded after the preparation of the canisters as the post-product weight, and recorded before the canister content test as the pre-test weight. The leakage rate was determined from the weight difference as calculated by the absolute value of the post-product weight minus the pre-test weight. The average of leakage rates was expected to be less than 3.5% of the net fill and no single canister to be more than 5% of the net fill per year (USP, 2017). Therefore, the leakage limit of Tio Control (Section 5.5.1) is average< 191 mg/year and single< 273 mg/year, and the leakage limit of Tio: SV010 (Section 5.5.2) and Tio: Leu (Section 5.5.3) is average< 206 mg/year and single < 294 mg/year. Three canisters of each formulation are randomly selected for the leakage test.

The environmental conditions where the canisters are stored could affect the chemical and physical stability of the drug substance, and therefore a canister content test accompanied the leakage test. According to Section 2.9, canisters were stored in accelerated conditions of 40°C/75% RH. The three canisters used in the leakage test were placed in a bespoke piercing tool to allow a slow evaporation of the propellant over 30 min. Once the propellant was fully evaporated, the canister valve was removed by an adjustable pipe cutter. The drug, attached to the valve and the canister, was recovered by 200 mL recovery solution. 1 mL was then withdrawn and further diluted by 9 mL BAC-IS in a 10 mL volumetric flask. Triplicate samples were analysed by HPLC. The total mass of the drug per canister was then calculated, and compared with the target weight in Section 5.4.2.

5.4.4 Moisture Test

Water can be introduced into a pMDI by moisture from the environment. As micronised material can be chemically or physically sensitive to the moisture content, it is essential to prevent ingress of water to maintain the long-term stability of the novel pMDI (FDA, 1998). According to Section 2.9, moisture content inside the canisters was monitored using a moisture test by Karl Fisher Coulometer (Model 831) at the beginning of canister life and at time points 0, 1, 3 and 6 months. The coulometer was calibrated by injecting 0.4 mL of Hydranal® water standard into the titration cell, and the syringe was weighed before and after the injection, where the water content should be in the range of 100 ± 10 ppm. The sample canisters described in Section 5.4.1 and 5.4.2 were actuated five time times in rapid succession into the titration cell, and triplicate tests per canister were performed. Each canister was weighed before and after testing, and the moisture level was automatically calculated in parts per million (ppm).

5.4.5 Stability Test

All the canisters were stored under accelerated conditions of 40°C/75% RH. 6 canisters were selected randomly from the batch at time points 0, 1, 3 and 6 months. Three of these were used for the leakage and content test, and the remaining 3 used for dose uniformity and particle size distribution tests. Uniformity of delivered dose was measured using a metal DUSA (Section 2.8.2), and aerodynamic particle size distribution was measured by a standard NGI (Section 2.8.3).

5.5 Results and Discussion

5.5.1 Tio Control

Five Tio control canisters were selected for the leakage test at time points of Release (0 month), 1, 3 and 6 Month, which is summarised in Table 5.4. There was no statistical difference between post production weight and pre-test weight at release (t-test, P> 0.05), 1 (t-test, P> 0.05), 3 (t-test, P> 0.05) and 6 months (t-test, P> 0.05) respectively. The absolute value of loss per year was far less than 191 mg per year and none was greater than 206 mg per year, which indicated the VARI® metering valve and the H&T Presspart® canister was compatible over the 6-month storage under the accelerated conditions.

Table 5. 4 Leakage Test for Tio Control Formulation (n=5; mean ± SD)

Period	Post Production (g)	Pre-test (g)	Weight Difference (mg)	Difference %	Leakage (mg/yr)
Release	12.72 ± 0.01	12.72 ± 0.01	0.2	0.0019	13.7
1 Month	12.69 ± 0.08	12.69 ± 0.08	-1.6	-0.0124	17.2
3 Month	12.73 ± 0.02	12.73 ± 0.02	-0. 9	-0.0069	-4.5
6 Month	12.67 ± 0.02	12.67 ± 0.02	-1.3	-0.0106	-2.3

(Post-production: weight recorded after the preparation of the canisters. Pre-test: weight recorded before the canister content test. Weight difference: the post-product weight minus the pre-test weight)

The moisture tests showed that the moisture level was 131.45 ± 9.00 ppm (Release), 194.27 ± 5.99 ppm (1 Month), 200.94 ± 4.62 ppm (3 Month), and 233.65 ± 8.61 ppm (6 Month). Correspondently, the water amount in each canister was calculated as 0.72 ± 0.05 mg (Release), 1.06 ± 0.03 mg (1 Month), 1.10 ± 0.03 mg (3 Month) and 1.28 ± 0.05 mg (6 Month). As shown in Figure 5.1, although the moisture maintains at a relative low level, less than 250 ppm, there is a trend of increase of moisture inside the canister over the storage. According to ANOVA and Tukey HSD test, the moisture value at 6 Month is the highest (P< 0.05) and at Release is the lowest (P<0.05). There is no statistical difference between 1 Month

and 3 Month (P> 0.05). Under the condition of 40°C/75% RH, it was found there was an 80% increase of moisture level over the 6-month storage.

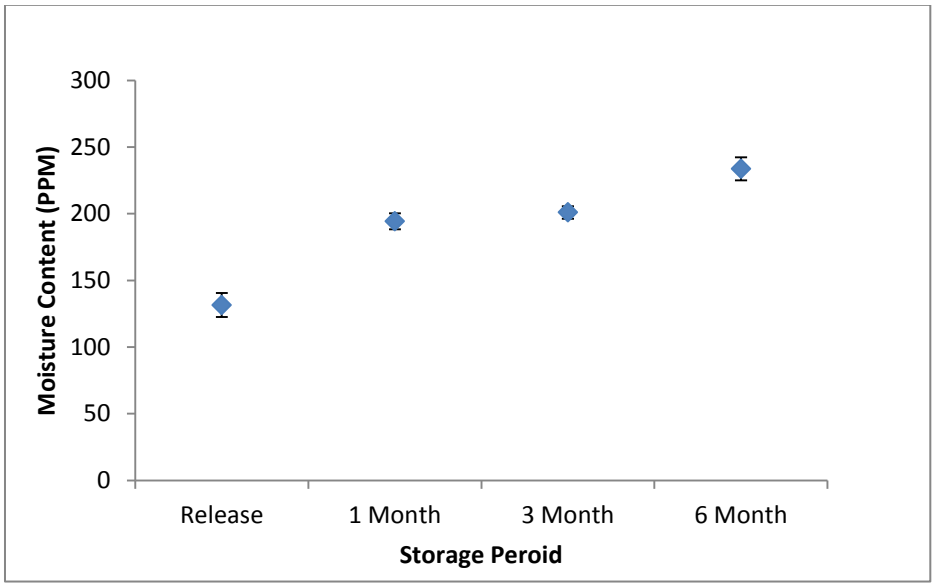

Figure 5. 1 Profile of Moisture Ingress of Tio Control Canisters over 6-Month Storage (n=5; mean ± SD)

The aerosol performance over the 6-month storage is summarised in Table 5.5. Ex-valve doses based on the NGI data are 7.95 ± 0.75 µg (Release), 9.84 ± 0.70 µg (1 Month), 11.18 ± 0.96 µg (3 Month) and 12.07 ± 1.29 µg (6 Month) respectively. There is a trend of dose increase with the increase of storage period, with the ex-valve dose at Release being significantly lower than the rest of time points (ANOVA and Tukey HSD test, P< 0.05). According to ANOVA and Tukey HSD test, the FPF at Release was also significantly lower than 1 Month (P< 0.05) and 6 Month (P< 0.05) but similar to 3 Month (P> 0.05). There is no statistical difference in FPF between 1 Month and 6 Month (P> 0.05). However, the Release group shows the lowest FPD (P< 0.05), and the rest of time points are similar (P> 0.05). Furthermore, there is no statistical difference in GSD regardless of the storage period. For MMAD, there was no significant difference between Release, 3 Month and 6 Month (P> 0.05), and 1 Month was significantly lower than Release (P< 0.05) and 1 Month (P< 0.05).

Compared with Release, the increased ex-valve dose at the time point of 6 Month suggests the potential dissolution of fine Tio particles and the growth of large Tio particles due to Ostwald ripening or particle aggregation. As large particles sediment quicker than small particles, this could cause more drugs are measured, which is also supported by increased ex-actuator doses at the beginning of canister life. However, the increased FPF and reduced MMAD of 6 Month against the Release indicate improved aerosol performance, which is different with the assumption based on ex-valve doses. The opposite finding in FPFs and ex-valve doses suggest the relevance between water ingress and aerosol performance. The rise of the water level could decrease the drug solubility in propellant, and depress the crystal growth in contrast to Ostwald ripening, which is proven in the research by Williams and Hu (2000). Alternatively, the higher FPF could be also caused by the change of vapor pressure and plume velocity, which lead to less of particles captured in upper stages of the NGI, which is illustrated in Figure 5.2. Some previous research revealed increase of the ethanol concentration in HFA propellants, e.g. HFA 134a and HFA 227, caused a decreased vapor pressure in a pMDI solution formulation (Williams and Liu, 1998; Vervaet and Byron, 1999). However, few studies have been done on the influence of moisture contents on the vapor pressure of propellants in a pMDI suspension system.

Table 5. 5 Aerosol Characteristics (ex-actuator) of Tio Control over 6-month Storage (n= 5; mean ± SD)

Excipient Size	Release	1 Month	3 Month	6 Month
Tio FPF (% < 5 μm)	20.70 ± 1.69	25.55 ± 2.56	23.28 ± 2.07	27.50 ± 1.82
Tio MMAD (μm)	5.43 ± 0.21	4.76 ± 0.25	5.40 ± 0.25	5.01 ± 0.23
Tio FPD (μg < 5 μm)	1.43 ± 0.17	2.15 ± 0.17	2.31 ± 0.34	2.86 ± 0.32
Tio GSD	2.05 ± 0.10	1.98 ± 0.06	1.93 ± 0.06	1.98 ± 0.07

As shown in Figure 5.2, similar distribution patterns are obtained at different time points, with the peak deposition on Stage 2 and 3. However, the IP deposition has a trend of reduction over the 6 month storage, which is opposite to the assumption

that particle growth or aggregation caused an increase in ex-valve doses. This finding could be explained by the change of vapor pressure as described in previous paragraph. According to ANOVA and Tukey HSD test, the IP deposition of the Release group is comparable to 1 Month ($P > 0.05$) but significantly higher than 3 Month ($P < 0.05$) and 6 Month ($P < 0.05$). When IP, Stage 1 and 2 deposition is combined as the "non-respiratory fraction" (particle diameter> 6.4 µm), the Release group is significantly higher than 1 Month ($P < 0.05$) and 6 Month ($P < 0.05$). When combining Stage 3 to MOC as the "respiratory fraction" (particle diameter< 3.99 µm), the Release group is significantly lower than 1 Month ($P < 0.05$) and 6 Month ($P < 0.05$). These findings serve to indicate that the storage period at 40°C/75% RH affects the drug particle size and vapor pressure of the formulation.

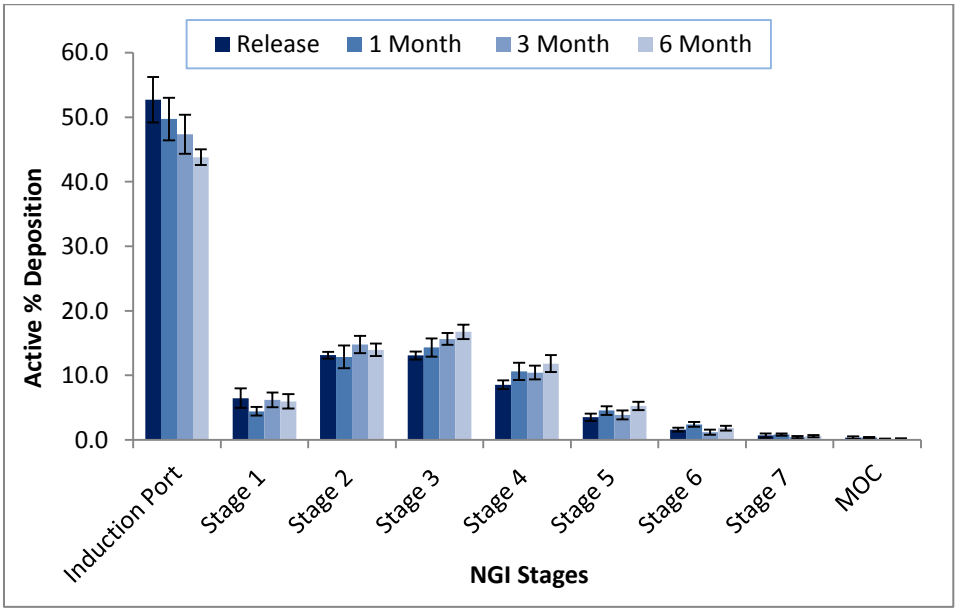

Figure 5. 2 NGI Distribution Pattern (%) of Tio Control over 6 Month Storage (n=5; mean ± SD)

Based on the findings from the dose uniformity test, it was found that the average metered weight (Table 5.6) was greater than the target of 35.01 mg per shot (Table 5.1). The right amount of formulations was filled in each canister, and excessive metering therefore lead to lack of sufficient suspension at the end of canister life

(Figure 5.3). Furthermore, large variations are observed from the metered weight, ex-valve and ex-actuator doses (Table 5.6).

Table 5. 6 Valve Performance of the Tio Control over 6 Month Storage (n=50; mean ± SD)

Period	Release	1 Month	3 Month	6 Month
Metered Weight (mg)	41.83 ± 1.12	40.80 ± 0.90	34.51 ± 12.35	38.74 ± 12.28
Emitted Dose (ex-valve) (μg)	8.60 ± 5.34	9.39 ± 4.91	7.25 ± 4.76	7.59 ± 5.98
Emitted Dose (ex-actuator) (μg)	5.48 ± 3.59	6.05 ± 3.70	5.41 ± 3.75	5.19 ± 4.08

(Metered Weight: canister weight difference between pre- and post-actuation, which includes the weight of the formulation and propellant; Emitted dose (ex-valve): the dose emitted from the valve; Emitted dose (ex-actuator): the dose emitted from the actuator)

As illustrated from Figure 5.3, the metering weight was consistent through the beginning and middle of canister life in all the samples tested which indicates good valve performance with Tio control. However, at the end of canister life, 4 out of 15 actuations in Release, and 3 out of 15 actuations in 1 Month, and all the 15 actuations in 3 and 6 Month show insufficient amount of the formulation left in the canister. Furthermore, as illustrated from Figure 5.4, big variations between replicates of ex-actuator doses were observed at the beginning of canister life, regardless of the storage period, and a dramatic dropping of doses was also found at the end of canister life. Even most of the actuations in Release and 1 Month demonstrate accurate metered weight at the end of canister life (Figure 5.3), delivered doses are much lower than the target range (Figure 5.4). All these findings suggest the formulation of Tio only is not a uniformly dispersed suspension. The right amount of Tio was filled in each canisters, but variable doses were delivered at the beginning of canister life, which lead to insufficient drug left at the end of canister life. To conclude, the Tio control group exhibits ingress of water during the 6-Month storage under accelerated conditions. The environment factors and length of storage have influence on the generation of fine particles. Without

the addition of secondary particle, the raw micronised Tio particles show poor physical stability, and cannot form a well dispersed suspension with manual shaking.

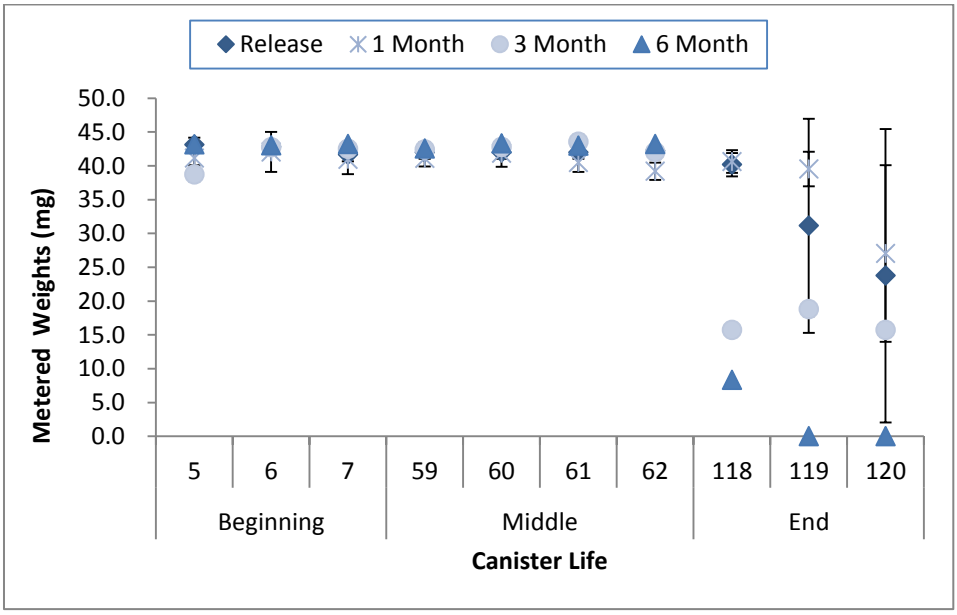

Figure 5. 3 Metered Weight Distribution of Tio Control over 6 Month Storage (n=5; mean ± SD)

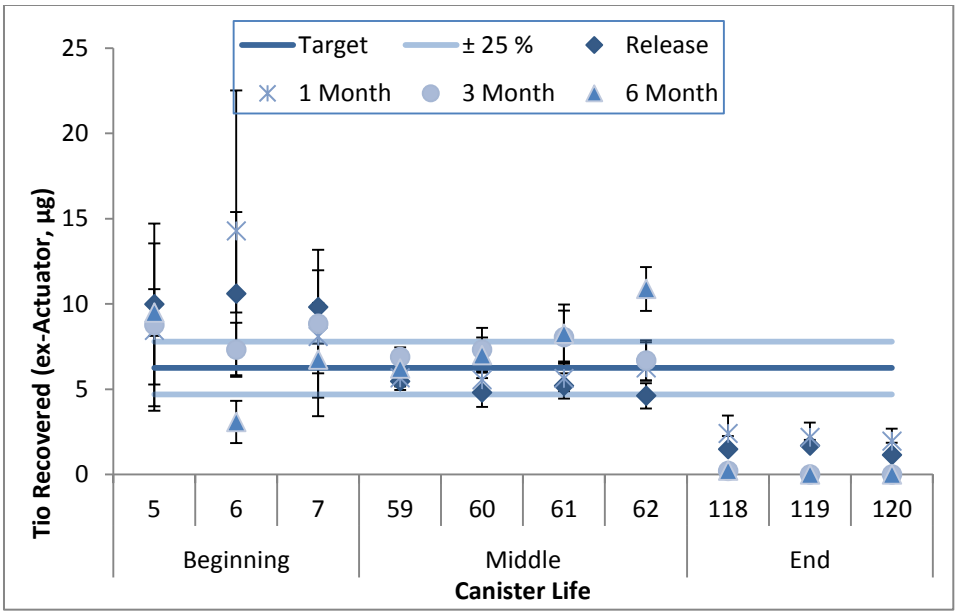

Figure 5. 4 Canister Life Content Uniformity of Tio Control over 6 Month Storage (n=5; mean ± SD)

5.5.2 Tio: SV010 Formulation

Table 5.7 illustrates the seal properties of pMDIs containing the novel Tio: SV010 formulation. There is less than 0.01% difference between post-production weight and pre-test weight with no significant difference regardless of storage period (ANOVA and Tukey HSD test, P> 0.05). Though the leakage rate varied over time, the values stays at low levels, where average of leakage is < 206 mg/year and none of single canister is > 294 mg/year, which can be neglected. The canister weights are considered as consistent during the storage, and no obvious leakage is identified in these novel products, which is similar to results seen with the Tio Control (Table 5.4).

Table 5. 7 Leakage Test for Novel pMDI Formulation of Tio: SV010 (n=3; mean ± SD)

Period	Post Production (g)	Pre-test (g)	Weight Difference (mg)	Difference %	Leakage (mg/yr)
Release	12.95 ± 0.07	12.95 ± 0.07	0. 2	0.0018	0.0
1 Month	13.08 ± 0.10	13.08 ± 0.10	0. 7	0.0051	8.1
3 Month	13.09 ± 0.05	13.09 ± 0.05	0. 5	0.0041	3.2
6 Month	13.13 ± 0.09	13.13 ± 0.09	0. 3	0.0025	1.3

(Post-product: weight recorded after the preparation of the canisters, Pre-test: weight recorded before the canister content test. The weight difference: the post-product weight minus the pre-test weight)

The water level of the novel Tio: SV010 formulation was 246.83 ± 5.02 ppm (Release), 288.32 ± 3.97 ppm (1 Month), 328.96 ± 5.10 ppm (3 Month) and 429.41 ± 23.72 ppm (6 Month) respectively. Accordingly, the water content per canister was 1.45 ± 0.03 mg (Release), 1.70 ± 0.02 mg (1 Month), 1.94 ± 0.03 mg (3 Month) and 2.53 ± 0.14 mg (6 Month). The water in the environment can diffuse through the valve components into the canisters. There is a trend of increase of water ingress over the 6-month storage. According to ANOVA and Tukey HSD test, moisture value was significantly increased at each time points ($P< 0.05$), and therefore the highest value was obtained at 6 Month, which shows a growth of 73% in 6 months. Compared with the Tio Control group (Figure 5.5), the novel Tio: SV010 shows a higher water level over the storage period. There is 88% (Release), 48% (1 Month), 64% (3 Month) and 84% (6 Month) increase of moisture in the novel formulation against the Tio Control at each time point, and all are statistically different (t-test, $P< 0.05$). However, the relative high moisture level in Tio: SV010 could be caused by batch-to-batch variation as environment humidity was not controlled during powder blending and canister filling.

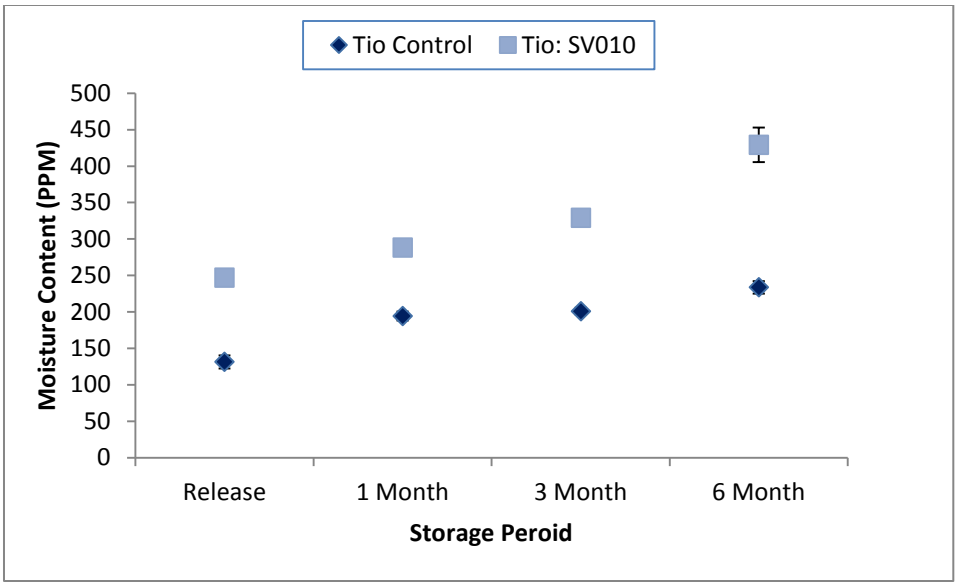

Figure 5. 5 Profile of Moisture Ingress of Tio Control and the Tio: SV010 Formulation in 6-Month Storage (Tio Control: n=5; mean ± SD, Tio: SV010: n=3; mean ± SD)

The total drug mass of each canister was calculated based on the HPLC methodology and compared with the target mass. As shown in Table 5.8, Tio contents in canisters are in the range of ± 5% of the target, and no drug loss and degradation is identified during the storage. The canister content shows no statistical difference at each time points (ANOVA, P> 0.05).

Table 5. 8 Canister Content Test for Novel pMDI Formulation of Tio: SV010 (n=3; mean ± SD)

Period	Canister Content (mg)	Target Mass (mg)	Difference (%)
Release	1.82 ± 0.11	1.89	96
1 Month	1.90 ± 0.07	1.89	101
3 Month	1.89 ± 0.18	1.89	100
6 Month	1.87 ± 0.05	1.89	99

The aerosol characteristics of the Tio: SV010 formulation is summarised in Table 5.9. The FPFs are slightly reduced with the increase of the storage period, but according to ANOVA and Tukey HSD test, there are no statistical difference between the four groups (P> 0.05). The same results are also observed for MMAD (P> 0.05), GSD (P>

0.05) and FPD (P> 0.05). The ingress of water in the Tio: SV010 formulation does not therefore appear to affect the dissolution of the drug in the propellants, and no particle size growth is obvious.

Table 5. 9 Aerosol Characteristics (ex-actuator) of Novel Tio: SV010 Formulation over 6 Month Storage (n= 3; mean ± SD)

Time Point	Release	1 Month	3 Month	6 Month
Tio FPF (% < 5 µm)	39.26 ± 3.15	39.66 ± 3.92	38.77 ± 1.12	32.92 ± 2.03
Tio MMAD (µm)	4.31 ± 0.09	4.29 ± 0.36	4.51 ± 0.30	4.42 ± 0.33
Tio FPD (µg < 5 µm)	3.99 ± 0.46	3.87 ± 0.49	4.23 ± 0.30	3.81 ± 0.39
Tio GSD	1.88 ± 0.03	1.91 ± 0.10	1.92 ± 0.15	2.04 ± 0.09

As shown in Figure 5.6, the aerosol performance was compared between the Tio control and Tio: SV010 at Release. According to ANOVA and Tukey HSD test, fine particle fraction (P< 0.05) and FPD (P< 0.05) of Tio: SV010 was significantly increased and MMAD was significantly decreased (P< 0.05). The addition of SV010 (< 20 µm) can therefore effectively prevent the aggregation of drug particles and improve the aerosol performance. Consequently, the performance of the novel Tio: SV010 formulation exhibits consistent performance during the accelerated storage, with good physical stability. The addition of SV010 therefore appears to provide advantages over Tio alone in delivery efficiency and resistance to environmental factors.

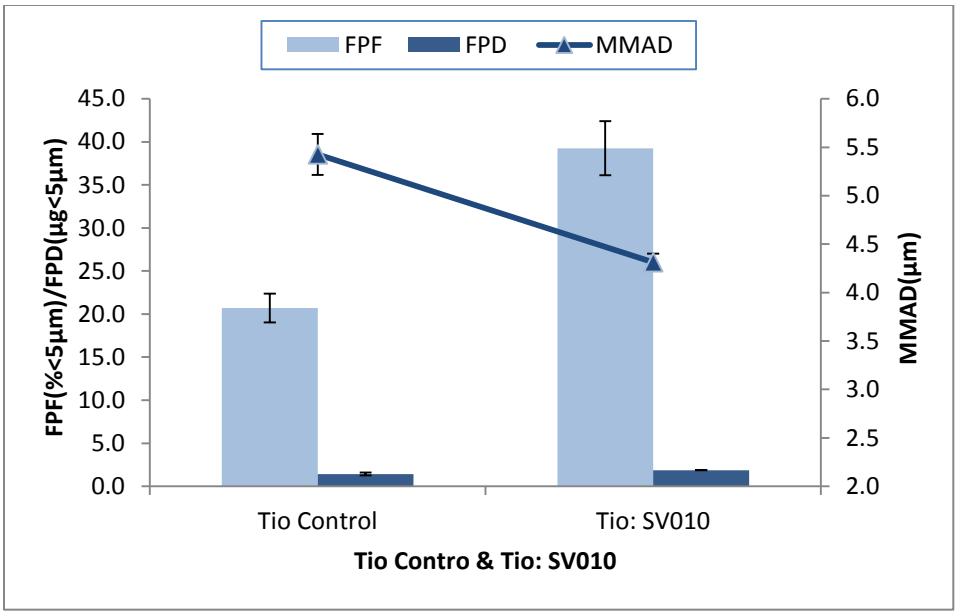

Figure 5. 6 Aerosol Characteristics (ex-actuator) of Tio Control and Tio: SV010 at Release **(Tio Control: n=5; mean ± SD, Tio: SV010: n=3; mean ± SD)**

As shown in Figure 5.7, similar distribution patterns of the Tio: SV010 formulation were obtained through the storage period, with the peak deposition appearing on Stage 3. According to ANOVA and Tukey HSD test, the IP deposition of the four groups are comparable ($P > 0.05$), which suggests plume velocity is constant and no particle size growth, e.g. aggregation, occurs during the 6-month storage. When the IP, Stage 1 and 2 are combined as the "non-respiratory fraction" (particle diameter > 6.4 µm), no statistical difference was found in the four groups ($P > 0.05$). However, when Stages 3 to MOC were combined as the "respiratory fraction" (particle diameter < 3.99 µm), the 6 Month group was significantly lower than the other groups ($P < 0.05$), which corresponds to the relative lower FPF (Table 5.7). Reviewing Figure 5.7 with Figure 5.2, it is seen that Tio: SV010 gives a lower IP deposition and non-respiratory fractions, and a higher respiratory fraction compared to Tio Control.

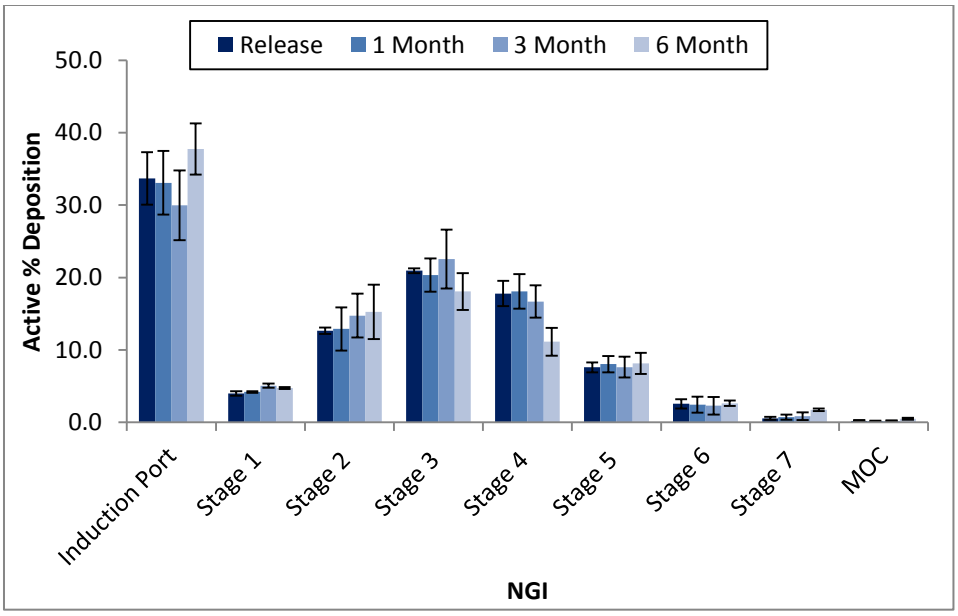

Figure 5. 7 NGI Distribution Pattern (%) of Tio: SV010 Formulation over 6 Month Storage (n=3; mean ± SD)

The valve performance of the novel pMDIs is illustrated in Table 5.10. The target emitted mass of the suspension per actuation is 35.07 mg, and the target ex-valve dose is 11.25 µg. The metered weight was consistent through the canister life regardless of the storage length (ANOVA, P> 0.05), which suggests that the VARI® KHFA metering valve is compatible with the novel Tio: SV010 formulation. The ex-valve dose was reproducible during the 6-month storage with no statistical difference (ANOVA, P> 0.05), which indicates that the novel Tio: SV010 formulation maintains a uniform suspension regardless of the length of storage. However, according to ANOVA and Tukey HSD test, ex-actuator doses of the Release group are significantly lower than the 1 and 3 Month groups (P< 0.05) but similar to 6 Month (P> 0.05). Kwok et al (2008) reported the mean charge of HFA 227 was negative at low water contents (<120 ppm) in a drug-free system, and the increase of water content could neutralise the negative charge of HFA 227. HFA 227 is charged positively when the moisture reached 400 ppm. The electrostatic retention was considered relevant to drug deposition in pMDI spacers by Kwok et al (2006) but few studies have been done on actuators. As the H&T Presspart® actuator used

in this research is made of polypropylene, which is negatively charged, the electrostatic deposition likely affects actuator retention of Tio (Chen et al, 2014). However, there is no trend of actuator deposition corresponding to the moisture level.

Table 5. 10 Valve Performance of the Novel Tio: SV010 pMDI (n=30; mean ± SD)

Period	Release	1 Month	3 Month	6 Month
Metered Weight (mg)	40.77 ± 1.06	41.04 ± 1.88	40.90 ± 1.25	41.36 ± 1.43
Emitted Dose (ex-valve) (μg)	12.03 ± 0.72	12.04 ± 0.92	12.44 ± 1.26	12.19 ± 1.58
Emitted Dose (ex-actuator) (μg)	8.26 ± 0.54	9.19 ± 0.90	9.48 ± 1.17	8.66 ± 1.99

(Metered weight: canister weight difference between pre- and post-actuation, which includes the weight of the formulation and propellant; Ex-valve emitted dose: the dose emitted from the valve; Ex-actuator emitted dose: the dose emitted from the actuator)

The distribution of ex-valve and ex-actuator doses over the canister life is illustrated in Figure 5.8 and Figure 5.9. The ex-valve dose were consistent over the canister life, independent of storage periods, but greater variations between replicates at 6 Month was observed. Average ex-actuator doses were reproducible at Release, 1 Month and 3 Month over the canister life respectively, which is identical with the finding in ex-valve dose distribution. However, these values are 32%-52% greater than the target dose of 6.25 μg, and therefore the metered dose of the novel Tio: SV010 need to be further modified. It is also noticed that the variability of ex-actuator doses between replications is amplified at 6 Months, which is suggested by greater standard deviations than ex-valve doses (Figure 5.8 and 5.9). There is no evidence of influence of moisture on solubility of Tio over the storage stability test (as described in Chapter 3), but the ingress of the moisture (> 400 ppm) could invert the polarity of HFA 227 and therefore lead to a variable actuator deposition (Kwok et al, 2008). To conclude, the novel Tio: SV010

formulation is compatible with the VARI® KHFA valve, H&T Presspart® canister and actuator, where it shows good in-vitro aerosol performance and maintains good physical stability of the suspension through the canister life up to 3-month storage under accelerated conditions.

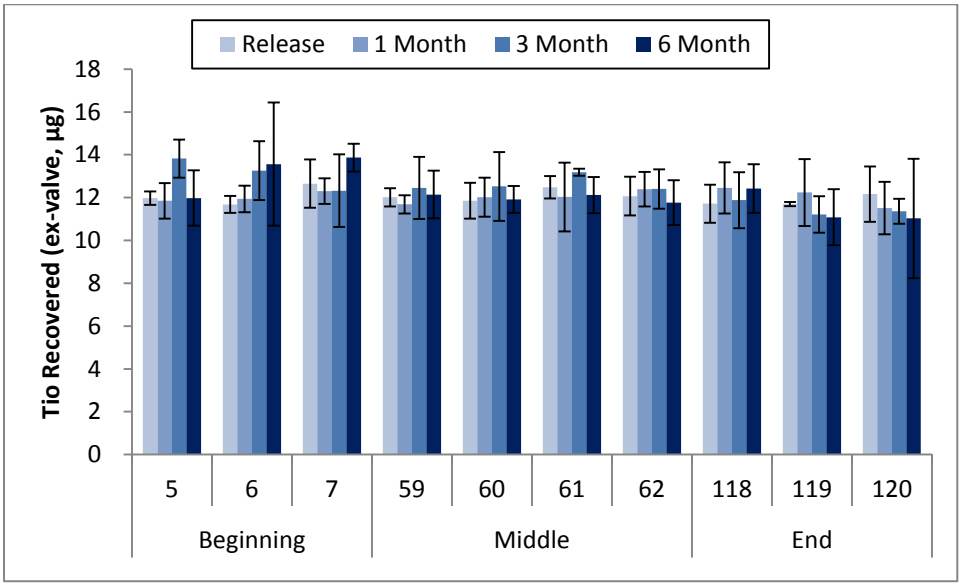

Figure 5. 8 Ex-valve Dose Distribution of Tio: SV010 Formulation over 6 Month Storage (n=3; mean ± SD)

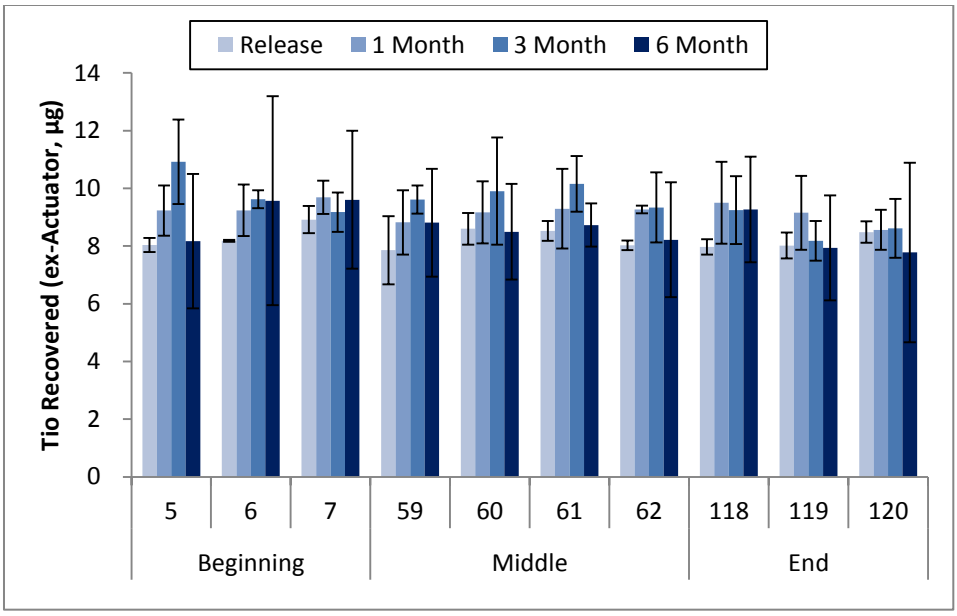

Figure 5. 9 Ex-actuator Dose Distribution of Tio: SV010 Formulation over 6 Month Storage (n=3; mean ± SD)

5.5.3 Tio: Leucine Formulation

Table 5.11 illustrates the leakage of pMDIs containing the novel Tio: leu formulation. The canister weights were consistent during the storage with negligible leakage rates, which is similar to Tio: SV010 (Table 5.7), and this finding suggests the seal properties of VARI® metering valve and the H&T Presspart® canister are compatible with the novel formulation regardless of the type of secondary particles, e.g. SV010 and leucine.

Table 5. 11 Leakage Test for Novel pMDI Formulation of Tio: Leu (n=3; mean ± SD)

Period	Post Production (g)	Pre-test (g)	Weight Difference (mg)	Difference %	Leakage (mg/yr)
Release	13.28 ± 0.05	13.28 ± 0.05	0. 9	0.0065	0.0
1 Month	13.15 ± 0.07	13.15 ± 0.07	1.2	0.0091	14.6
3 Month	12.92 ± 0.41	12.92 ± 0.41	0.5	0.0036	2.8
6 Month	13.15 ± 0.05	13.15 ± 0.05	0. 6	0.0043	2.3

The moisture levels of the stored canisters were 154.42 ± 9.16 ppm (Release), 234.23 ± 1.93 ppm (1 Month), 292.20 ± 22.7 ppm (3 Month) and 383.99 ± 4.54 ppm (6 Month) respectively. Accordingly, the water content per canister was calculated as 1.00 ± 0.12 mg (Release), 1.50 ± 0.04 mg (1 Month), 1.89 ± 0.09 mg (3 Month) and 2.41 ± 0.05 mg (6 Month). As water diffuses through the valve component, the moisture level was increased at increasing storage times, with significant differences identified at each time points (ANOVA and Tukey HSD test, $P< 0.05$). As shown in Figure 5.10, the moisture ingress of Tio: Leu was compared against the Tio Control and Tio: SV010. Tio: Leu demonstrates an intermediate moisture level between the Tio Control and Tio: SV010 with statistical difference at each time point (ANOVA and Tukey HSD test, $P< 0.05$). The increase of the moisture level is identified in both Tio: SV010 and Tio: Leu against the Tio control, and this could be due to ingress of moisture from the environment during formulation preparation, e.g. sieving and blending. Regardless, the addition of leucine as the secondary excipient is found to maintain a low moisture level under 400 ppm in the canister.

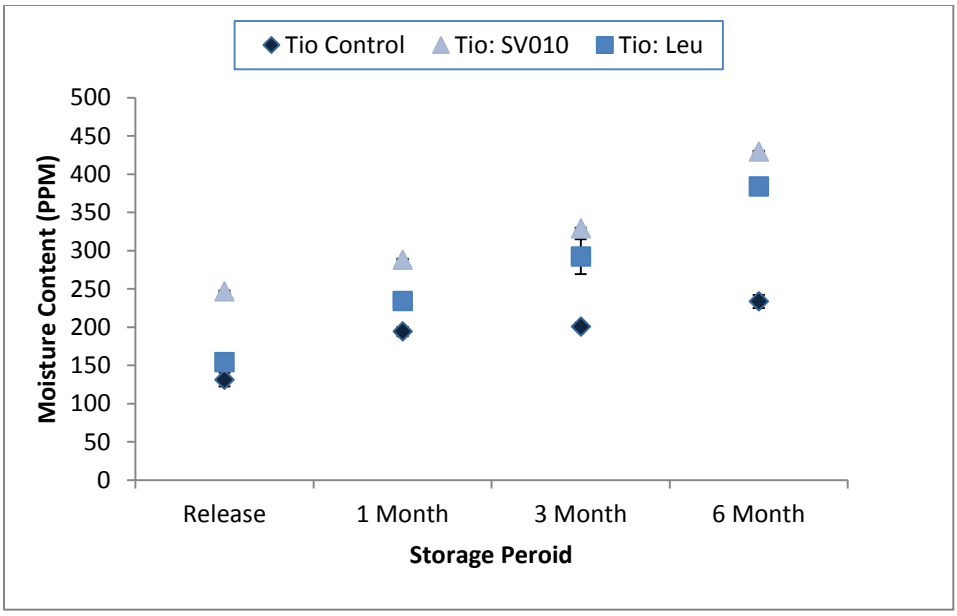

Figure 5. 10 Profile of Moisture Ingress of Tio Control, Tio: SV010 and Tio: Leu Formulations in 6-Month Storage (Tio Control: n=5; mean ± SD, Tio: Leu: n=3; mean ± SD)

The canister content for the formulation of Tio: Leu is illustrated in Table 5.12. The total drug mass per canister was in the range of 85% and 95% of the target, whereas Tio: SV010 was in the range of ±5% of the target. The difference in powder density and particle shape could incur the powder segregation subsequent handling of complete powder blending as described in Section 2.5. The powder segregation could further affect the uniformity of the blend especially with a period of storage. According to ANOVA and Tukey HSD test, the canister content is significantly higher at 3 Month ($P < 0.05$), and the rest of groups are comparable ($P > 0.05$). There is no indication of drug loss due to chemical degradation.

Table 5. 12 Canister Content Test for Novel pMDI Formulation of Tio: Leu (n=3; mean ± SD)

Period	Canister Content (mg)	Target (mg)	Difference (%)
Release	1.31 ± 0.03	1.51	87
1 Month	1.35 ± 0.04	1.51	89
3 Month	1.43 ± 0.01	1.51	95
6 Month	1.35 ± 0.02	1.51	90

The aerosol characteristics of Tio: Leu is summarised in Table 5.13. According to ANOVA and Tukey HSD test, there are no statistical differences in FPF ($P > 0.05$), FPD ($P > 0.05$) and MMAD ($P > 0.05$) regardless of the storage period. GSD at 6 Month is identified as the highest valve ($P < 0.05$), and the other groups are similar ($P > 0.05$). Over the 6-month storage, the aerosol performance is generally considered as being consistent though the polydispersity of drug particle distribution increased about 14% at 6 Month compared with Release. The aerosol performance of Tio: Leu is compared with the control group in Figure 5.11. According to ANOVA and Tukey HSD test, the Tio: Leu illustrates a significant increase of FPF ($P < 0.05$) and FPD ($P < 0.05$), and a significant decrease of MMAD ($P < 0.05$). Furthermore, when compared against the Tio: SV010 formulation, Tio: Leu also demonstrates improved aerosol performance with increased FPFs (t-test, $P < 0.05$) and decreased MMADs (t-test, $P < 0.05$). These findings suggest the addition of leucine as the secondary particle could improve the efficiency of generation of fine particles regardless of the influence of the increased water content.

Table 5. 13 Aerosol Characteristics (ex-actuator) of Tio: Leu Formulation over 6 Month Storage (n=3, mean ± SD)

Time Point	Release	1 Month	3 Month	6 Month
Tio FPF (% < 5 µm)	55.61 ± 1.50	57.33 ± 0.87	55.16 ± 2.89	52.54 ± 1.88
Tio MMAD (µm)	3.13 ± 0.13	3.03 ± 0.25	3.41 ± 0.27	2.90 ± 0.24
Tio FPD (µg < 5 µm)	3.64 ± 0.23	4.21 ± 0.16	3.55 ± 0.43	3.86 ± 0.23
Tio GSD	1.90 ± 0.09	1.85 ± 0.05	1.82 ± 0.03	2.17 ± 0.06

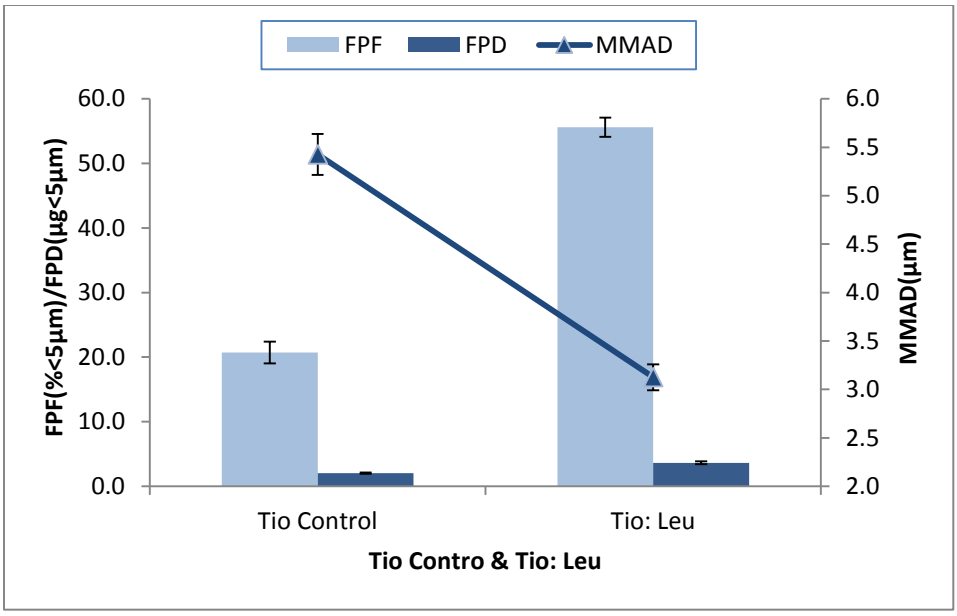

Figure 5. 11 Aerosol Characteristics (ex-actuator) of Tio Control and Tio: Leu at Release (Tio Control: n=5; mean ± SD, Tio: Leu: n=3; mean ± SD)

The aerodynamic particle size distribution of Tio: Leu over 6 month storage is shown in Figure 5.12. According to ANOVA and Tukey HSD test, the very similar IP depositions suggests the novel formulation avoids the aggregation of Tio particles over the 6-month storage. When the deposition in IP, Stage 1 and 2 are combined as the non-respiratory fraction (particle diameter > 6.4 µm), no statistical difference is found in the four groups (P> 0.05). The same result was obtained when Stages 3 to MOC were combined as the respiratory fraction (particle diameter < 3.99 µm) (P> 0.05). The grouped data suggests that the distribution patterns are comparable regardless of the storage time. However, at 6 Month less particles are deposited at Stage 4, and more at the lower stages, e.g. Stage 7, which indicates a broader particle size distribution which is also demonstrated by the increased GSD (Table 5.11). Consequently, the similar aerosol performance is obtained despite an increase in water content up to 300 ppm (at 3 Month) through the storage period.

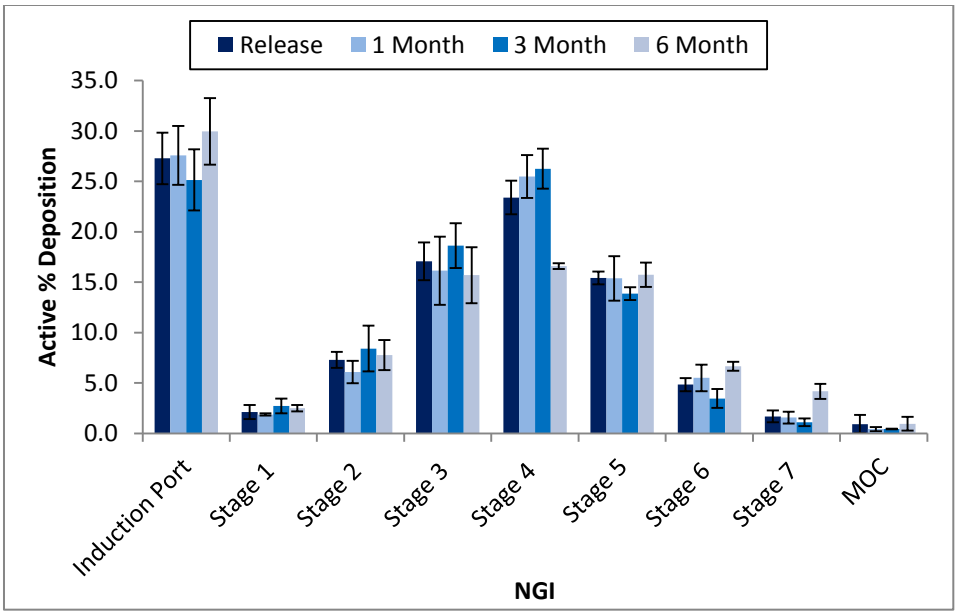

Figure 5. 12 NGI Distribution Pattern (%) of Tio: Leu Formulation over 6 Month Storage (n=3; mean ± SD)

Table 5.14 illustrates the valve performance of the novel formulation containing leucine. The target metered weight of the suspension is 35.05 mg, whereas the obtained values are 15% - 19% higher than the target. The modified metering dose of Tio is 9 µg, whereas the obtained values are 2% - 13% lower than the target but in the acceptable range of ± 25% of the target. There is no trend of drug loss during the 6-month storage.

Table 5. 14 Valve Performance of the Novel Tio: Leucine pMDI (n=30; mean ± SD)

Period	Release	1 Month	3 Month	6 Month
Metered Weight (mg)	41.73 ± 2.49	40.61 ± 1.38	40.63 ± 1.50	40.37 ± 1.02
Emitted Dose (ex-valve) (µg)	7.82 ± 0.56	8.80 ± 0.46	8.66 ± 0.70	8.39 ± 0.58
Emitted Dose (ex-actuator) (µg)	5.47 ± 0.44	6.84 ± 0.46	6.12 ± 0.56	6.03 ± 0.46

(Metering weight: canister weight difference between pre- and post-actuation, which includes the weight of the formulation and propellant; Ex-valve emitted dose: the dose emitted from the valve; Ex-actuator emitted dose: the dose emitted from the actuator)

The uniformity of ex-valve and ex-actuator doses is illustrated in Figure 5.13 and 5.14. The ex-valve and ex-actuator dose was reproducible over the canister life regardless of the storage length. As shown in Figure 5.13, 1 out of 30 actuations in the Release and 6 Month respectively was outside the range of ± 25% of the target of ex-valve doses, which indicate Tio particles were suspended uniformly over the canister life, and accelerated conditions had no influence of the physical stability of the novel formulation. However in the formulation of Tio: SV010, the 6 Month group showed a lack of suspension uniformity where great variations between replicates were found at the beginning and end of canister life (Figure 5.8). The uniformity of ex-actuator doses of Tio: Leu was shown in Figure 5.14, and only 1 out of 30 actuations in the Release was outside the range of ± 25% of the target, whereas the 6 Month group of Tio: SV010 presented great variability of replicates over the canister life. Therefore, the consistent ex-valve and ex-actuator doses demonstrated that the Tio: Leu formulation is physically stable and compatible with the hardware components used in this batch. Furthermore, Tio: Leu, compared with Tio only, demonstrates significant improvement in dose consistency and effectively prevents the lack of drug content at the end of canister life. To conclude, the addition of leucine (< 20 μm) as the secondary particle retains good aerodynamic performance and physical stability of Tio suspension over 6-month storage under accelerated conditions.

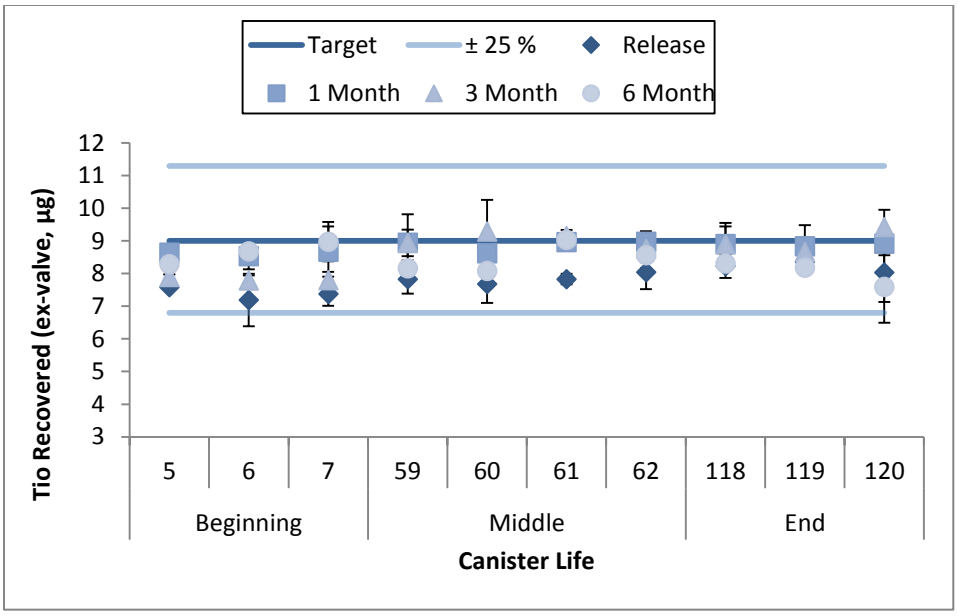

Figure 5. 13 Ex-valve Dose Distribution of Tio: Leu Formulation over 6 Month Storage (n=3; mean ± SD)

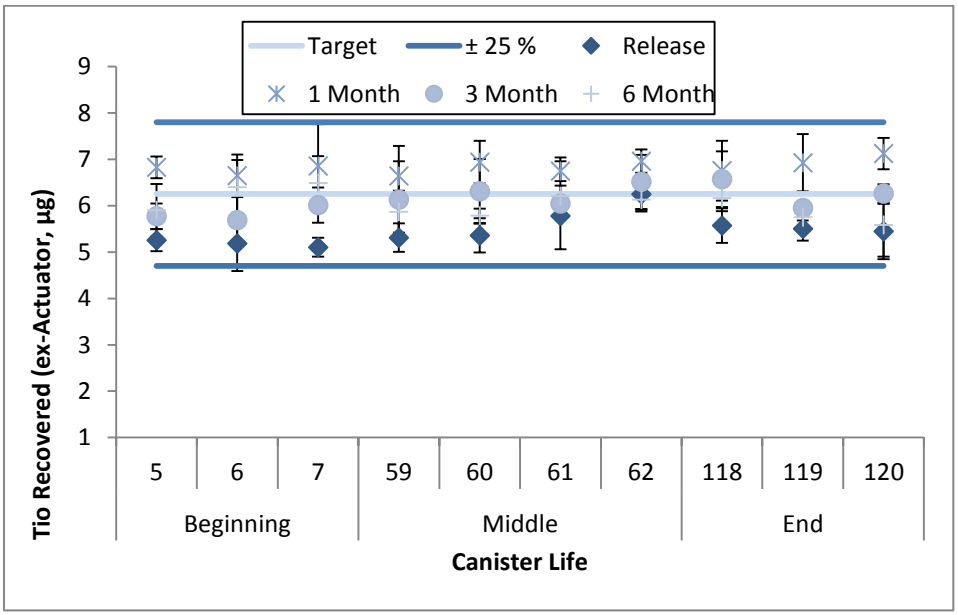

Figure 5. 14 Ex-actuator Dose Distribution of Tio: Leu Formulation over 6 Month Storage (n=3; mean ± SD)

5.5.4 Comparison of Optimised Formulations with Commercialised Products

The optimised novel formulations comprising Tio: SV010 (< 20 µm) (Section 5.5.2) and Tio: Leu (< 20 µm) (Section 5.5.3) were compared with reference commercial products Tiova® (Section 3.5.5), Handihaler® (Section 3.5.3) and Respimat® (Section 3.5.4). The loaded dose and ex-device doses are based on experimental design, e.g. Tio: SV010 and Tio: Leu, and patient information leaflets (PILs), e.g. Tiova®, Handihaler® and Respimat®, as shown in Table 5.15. Tiova® meters 11.25 µg Tio per actuation with two actuations providing one medicinal dose. One capsule of Handihaler® contains 22.5 µg Tio to target one medicinal dose of 12.5 µg of Tio, with two inhalations being recommended to empty each capsule. Respimat® delivers 3.124 µg Tio, with two inhalations providing one medicinal dose of 6.25 µg of Tio. The novel formulation of Tio: SV010 meters 11.25 µg of Tio per actuation with two actuations providing one medicinal dose of 12.5 µg of Tio. The novel formulation of Tio: Leu meters 9 µg of Tio per actuation with two actuations providing one medicinal dose of 12.5 µg of Tio.

Table 5. 15 Summary of Theoretical Doses per Actuation/Capsule of Novel Formulations and Reference Products (n=3; mean ± SD)

Products	SV010	Leucine	Tiova®	Handihaler®	Respimat®
Tio Loaded Dose (µg)	11.25	9	11.25	22.5	3.124
Tio Ex-device Dose (µg)	6.25	6.25	n/a	12.5	n/a

(Loaded dose: the dose metered by the valve of pMDIs, e.g. Tio: SV010, Tio: Leu and Tiova®, capsule of Handihaler®, dosing chamber of Respimat®. Ex-device dose: the dose emitted from the actuator of pMDIs, mouthpiece of Handihaler® and Respimat®)

As shown in Table 5.16, experimental doses and aerosol characteristics for Tio: SV010, Tio: Leu, Tiova®, Handihaler® and Respimat® were calculated based on the NGI data. For the pMDI formulation including Tio: SV010, Tio: Leu and Tiova®, all these three formulations showed consistent loaded (ex-valve doses) and ex-device

(ex-actuator doses) doses with small standard deviations. According to ANOVA and Tukey HSD test, Tio: SV010 shows the lowest FPF (P< 0.05), whilst the Tio: Leu formulation is comparable to Tiova® (P> 0.05). Tiova® demonstrates the greatest FPD (P< 0.05) and Tio: SV010 the least (P< 0.05). Furthermore, compared with Tiova®, it was found that the addition of secondary particle significantly increased the MMAD (P< 0.05) and GSD (P< 0.05).

Table 5. 16 Summary of Experimental Doses and Aerosol Characteristics of Novel Formulations and Reference Products (SV010/Leu/Tiova®: n=3; mean ± SD, Handihaler® and Respimat®: n=9; mean ± SD)

Formulation	SV010	Leucine	Tiova®	Handihaler®	Respimat®
Tio Loaded Dose (μg)	11.97 ± 0.32	8.43 ± 0.35	9.90 ± 0.22	11.06 ± 0.57	3.89 ± 0.26
Tio Ex-device Dose (μg)	10.15 ± 0.38	7.18 ± 0.33	9.25 ± 0.29	9.89 ± 0.72	3.51 ± 0.16
Tio FPF (% < 5 μm)	39.26 ± 3.15	53.44 ± 1.84	52.47 ± 0.57	40.60 ± 3.16	53.47 ± 6.57
Tio MMAD (μm)	4.31 ± 0.09	3.40 ± 0.09	2.70 ± 0.09	3.64 ± 0.19	2.72 ± 0.52
Tio FPD (μg < 5 μm)	3.39 ± 0.46	3.84 ± 0.25	4.85 ± 0.12	4.03 ± 0.54	1.88 ± 0.29
Tio GSD	1.88 ± 0.03	1.94 ± 0.06	1.60 ± 0.03	1.83 ± 0.05	3.31 ± 0.55

(Loaded dose: the dose metered by the valve of pMDIs, e.g. Tio: SV010, Tio: Leu and Tiova®, capsule of Handihaler® or dosing chamber of Respimat ®, Ex-device dose: the dose emitted from the actuator of pMDIs or mouthpiece of Handihaler® and Respimat®)(According to ANOVA and Tukey HSD test, in the group of Tio: SV010, Tio: Leu and Tiova®, Tio: SV010 shows the lowest FPF (P< 0.05), and Tio: Leu is similar to Tiova® (P> 0.05). For FPD, Tiova®>Tio: Leu>Tio: SV010 with statistic difference (P<0.05); in the group of Tio: Leu, Handihaler® and Respimat®, Handihaler® shows the lowest FPF (P< 0.05), while Tio: Leu and Respimat® are comparable (P> 0.05). For FPD, Tio: Leu is similar to Handihaler® (P> 0.05), which are double the value of Respimat®.)

For the pMDI formulation, the particle distribution patterns for Tio: SV010, Tio: Leu and Tiova® formulations is shown in Figure 5.15. According to ANOVA and Tukey HSD test, it was found that Tio: Leu has the lowest IP deposition ($P< 0.05$), whereas Tiova® has the highest IP deposition ($P< 0.05$). When deposition in the IP, Stage 1 and 2 are combined as the non-respiratory fraction (particle diameter > 6.4 μm), Tio: Leu still has the lowest deposition ($P< 0.05$) but Tio: SV010 has the highest ($P< 0.05$). Correspondingly, when Stages 3 to MOC are combined as the respiratory fraction (particle diameter < 3.99 μm), Tio: Leu is significantly greater than Tio: SV010 ($P< 0.05$) and Tiova® ($P< 0.05$). Therefore, Tio: Leu demonstrates comparable or improved aerosol performance against Tiova® and Tio: SV010, and the lowest IP deposition also suggests clinical potential to reduce side effects relevant to the oropharyngeal deposition

The ex-valve dose uniformity for Tio: SV010, Tio: Leu and Tiova® formulations is illustrated in Figure 5.16. Over the canister life, the Tio: Leu and Tio: SV010 formulations showed reproducible results. However, the ex-valve doses of Tiova® was consistent at the beginning and middle of canister life, but appeared to be more varied towards the end of canister life. The same pattern was found in the canister life content uniformity (shown in Figure 5.17). The ex-actuator dose of Tio: Leu and Tio: SV010 remained consistent but Tiova® increased at the end of canister life. To conclude, the secondary particles were found to play a role in maintaining the suspension uniformity. The novel formulations, Tio: Leu and Tio: SV010 demonstrated good physical stability over the canister life.

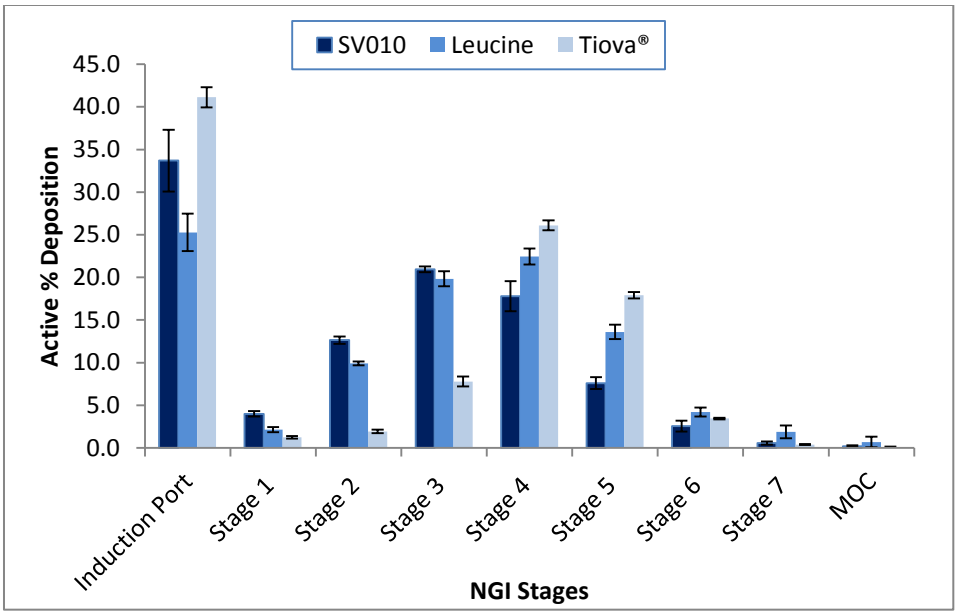

Figure 5. 15 NGI Distribution Pattern (%) of pMDI canisters including Tio: SV010, Tio: Leu and Tiova® (n=3; mean ± SD) (According to ANOVA and Tukey HSD test, for IP deposition: Tio: Leu< Tio: SV010< Tiova® (P< 0.05); for non-respiratory fraction (> 6.4 µm): Tio: Leu< Tio: SV010< Tiova® (P< 0.05); for respiratory fraction (< 3.99 µm): Tio: Leu is significantly greater than Tio: Leu and Tiova® (P< 0.05))

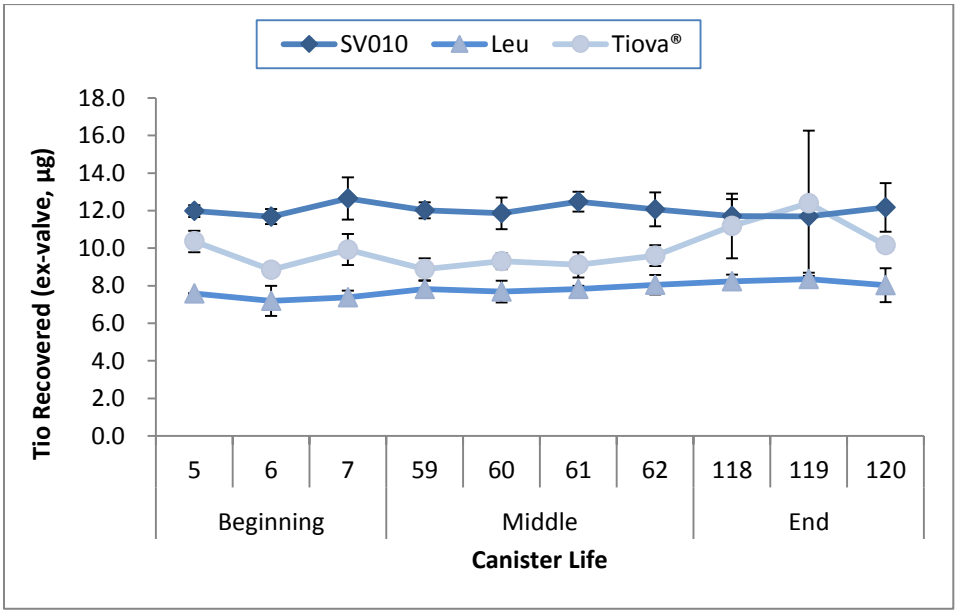

Figure 5. 16 Ex-valve Dose Uniformity of pMDI canisters including Tio: SV010, Tio: Leu and Tiova® (n=3; mean ± SD)

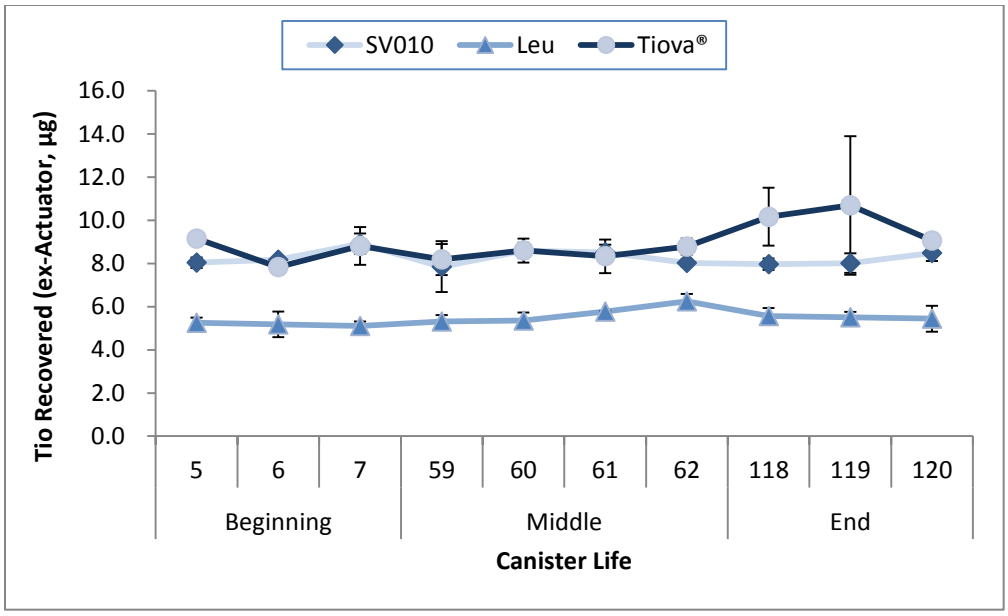

Figure 5. 17 Canister Life Content Uniformity of pMDI canisters including Tio: SV010, Tio: Leu and Tiova® (n=3; mean ± SD)

Based on the above findings, the novel formulation Tio: Leu has shown some advantages over other pMDI products. Different Tio inhalation systems including Tio: Leu (pMDI), Handihaler® (DPI) and Respimat® (SVLI) were then compared with respect to aerodynamic particle size distribution (APSD). As referred to Section 3.5.3, the Handihaler® is operated at the flow rate of 39 L/min, and as referred to Section 5.5.3 and 3.5.4, Tio: Leu and Respimat® are operated at 30 L/min under ambient conditions. Effective cut-off diameters of the NGI stages are calculated based on the flow rate, referred to Section 2.8.3. d50 values of each stage at 30 L/min and 39 L/min has been described in Table 3.5 and Table 3.4 in Chapter 3.

The aerosol characteristic Tio: Leu, Handihaler® and Respimat® formulations is shown in Table 5.15. According to ANOVA and Tukey HSD test, Handihaler® demonstrates the lowest FPF ($P< 0.05$), which is comparable to Tio: SV010 (t-test, $P> 0.05$), whilst Tio: Leu was comparable with Respimat® ($P> 0.05$). Respimat® has the

lowest MMAD (P< 0.05) and Handihaler® has the lowest GSD (P< 0.05). FPDs of Tio: Leu and Handihaler® have no statistical difference, and are double the value of Respimat®, which suggests the dose delivered by one actuation of Tio: Leu (pMDI) is equal to one Handihaler® (DPI) capsule and two inhalations of the Respimat® (SVLI). The aerodynamic particle size distribution for Tio: Leu, Handihaler® and Respimat® formulations is illustrated in Figure 5.18. The slow velocity and fine mist of plume generated by Respimat® lead to a low IP deposition and high MOC deposition, with more than 20% of the emitted dose less than 0.45 µm. The high proportion of extremely fine particles identified in in-vitro test support pharmacokinetic studies where peak plasma concentrations of Respimat® is 35% higher than Handihaler® (van Noord et al, 2009). The high delivery efficiency Respimat® could lead to greater systemic exposure than Handihaler®, and the all-cause mortality caused by Respimat® was considered as a possibility of dose-response effect (Singh et al, 2012). The distribution pattern of Tio: Leu is similar to Handihaler® but with an obvious decrease of IP deposition. Based on these findings, the fine and slow mist of particles generated by Respimat® results in a high fraction of material below 0.45 µm. Handihaler® generates a uniformly dispersed formulation but aerosol performance is reduced and IP deposition is high. The novel Tio: Leu formulation demonstrates similar delivery efficiency, with respect to FPF, as Respimat®, yet avoids generating extremely fine particles.

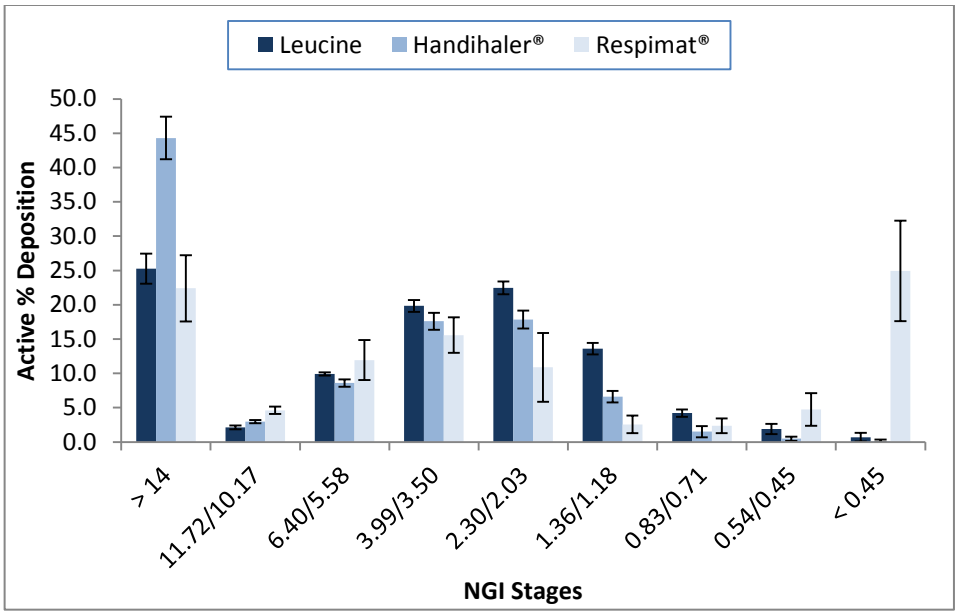

Figure 5. 18 NGI Distribution Pattern (%) of Different Inhalation System Including Tio: Leu, Handihaler® and Respimat® (Tio: Leu: n=3; mean ± SD, Handihaler® and Respimat®: n=9; mean ± SD) (Respimat® demonstrates a low IP deposition and high MOC deposition. Distribution pattern of Tio: Leu is similar to Handihaler®, with an obvious decrease in IP deposition.)

5.6 Conclusion

The Tio Control (Tio only) formulation demonstrated poor aerosol performance with a low FPF and variable metered doses. Under the accelerated conditions (40°C/75%), environment factors adversely influenced performance of the formulation even though moisture is maintained at a low level <300 ppm (Table 5.1). A reduction in drug concentration, indicated by decrease of ex-valve doses at the end of canister life (Figure 5.4), suggests aggregation of drug particles and loss of drug on the surface of device components. The low delivery efficiency is also associated with poor suspension uniformity.

The novel formulation Tio: SV010 (<20 µm) increases the physical stability of micronised Tio particles in HFA 227. Compared with the Tio Control, consistent aerosol performance is demonstrated with increased FPFs and FPDs, and decreased MMAD regardless of the storage period (Table 5.9 and Figure 5.6). Although the moisture level of Tio: SV010 is nearly doubled against the Tio Control at each time point (Figure 5.5), reproducible ex-valve doses (Figure 5.8) indicate good suspension uniformity and constant performance of the VARI® KHFA metering valves up to 3-month storage under accelerated conditions (40°C/75%). However, variable actuator deposition is observed, which is indicated by the increased variation of ex-actuator doses (Figure 5.9), and the novel Tio: SV010 (<20 µm) pMDI also resulted in 32%-52% extra delivery of Tio in ex-actuator doses and so a further deduction of metering doses is required.

The addition of leucine (<20 µm) as the secondary particle also significantly increased the physical stability of micronised Tio particles in HFA 227. Compared with SV010 (<20 µm) (Table 5.16), improved aerosol performance was observed, as FPFs are increased and MMADs are decreased. The addition of leucine (<20 µm)

appears to prevent particle aggregation inside the suspension more effectively and improve the detachment of the drug from leucine particles during atomisation, which is indicated by consistent ex-valve doses (Figure 5.13) and low IP depositions (Figure 5.12). The dose uniformity and reproducible aerosol performance demonstrate good compatibility between the novel formulation Tio: Leu (<20 µm) and hardware components, e.g. 25 µL VARI® KHFA metering valve and 0.25 mm H&T Presspart® actuator. Over 6-month storage under accelerated conditions, although an increase of moisture is observed (Figure 5.10), leucine (<20 µm) can effectively maintain the suspension uniformity and aerodynamic performance.

The optimised formulation of Tio: SV010 and Tio: Leu demonstrated good dose uniformity over canister life, and improved aerosol delivery efficacy with high FPF (39% -55%). Through accelerated stability test, Tio: Leu and Tio: SV010 maintained consistent dose distribution and aerosol performance, e.g. Tio: SV010: FPF (33% - 40%) and Tio: Leu: FPF (53% - 57%). The in-vitro performance of the optimised formulation was further evaluated in comparison with commercialised products. As different aerosol generation mechanisms are applied, different deposition profiles are observed with these products. In the pMDI group, the novel formulation Tio: Leu (<20 µm) demonstrated similar delivery efficiency with Tiova® (Table 5.16), however with improved dose uniformity over the canister life (Figure 5.16 and 5.17). Compared with Handihaler® and Respimat®, Tio: Leu (<20 µm) demonstrated improved aerosol performance compared with its DPI counterpart and comparable FPF with its SVLI counterpart along with avoiding the generation of extremely fine Tio particles (Table 5.16 and Figure 5.18).

5 Conclusions and Future Work

This thesis focused on investigations of particle interaction between suspended drug particles and associated secondary particles in a pMDI suspension using in-vitro methods. An anticholinergic agent, tiotropium (Tio) was investigated under the scope of secondary particulate theory. Tio is a high potency drug, which needs a low medicinal dose, 12.5 µg/ 10 µg/ 6.25 µg as the form of tiotropium bromide. Tio is clinically administrated using a DPI, Spiriva Handihaler® or Braltus Zonda® and a SVLI, Spiriva Respimat®. Though TIOSPIR trial suggested the similar safety and exacerbation efficacy profiles between Handihaler® 18 µg and Respimat® 5 µg, meta-analysis and pooled analyses indicated increased the stroke or cardiovascular adverse events, which were associated with these two delivery systems (Singh et al, 2008; Singh et al, 2011; Wise et al, 2013). The safety concern of inhaled Tio was possibly relevant to systemic absorption or device-related factors which could lead to a higher delivered dose or systemic concentration (Sharafkhaneh et al 2013). As an alternative formulation strategy and delivery system, re-formulation of Tio in a pMDI suspension could provide advantages over the current products such as safety, efficacy, cost and some patient preference for pMDIs. To investigate the effect of the addition of secondary particles, micronised Tio particles were pre-blended and sieved by an established preparation method which was described in Section 2.4 and 2.5. A variety of secondary particulates, e.g. lactose and leucine, were selected and different formulation and hardware parameters were investigated on the influence of suspension stability and aerodynamic performance. The particle interaction, e.g. drug/device, drug/secondary particulates, were demonstrated indirectly by the in-vitro tests, which were described in Section 2.8. Through the optimisation of the formulation and hardware factors, two novel Tio pMDI formulations, Tio: SV010 (<20 µm) and Tio: Leu (<20 µm) were found with certain advantages over the commercialised products including Spiriva Handihaler®, expressed as Handihaler®, Spiriva Respimat®, expressed as Respimat® and a pMDI product, Tiova®.

Initially, this study has revealed a modified and validated RP-HPLC method for the sensitive and quantitative analysis of Tio in low concentrations within the range of 0.126 and 2.000 µg/mL (Section 2.10). However, further method development for analysis of Tio and relevant chemical entities is required for impurity identification and chemical stability study. For the potential of development of Tio in a suspension formulation, the method of solubility test was adapted from Williams et al (1999). The Tio was proven to be practically insoluble in HFA 134a and HFA 227, indicated by an un-detectable level over 6-month storage under the accelerated condition of 40 °C/75 RH (Section 3.5.1). As HFA propellants currently used in pMDI products still have the impact potential on global warming, a phase-out process is coming to the fore (Kigali Amendment, 2016). Replacement with some potential new propellants e.g. HFA 152a in the future will contribute to minimise the environment impact (Noakes and Corr, 2016), and therefore, the possibility of formulating Tio in this novel propellant is also a future research focus. In the preliminary studies, the feasibility of Tio used with co-suspending of a secondary particle, L-leucine, in a suspension system has been verified. Good physical stability of Tio particles was maintained even at a low level of secondary particle concentration. The dose uniformity was improved against Tio alone as the control group over 3 month storage under accelerated conditions of 40 °C/75 RH (Figure 3.5).

Three commercialised Tio products including Handihaler®, Respimat® and Tiova® were analysed as the reference to the novel Tio formulation. The FPF and FPD of Handihaler® was consistent in the range of 39 and 46 L/min (Table 3.9). As a dry powder inhaler with a high intrinsic resistance, the performance of Handihaler® was less sensitive with change in flow rate and the recommended test flow rate for the NGI test was 39 L/min. Respimat® is defined as a small volume liquid inhaler, and the aerosol performance was sensitive to flow rate, humidity and temperature.

The flow rate of 30 L/min under ambient conditions showed a significant increase in FPF and FPD, and a significant decrease in MMAD (Table 3.10 and 3.11), which was recommended for the NGI test. Tiova®, as a pMDI, was evaluated by standard British Pharmacopoeia methods for in-vitro testing. The FPF of Tiova® (Table 3.12) was comparable to Respimat® (Table 3.11), and significantly higher than Handihaler® (Table 3.9). However, the FPD of Tiova® in two actuations, which delivered a medicinal dose of Tio, was double the values of Handihaler® and Respimat®. As there was no available data about clinical or equivalent studies of Tiova®, these findings based on in-vitro testing were used as the reference to our novel pMDI formulations.

Through the screening test of hardware components, the influence of hardware parameters, e.g. canister volume (14/19 mL), canister coating (plain/FCP), valve volume (25/35/50 µL) and actuator orifice (0.25/0.30/0.40/0.46 mm), on the in-vitro performance of the novel formulation was investigated. It was found that aluminium canisters with larger canister volume (19 mL) was observed with reduced ex-actuator doses at end of canister life (Figure 4.2), which could be due to increased adhesion of the drug particles over the canister life. Although there was no difference in FPF between 14 and 19 mL canisters, increased IP deposition suggested a potential of particle aggregation associated with large canister volume (Table 4.1 & Figure 4.1). Furthermore, the novel formulation of Tio: SV003 demonstrated significant improvement in aerosol performance against Tio alone when either canister volume was used, whereas suspension uniformity was more consistent with 14 mL canisters. Although the interaction between the drug and canister surface is somehow formulation dependent, choosing appropriate canister volume is based on the dosing requirement, and a small volume, e.g. 14 mL in this research, is preferable. New canister coating materials were introduced into recent MDI development to overcome formulation challenges such as drug deposition or

degradation (Dohmeier et al, 2009; Stein et al, 2014). In this research, the novel formulation of Tio: Leu was more sensitive to canister coating materials than Tio: SV010, as a significant increase in FPF was identified with Tio: Leu in FCP coated canisters against plain canisters (Figure 4.14 & 4.17). Nevertheless in both formulations, FCP coated canisters showed better through can life performance, which could be due to reduced interaction between Tio particles and the canister inner surface, aluminium, and indicated improved physical stability of the formulation (Figure 4.16 & 4.19) without changing the aerosol performance (Table 4.10 & 4.11). Regarding the valve volume, smaller metering volume i.e. 25 µL showed significant improvement of aerosol performance against 50 µL, indicated by increased FPF and reduced MMAD (Table 4.4). As the metered dose per actuation was kept the same, variable Tio and SV003 particle concentrations were found in 25, 35 and 63 µL KHFA metering valves. Therefore, the initial droplet size and number could also be affected by the ingredient concentration during the atomisation. Furthermore, the less filled volume of propellants in 25 µL valves could shorten the period of the atomisation process, which could lead to more efficient droplet evaporation. However, all the assumptions regarding the initial droplet size need to be further investigated by phase Doppler anemometry which was well described by Myatt et al (2015). When the excipient ratio was decreased from Tio: SV003 1: 25 to Tio: SV003 1: 5, FPF was reduced with an increased in MMAD. This finding suggested the more SV003 was added and the better aerosol performance was achieved but along with the actuator clogging issue. Therefore, actuators with smaller orifice size were recommended for the pMDI suspension with a low drug concentration, and a low ratio of excipients such as secondary particles. Regarding the actuator orifice, the actuator orifice was relevant to the atomisation process, as the smaller orifice size suggested the advantages to avoid drug loss inside the actuators and IP stages, and generate more fine particles. A further study of plume geometry and velocity study by X-ray phase contrast imaging or high-speed laser image analysis will be useful to give a better

understand of this aerodynamic process, which has been well described by Mason-Smith et al (2017) and Chen et al (2017).

Through the screening test of formulation parameters, e.g. propellant type (HFA 134a/227), excipient ratio (1:2.5/1:5/1:10/1:25), excipient type (SV003/SV010/L-leucine) and excipient particle size (<20/<38/<63 μm), the potential particle interaction between Tio and secondary particles were further investigated by in-vitro evaluation. The different physico-chemical properties of HFA 134a and HFA 227, e.g. density, viscosity and vapor pressure, were relevant to delivery efficiency of the novel formulations. The ideal formulation is featured with adequate sedimentation rate and easily to re-disperse. The high density and viscosity of HFA 227 may contribute to the uniform dispersion of Tio particles over the canister life which was indicated by reproducible ex-actuator doses (Figure 4.9). The low vapour pressure of HFA 227 may contribute to greater FPF and FPD, and a less IP deposition as shown in Table 4.6 and Figure 4.7. An aerodynamic analysis of spray patterns, e.g. particle size and velocity, by phase Doppler anemometry and high-speed laser image analysis might be useful to understand the aerodynamic behaviour of different propellants. Another feature was found in this study that the actuator deposition was higher in HFA 134a, and the relevance between actuator deposition and plume geometry study may be useful in the future to give a better understanding of the atomisation process. Furthermore, the highly electronegative mantle of HFAs may lead to strong interactions between the drug and the actuator. It will be helpful to conduct an electrostatic study by an electrical low-pressure impactor (ELPI) to evaluate the relevance between the electrostatic charge of droplets and actuator materials (Chen et al, 2014). The new HFA propellant, HFA 152a, has revealed some advantageous properties against HFA 134a and HFA 227, which will be investigated with the novel Tio formulation in the future.

Several secondary participate, e.g. SV003, SV010 and L-leucine, were investigated in this study. Regarding the excipient ratio, in the range of Tio: SV003 1: 2.5 and Tio: SV003 1: 25, no significant improvement was found in the dose uniformity (Figure 4.21) and the aerosol performance (Table 4.13) of the novel formulation. This could be due to the saturated association between Tio and lactose, which could be further investigated by microscopic measurement using scanning electron microscope (SEM). Another explanation could be due to the poor performance of 50 µL valve that compensated the difference in aerosol performance with different excipient ratio. This assumption is supported by Section 4.5.2 that a decrease in FPF and increase in MMAD is found in Tio: SV003 1:5 than Tio: SV003 1: 25 (Figure 4.4). Furthermore, with a decrease in the valve volume from 50 µL to 25 µL (Figure 4.22), the aerosol performance was also improved with an increased FPF and decreased MMAD. As increases in the excipient ratios enhanced the possibility of device blockage, and therefore, Tio: secondary particle 1:5 was recommended for use with the novel Tio formulation equipped with 0.25 mm actuators. Regarding the excipient type, SV003, SV010 and L-leucine were found to maintain or improve the aerosol performance of the Tio suspension. The reduced agglomeration was also indicated by lower IP deposition which was shown in Figure 4.26. SV010 and leucine were selected as the two most prospective secondary particles, where difference in aerosol characteristics, e.g. FPF and MMAD was also identified (Table 4.16). Therefore, a study regarding surface characteristics and morphology of secondary particles, e.g. shape and roughness, by SEM might be helpful to give a better understanding of their aerosol performance. Regarding the influence of particle size, smaller secondary particles (<20 µm) were found to improve the aerodynamic characteristics of Tio particles indicated by significantly increased FPF and FPD and decreased IP deposition (Table 4.18 and Figure 4.30). With the decrease in particle size, the sieving process also increased the number of fine secondary particles. The generated high ratio of fine secondary particles might decrease the adhesive bond of drug particles to larger secondary particles by

covering the high-energy binding points and facilitate the de-association during atomisation. Furthermore, the strong adhesion of the drug particles to excipients could hinder the de-association process during the atomisation process. Both of these processes are considered related to the generation of fine drug particles. Therefore, further SEM investigation would be useful to understand the association between drug and excipient particles, and gain an understanding of changes of surface characteristics before and after the grinding process. The direct measurement of particle interactions between drugs and secondary particles is accessible by using in situ atomic force microscopy (AFM), which was well described by Rogueda et al (2011).

Based on evaluation of formulation and hardware parameters, two optimised Tio formulations were prepared at 1:5 (w/w) ratio of Tio: secondary excipients (SV010 or leucine) (< 20 µm), which was equipped with 25 µL VARI® KHFA metering valves, 14 mL FCP coated H&T Presspart® canisters and 0.25 mm VARI® actuators. The formulation of Tio: SV010 demonstrated improved aerosol performance, indicated by increased FPFs and FPDs, and decreased MMAD against Tio alone (Table 5.5 & 5.9). Ex-actuator doses were also more reproducible than Tio alone over the canister life (Figure 5.4 & 5.9). These results suggested the addition of SV010 could prevent the potential particle aggregation and improve physical stability of Tio particles. Even through the 3 month storage under accelerated conditions (40°C/75%), dose uniformity and aerosol performance were consistent for Tio: SV010. The formulation of Tio: Leu also demonstrated significant improvement in aerosol performance against Tio alone and Tio: SV010. Up to 6 month storage under accelerated conditions (40°C/75%), there was no significant difference in aerosol characteristics. The ex-valve and ex-actuator doses were also consistent over the canister life, which was also independent of storage period. Both SV010 and leucine were proven to promisingly improve the physical stability and obtain

consistent delivery of the micronised Tio suspension. When compared with the commercialised products consisting of Handihaler®, Respimat® and Tiova®, Tio: Leu demonstrated increased FPF than Handihaler®, which was comparable to Respimat® but with a significant reduction in production of extreme fine Tio particles. The ex-valve and ex-emitted dose uniformity of Tio: Leu were also more consistent than Tiova®. All these findings demonstrated that the optimised novel formulation of Tio: Leu show an improved or comparable aerosol performance with a maintenance of good formulation physical stability over the product shelf life.

Through this early study, it was found that a complicated matrix of factors could contribute to the formulation performance of pMDIs. Particle size fraction, propellant types and actuator orifice were identified with critical influence on the suspension physical stability and aerosol performance. The addition of secondary particles into a suspension formulation indicated potential particle interaction by in-vitro measurements e.g. dose uniformity and aerosol particle size test. Through a screening of variable formulation and hardware parameters, a stable, efficient and promising Tio pMDI system was developed which was comparable to commercialised products, which could provide an alternative, convenient and costless option for patients, and avoid the requirement for critical inspiration flow to achieve a therapeutic effect. The secondary particles were proven to be effective in stabilising the Tio suspension. Based on the assumption, the addition of inert particles in a suspension pMDI formulation could introduce steric obstruction between drug particles to prevent the interaction between active and active or device component to avoid deposition. The in-vitro evaluation has indirectly proven that the potential association and de-association between drug particle sand secondary particles could increase physical stability and aerodynamic behaviour of a suspension system. However, each formulation was manufactured once and reproducibility of this novel approach needs to be further verified by assessing

batch variability. The principles discovered in this early work will also need to be explained based on the future research on physical characteristics of secondary particles e.g. roughness and shape, aerodynamic analysis of droplets, e.g. droplet size and plume velocity, and direct measurement of particle interactions by available technologies and equipment, e.g. SEM, AFM, X-ray phase contrast imaging and high-speed laser image analysis. The evidence suggested the influence of particle size of secondary particles on aerosol performance of the novel formulation but discrete particle size fractions were not used in this research. Furthermore, the experiment design focused on investigation of single formulation and hardware factor, which was lack of evidence of the interactions between these variables.

The common challenges with pMDIs are the lack of reproducibility of the inhaled dose to targeted deposition sites and drug loss through inertial impaction due to the high velocity of airflow. To improve the clinical performance of pMDIs, breath actuated devices, novel formulations e.g. spray-dried lactose, liposome, lipid-based porous particle (Pearl therapeutics) and add-on devices i.e. spacer are also applied to target different group of people such as elders or paediatrics. The alternative approach described in this research by using the secondary particulate technique, is demonstrated to prevent particle growth and caking which may happen during the shelf life of products, and improved the physical stability and aerosol performance of the formulation. The applicability of this novel delivery system to other compounds which have presented difficulties to formulate in HFA systems need to be further investigated. Furthermore, secondary particles were also used as the bulk agent to make easier manufacture and handle. Good uniformity of the blending was observed and it was possible to use the secondary particle as a diluent. With the addition of other excipient e.g. binder or disintegrant, the novel formulation could be compressed into compatible tablets, which was described by

Taylor et al (2015), Tran et al (2016) and Warren et al (2017), and the simplicity of technology could be beneficial for industry manufacturing which simplifies the filling process by avoiding the problems of maintaining homogenised suspensions under high pressure.

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