Effect of Inhalation Manoeuvre Parameters In-Vitro and Ex-Vivo on the Dose Emission and the Aerodynamic Characteristics of Formoterol and Indacaterol from Marketed Dry Powder Inhalers

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A thesis submitted to the University of Huddersfield in partial fulfilment of the requirements for the award of the degree of Doctor of Philosophy

HUDDERSFIELD UNIVERSITY

OCTOBER - 2017

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Abstract

Dry powder inhalers (DPIs) are breath actuated devices relying on the inhalation effort generated by the patients during the inhalation manoeuvre to de-aggregate and disperse the powder formulation and release the dose for lung deposition. The aerodynamic dose emission characteristics vary among patients depending on the maximum inhalation flow (MIF), the inhalation volume (Vin) and the initial acceleration of the inhalation manoeuvre (ACIM). The compendial method for *in-vitro* testing of DPIs uses a vacuum pump to represent the patient's inhalation manoeuvre. Furthermore, the pharmacopoeia recommended the use of a MIF corresponding to 4 kPa pressure drop with a Vin of 4 L. The vacuum pump square wave profile cannot be replicated by humans, and it is by no mean representing the actual patient's inhalation manoeuvre. Additionally, most of the patients and even healthy volunteer are unable to achieve the pharmacopoeia recommended inhalation parameters of MIF and Vin when inhaling through different DPIs.

In this thesis, a wide range of MIFs and Vins were used to investigate the dose emission and aerodynamic characteristics of formoterol and indacaterol using the *in-vitro* methodology. Furthermore, an *ex-vivo* methodology using COPD patients' inhalation profiles and simulated patients' inhalation profiles was used instead of a vacuum pump to study the performance of the indacaterol Breezhaler®. The aim of the study was to initially investigate the effect of MIF and Vin on the dose emission and the aerodynamic dose emission of indacaterol (Onbrez Breezhaler® and formoterol (Aerolizer® and Easyhaler®) over a MIF range of 28.3, 60, 90 and 120 L/min and a Vin range of 0.5, 0.75, 1, 1.5 and 2 L. Secondly, to assess the effect of all three inspiratory parameters MIF, Vin and ACIM on the aerodynamic dose emission of indacaterol Breezhaler® using the *ex-vivo* methodology with both COPD patients' inhalation profiles and simulated patient's inhalation profiles. The formoterol dose emitted after the first inhalation (ED1) from the Aerolizer® significantly increased ($p < 0.05$) with increasing the

MIF and the Vin. Conversely, the dose emitted after the second inhalation (ED2) and the residual amount (RA) decreased significantly ($p < 0.05$) with increasing the MIF and Vin. The ED1 and RA (% of the nominal dose) at low MIF of 28.3 L/min and Vin 0.5 L were 45.91%, 34%. At high MIF 120 L/min and a Vin of 2 L, the ED1 and RA were 70.75%, 22.91%. The formoterol dose emission from Easyhaler® showed that increasing the Vin and MIF resulted in a significant ($p < 0.05$) increase in the total emitted dose (TED), the fine particle dose (FPD) and a significant decrease in the Mass median aerodynamic diameter (MMAD) over a MIF range of 28.3, 60 and 90 L/min with a Vin range of 240, 750, 1500, 2000 mL. The increase in the Vin above 750 mL showed no improvement in the aerodynamic characteristics of formoterol from Easyhaler®. Similarly, the dose emission of indacaterol from Breezhaler® showed that the ED1 significantly increased ($p < 0.05$) with increasing the MIF, while both the ED2 and RA decreased. At a low MIF of 28.3 L/min and a Vin of 0.5 L, the ED1 and RA (% of the nominal dose) were 66.5% and 20.4%. In contrast, at a high MIF of 120 L/min and a Vin of 2 L, the ED1 and RA were 95.2% and 4.1% respectively. The dose strength study of indacaterol 150 µg and 300 µg showed that despite the difference observed in the carrier particle size, size distribution (SEM) and drug to carrier ratio yet both formulations produced a good drug content uniformity with a % CV < 1.8% and 100% dose recovery. The results of the dose emission and the aerodynamic characteristics followed the same trend for both dose strengths. The increase in the MIF and Vin resulted in an improvement in the aerodynamic characteristics of both formulations. The Breezhaler® device was able to generate particles within the extrafine range (≤ 3 µm) when a MIF of 60 L/min and above was used.

Secondly, the performance of 150µg indacaterol Breezhaler® was evaluated using an *ex-vivo* method with COPD patients' inhalation profiles. The results showed the importance of increasing the MIF on the dose emission characteristics of indacaterol dose emitted from Onbrez Breezhaler®. At a low MIF of 28.3 L/min, the TED and FPD were 92.0 µg ± 4.2 and

28.7 µg ±1.6 while at a high MIF of 87.8 L/min the TED and FPD were 125.1 µg ± 1.4 and 45.6 µg ± 0.8. The impact of the Vin and ACIM was difficult to be determined using COPD patients' profiles. Therefore, simulated inhalation profiles (changing one parameter at a time) were used to identify the extent of each parameter. The results of the study showed that increasing both the Vin and the ACIM showed an impact on the dose emission characteristics of indacaterol. An increase in the Vin from 1 L to 3 L resulted in an increase in the TED and FPD with less impact on the MMAD. Similarly, increasing the ACIM from 2 L/s^2 to 8 L/s^2 increased the TED, FPD and more observed effect of the MMAD than the Vin. The results of *in-vitro* studies showed that a MIF of 30 L/min and a Vin of 750 mL were sufficient to de-aggregate the formoterol dose from a reservoir based Easyhaler®. The dose emission from capsule based inhalers Breezhaler® and Aerolizer® require a higher MIF > 60 L/min and patients are recommended to inhale twice in order to empty the capsule especially those with limited lung capacity. The use of an *ex-vivo* methodology is important for identifying the effect of all inhalation manoeuvre parameters. Although the MIF is the dominant factor in the dose emission from DPIs, increasing both ACIM and Vin resulted in an improvement in the aerodynamic characteristics of indacaterol Breezhaler® that explains the recommendations of forceful, deep and prolonged inhalation manoeuvre for patients using DPIs.

Publications and presentations:

Sections of this PhD thesis have been already published in scientific Journals and presented at international conferences as follow:

Journal Publications:

- Abadelah, M., Hazim, F., Chrystyn, H., Bagherisadeghi, G., Rahmoune, H. and Larhrib, H., 2017. Effect of maximum inhalation flow and inhaled volume on formoterol drug deposition *in-vitro* from an Easyhaler® dry powder inhaler. *European Journal of Pharmaceutical Sciences*, *104*, pp.180-187.
- Abadelah, M., Chrystyn, H., Bagherisadeghi, G., Abdalla, G. and Larhrib, H., 2017. Study of the Emitted Dose after Two Separate Inhalations at Different Inhalation Flow Rates and Volumes and an Assessment of Aerodynamic Characteristics of Indacaterol Onbrez Breezhaler® 150 and 300 μg. AAPS PharmSciTech, pp.1-11.

Conference Presentations:

- Abadelah, M., Chrystyn, H., Larhrib, H., (2015) Effect of some inspiratory parameters on the dose emission from the Foradil Aerolizer®. *AAPS Annual Meeting and Exposition and Exposition, Orlando –Florida, USA*

- Abadelah, M., Chrystyn, H., Larhrib, H., (2016) Dose Emission of Indacaterol from an Indacaterol Breezhaler®. *AAPS Annual Meeting and Exposition and Exposition, Denver –Colorado, USA*

- Abadelah, M., Chrystyn, H., Larhrib, H., (2016) Effect of inhalation manoeuvre parameters on the dose emitted from Onbrez Breezhaler® using inhalation profiles of patients with chronic obstructive pulmonary disease (COPD). *27th Drug Delivery to the Lungs Conference. Edinburgh- UK*

- Abadelah, M., Chrystyn, H., Larhrib, H., (2017) "The effect of the inhalation manoeuvre parameters on the indacaterol drug deposition from Onbrez Breezhaler® dry powder inhaler. *PDDS Conference. Valencia - Spain*

Acknowledgements

I would like to express my sincere gratitude to my supervisory team Prof Henry Chrystyn and Dr El Hassane Larhrib for their excellent guidance and endless support and help throughout my PhD. I came to know about many new things and I learnt a lot from them, sincerely big THANK YOU. I would extend my thanks to all technicians at the University of Huddersfield/ Applied science department for their support and guidance especially Dr Richard Hughes and Mr James Rooney.

I am using this opportunity to express my thanks and appreciations to my brother Dr Gaballa Abdalla and his family for their help, sacrifice and support throughout my study in the UK. I am sharing with him and his family great memories will stay with me as long as I am alive. In addition, I would like to thank my friend Dr Ramadan Diryak who has been with me throughout all stages of my study in the UK and we shared all good times and laughs. Finally, I would like to thank the Libyan government for giving me the excellent opportunity to pursue my PhD degree and they have provide me with both academic and financial support.

University of Huddersfield researchers, lecturer and technicians thank you for the excellent atmosphere to complete my PhD.

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List of Abbreviations

∆P	Pressure drop
°C	Degree Celsius
ACI	Andersen Cascade Impactor
ACIM	Initial Acceleration rate of inhalation manoeuvre
AIT	Alberta Idealised Throat
AUC	Area Under Curve
BA-pMDI	Breath actuated- pressurized meter dose inhalers
BMI	Body mass index
BP	British Pharmacopoeia
BRS	Breath Simulator
CFC	Chlorofluorocarbons
cmH20	Centimetre of water
COPD	Chronic obstructive pulmonary disease
DPIs	Dry powder inhalers
DUSA	Dose unit sampling apparatus
EFPD	Extra fine particle dose
EP	European Pharmacopoeia
ERV	Expiratory reserve volume
FEV1	Forced expiratory volume in one second
FPD	Fine particle dose
FPF	Fine particle fraction
FRC	Functional residual capacity
FVC	Forced vital capacity
g	Gram
GSD	Geometric standard deviation
HFA	Hydrofluoro-alkanes
HPLC	High performance liquid chromatography
ICH	International Committee on Harmonisation
ICS/LABA	Inhaled corticosteroids and long-acting β2-agonists
IgE	Immunoglobulin E
IP	Inhalation profile
kPa	Kilopascal
kPa	Kilopascal (1 Pascal= force of 1 Newton per square meter)
L	Litre
L/min	Litre per minute
LOD	Limit of detection
LOQ	Limit of quantification
LPM	Large particle mass
m/s	Metre over second
MDI	Meter dose inhaler
mg	Milligram

MI	Mixing inlet
MIF	Maximum inhalation flow
mL	Millilitre
mL/min	Millilitre per minutes
mM	Millimolar
MMAD	Mass median aerodynamic diameter
MP	Mouth piece
ng/mL	Ninogram per millilitre
NGI	Next generation impactor
nm	Nanometre
PEF	peak expiratory flow
PIL	Patient information leaflet
pMDIs	Pressurized meter dose inhalers
PS	Pre-separator
Q	Flow rate
R	Resistance
RA_{cap}	Residual amount in capsule
RA_{Dev}	Residual amount in inhaler device
RSD	Relative standard deviation
RV	Residual volume
SD	Standard deviation
SMIs	Soft mist inhalers
TEA	Triethylamine
TED	Total emitted dose
Ti	inhalation time in seconds
TLC	Total lung capacity
Tp	Time to peak inhalation profile
TRA	Total residual amount
TRD	Total recovered dose
USP	United states Pharmacopoeia
USP - IP	United States pharmacopoeia induction port
Vin	Inhaled volume
µg	Microgram
µg/mL	Microgram per millilitre
µL	Microliter
µm	Micrometre

Thesis structure

This thesis comprises of 8 chapters:

Chapter 1: Presents a general introduction with an emphasis on the importance of the pulmonary route, types of inhaler delivery devices for drug administration, factors affecting the dose de-aggregation and lung deposition, common methods for determining the dose emission and APSD of inhaled products.

Chapter 2: Provides a review of the respiratory systems, respiratory diseases, types of inhaler devices, factors affecting dose emission from the inhalers, factors affecting dose deposition in the lungs, DPI resistance, and using the literature to highlight the importance of different inspiratory parameters. We also discussed recent modifications in the Pharmacopeia methods to study the APSD of inhaled products.

Chapter 3: Provides a list of laboratory materials and instrumentation that were used in the experimental *in-vitro* and *ex-vivo* studies. It also provides a detailed explanation of the HPLC method development and validation for the quantification of indacaterol and formoterol.

Chapter 4: Presents the *in-vitro* dose emission performance of both formoterol Aerolizer® and indacaterol Breezhaler® after two separate inhalations using different MIFs of (28.3 L/min, 60 L/min, 90 L/min and 120 L/min), and different Vins of (0.5 L, 0.75 L, 1 L, 1.5 L, and 2 L).

Chapter 5: Outlines the *in-vitro* assessment of formoterol aerodynamic dose emission characteristics from an Easyhaler® using dose unit sampling apparatus (DUSA) and ACI at different MIFs and Vins.

Chapter 6: Presents the *in-vitro* assessment of dose strength indacaterol 150 µg and 300 µg dose emission using DUSA after two inhalations and investigation of the aerodynamic characteristics using ACI at different MIFs and Vins.

Chapter 7: Discusses the use of the *ex-vivo* methodology to determine the effect of real life recorded and simulated COPD patients' IPs on the aerodynamic dose emission characteristics of indacaterol Breezhaler®.

Chapter 8: Presents a general discussion and conclusion to the study carried out in this thesis as a whole, and finally making some recommendations for future research.

Chapter 1. General introduction

1.1 Introduction

The inhalation route of drug delivery is considered as one of the most efficient routes of drug administration because this route has been widely accepted for localised treatment of respiratory diseases such as asthma and chronic obstructive pulmonary disease (COPD) (Bisgaard et al., 2001). For local effect, the orally inhaled drugs are delivered directly to the site of action to provide rapid onset of action while using small doses, thus providing fewer systemic side effects (Rau, 2006). The lung has also been used as a portal for systemic delivery due to its large alveolar epithelial absorption area, rich blood supply, and low enzymatic activity when compared to the oral route of drug administration (Patton et al., 2004).

The main types of inhaler available for treating lung diseases can be classified into three main categories: nebulisers, pressurised metered dose inhalers (pMDIs), dry powder inhalers (DPIs), and recently, soft mist inhalers (SMIs). Nebulisers are not true competitors to pMDIs and DPIs for outpatient use as they are bulky non-portable, a large amount of medication is wasted, and treatment times are long (Grossman, 1994, Islam and Gladki, 2008).

The pMDI was the first inhaler device to deliver bronchodilators with a multi-dose capability and consistent and reproducible dosing characteristics, and it was relatively inexpensive (Boyd, 1995, Vaswani and Creticos, 1998). Although pMDIs are popular and the most commonly used inhalers in delivering therapies for asthma and COPD, they have some major drawbacks, such as poor coordination between dose actuation and patient inhalation. Furthermore, the fast-moving plume aerosol results in more dose being deposited in the oropharynx even with good inhalation technique (Crompton, 1981, Purewal and Grant, 1997, Dolovich, 1999, Lavorini and Corbetta, 2008, Roche et al., 2013).

Higher lung deposition and lower oropharyngeal deposition were achieved with some new formulations using a drug solution in hydrofluoroalkane (HFA) HFA-134a propellant compared to a drug suspension. An example of a drug solution is beclomethasone dipropionate-HFA-134a, ethanol (Qvar, TEVA). The high vapour pressure of HFA coupled with the small droplet size from the pMDI solution is the driving force to enhance peripheral and total lung deposition. The correct pMDI technique involves firing the pMDI while breathing in deeply and slowly and then following inhalation with a breath-holding pause to allow particles to sediment on the airway surfaces (Newman, 2005, Gibaldi, 2007, Usmani, 2015).

The complexity associated with pMDI formulations (*e.g.*, poor solubility of surfactants in HFA propellants, the use of co-solvent, partial solubility of some drugs in HFA, stability issues for fragile molecules, a small amount of the drug can be delivered due to the small volume of the metering chamber), the cold Freon effect, the misuse of pMDIs, poor understanding of how to use a pMDI, and some minor greenhouse effects associated with HFAs may become an issue in the future. All these factors have pushed the inhalation scientists to think about a safe and viable alternative to deliver a wider range of drugs for both local and systemic effects while still delivering a large dose of drugs such as tobramycin. DPIs have the potential to overcome all the above problems. All currently marketed DPIs are breath-activated and do not require propellants to generate the aerosol. It is the interaction of the patient's inspiratory flow with the resistance of the inhaler device that causes de-aggregation of the powder formulation to deliver the drug to the lungs (Clark and Hollingworth, 1993, Chrystyn, 2003, Telko and Hickey, 2005). Each DPI has its unique internal resistance; the difference in their resistance is due to the internal design of DPIs (Steckel et al., 1997, Chrystyn, 2003). DPIs can be classified into three main categories: low, medium, and high resistance according to the pressure drop generated across the inhaler

device when inhaling through a DPI. The turbulent energy inside the inhaler is represented by the pressure drop (Δp) that occurs during the inhalation manoeuvre. The relationship between the intrinsic resistance and the inhalation flow has been defined as $\sqrt{\Delta P} = Q*R$, where the pressure drop is directly proportional to both the flow rate (Q) and the intrinsic resistance (R) (Clark and Hollingworth, 1993, Dal Negro, 2015). The flow rate required for a set inspiratory effort through a low resistance device will be faster when compared to a high resistance device. The fast inhalation flow will result in high turbulent energy, and this will result in an improvement in the quality of the emitted dose. All commonly used DPIs show flow rate dependency of dose emission, and the extent of flow rate dependency varies from device to device (Chrystyn, 2009).

In fact, high resistance devices show less flow rate dependency than low resistance devices. The former was developed to provide consistent dosing irrespective of the inhalation flow and to limit the oropharyngeal deposition caused by the high velocity of the inhaled particles and its associated side effects, especially with some inhaled drugs such as corticosteroids. Each DPI requires a minimum threshold inspiratory effort to de-aggregate the formulation and to achieve particles within the respirable range $\leq$ 5 μm. High resistance devices such as Handihaler® and Easyhaler® would require less flow than low resistance devices such as Breezhaler® and Aerolizer® (Pedersen et al., 1990, Assi and Chrystyn, 2000, Al-Showair et al., 2007b, Azouz et al., 2015b, Price and Chrystyn, 2015). ERS/ISAM task force state that "patients using DPIs should inhale as long, deep and for as long as they can in order to maximise the dose emission and lung deposition"(Laube et al., 2011). The forceful inhalation from the start of the inhalation manoeuvre (fast acceleration) was found to be important for the promotion of the dose de-aggregation and therefore in increasing the benefits from every single dose (Everard et al., 1997, Chrystyn and Price, 2009).

Patients' failure to use their DPI device correctly can result in a reduction in the dose reaching the lungs. Studies have shown that approximately 40% ± 20 of patients with asthma and COPD have experienced a problem in using their inhalers correctly (Rau, 2006, Bartolo et al., 2017, Chrystyn et al., 2017). Regardless of the inhaler device used, patient misuse is common, and more errors are observed with DPIs than with pMDIs. For good airway disease management, it is very important for patients to use their device correctly, as failure to do so will result in poor asthma and COPD control (Lavorini et al., 2008, Noyce, 2017). The inhaled dose efficacy depends on the aerodynamic characteristics of the emitted dose; the latter is dependent on the inter-relation between the formulation, the inhaler device used, the inter- and intrapatient variability, and the inhalation technique (Bonini and Usmani, 2015, Ibrahim et al., 2015).

In the Pharmacopoeia method for the *in-vitro* dose emission testing of DPIs, a vacuum pump is used to generate an inhalation flow with a constant MIF that corresponds to a 4 kPa pressure drop with an inhaled volume of either 2 L (FDA, 1998) or 4 L (USP, 2014). The airflow square wave profile generated by the vacuum pump cannot be replicated by a human and is not representative of the bell-shaped patient inhalation profiles (IPs) in real-life use (Copley et al., 2014, Azouz et al., 2015b).

A clinical study showed that the majority of patients with asthma and COPD could neither achieve the required flow rate corresponding to a pressure drop of 4 kPa across the inhaler nor achieve the 4 L Vin (Azouz et al., 2015a, Chrystyn et al., 2015). In the routine use of the inhalers, patients' inspiratory parameters vary due to different factors caused by the size of the lungs, which in turn are related to the age and gender in addition to the degree of obstruction in the airways (Stocks, 1995, Al-Showair et al., 2007b). Not all patients inhale in the same manner, and each patient will achieve different respiratory parameters depending on the

education they receive on how to use their inhaler properly and their lung capacity. For instance, patients with COPD and asthma are unable to achieve a 4 L inhaled volume across the device. It has been reported that patients with asthma and COPD inhaling through DPIs were able to achieve an average inhaled volume of 2 L (Hawksworth et al., 2000).

The IPs differ according to the DPI in use. For example, a study of blister- and reservoir-based DPIs showed that dose de-aggregation occurs immediately at the start of the inhalation manoeuvre, whereas capsule-based devices required a greater inhalation time to empty the dose effectively (Chrystyn and Price, 2009). For DPIs, three important inhalation manoeuvre parameters were identified to be important for drug aerosolisation and deposition to the lungs, namely MIF, Vin and ACIM (Laube et al., 2011, Bagherisadeghi et al., 2017). Although the impact of the MIF has been extensively studied since the emergence of DPIs, researchers have only recently realised the importance of the Vin and ACIM, especially with the technical advances in the inhalation science with patients' inhalation recording and the breath simulators. Recently, several studies have highlighted the importance of the Vin and ACIM in the overall aerodynamic dose emission from DPIs (Yakubu et al., 2013, Chrystyn et al., 2015, Buttini et al., 2016a, Bagherisadeghi et al., 2017). The studies showed that increasing the Vin and ACIM resulted in high fine particle dose (FPD) and a reduction in the mass median aerodynamic diameter (MMAD) of the inhaled particles.

The use of a vacuum pump does not replicate the patient's IP in real life, which results in a poor *in-vitro in-vivo* correlation. Researchers have recently introduced a new *in-vitro* method that is more representative of a patient's real-life use. Breath simulator-based methods (BRS) have been introduced recently to study the aerodynamic characteristics of the inhaled products using previously recorded patient IPs (Copley et al., 2014). This was termed *ex-vivo* methodology, as the profiles are generated *in-vivo* and replayed *in-vitro* using the breath

simulator.The BRS is connected to the Andersen cascade impactor (ACI) by using a mixing inlet (MI) between the Alberta idealised throat (AIT) and the ACI. Such *ex-vivo* methodology is becoming popular for testing the performance of orally inhaled products (OIPs). The use of real patient IPs measured during routine operation of the DPI has therefore enhanced the investigation of the aerodynamic characteristics of the emitted dose from different marketed DPIs (Nadarassan et al., 2010, Olsson et al., 2013, Chrystyn et al., 2015, Bagherisadeghi et al., 2017).

In this thesis, *in-vitro* and *ex-vivo* methodologies were used to assess the effect of MIF, Vin, and ACIM on the dose emission and the aerodynamic characteristics of an emitted dose of formoterol and indacaterol. Three commercial DPI devices were used, namely, Onbrez Breezhaler® two strengths (150 µg and 300 µg indacaterol), Easyhaler® (12 µg formoterol), and Foradil Aerolizer® (12 µg formoterol). Patient IPs and simulated IPs were used in this thesis to study aerodynamic dose emission characteristics from 150 µg indacaterol Breezhaler®.

1.2 Aims and objectives

1.2.1 Aims

The aims of this study were:

- To investigate the effect of MIF and Vin on the dose emission and the aerodynamic characteristics of the emitted dose of both formoterol and indacaterol from Aerolizer®, Easyhaler®, and Onbrez Breezhaler® using *in-vitro* methods
- To assess the effect of the three main inhalation manoeuvre parameters in the patient IPs (MIF, Vin, and ACIM) on the aerodynamic characteristics of the emitted dose of indacaterol from Onbrez Breezhaler® using COPD patients' IPs and simulated COPD patients' IPs.

1.2.2 Objectives

The objectives of this study were:

- To measure the dose emission and dose delivery uniformity (n=16) of formoterol from a Foradil Aerolizer® powder formulation at different MIFs (28.3, 60, 90, and 120 L/min) and Vins (500, 750, 1000, 1500, and 2000 mL) after one and two inhalations.

- To evaluate the effect of MIF and Vin on the dose emission and aerodynamic particle size distribution (APSD) of formoterol emitted from an Easyhaler® using different MIFs and Vins.

- To measure the dose emission and dose delivery uniformity (n=16) of indacaterol from an Onbrez Breezhaler® at different MIFs (28.3, 60, 90, and 120 L/min) and Vins (500, 750, 1000, 1500, and 2000 mL) after one and two inhalations.

- To assess the effect of dose strength (150 µg and 300 µg) on the dose emission and APSD of indacaterol using different MIFs and Vins after one and two inhalations.

- To determine the amount of indacaterol delivered from an Onbrez Breezhaler® using COPD patients' IPs using *ex-vivo* methodology.

- To use *ex-vivo* methodology with simulated patients' IPs to identify the effect of each inspiratory parameter (MIF, Vin, and ACIM) on the aerodynamic dose emission characteristics of indacaterol Breezhaler®.

Chapter 2. Literature review

2.1 Respiratory system

The respiratory system of a human being consists of a branching system, whereas 23 bifurcations of air channels comprised the whole system, starting from the mouth to the tiny clusters of alveoli. It is responsible for the supply of oxygen to the blood through inspiration. Therefore, it will be distributed to various parts of the body, and in the process of oxygen supply carbon dioxide is removed by the reverse mechanism of expiration, the gaseous exchange is taking place in the alveoli (Bisgaard et al., 2001).

2.1.1 The Lungs

The lungs are the main organ of the respiratory system. It has a spongy texture with a cone-like structure. The lungs are different in the structure, whereas the left lung is comprised of only two lobes comparing to three lobes to the right lung. Moreover, the left lung is smaller due to the presence of the heart (Waldron, 2007).

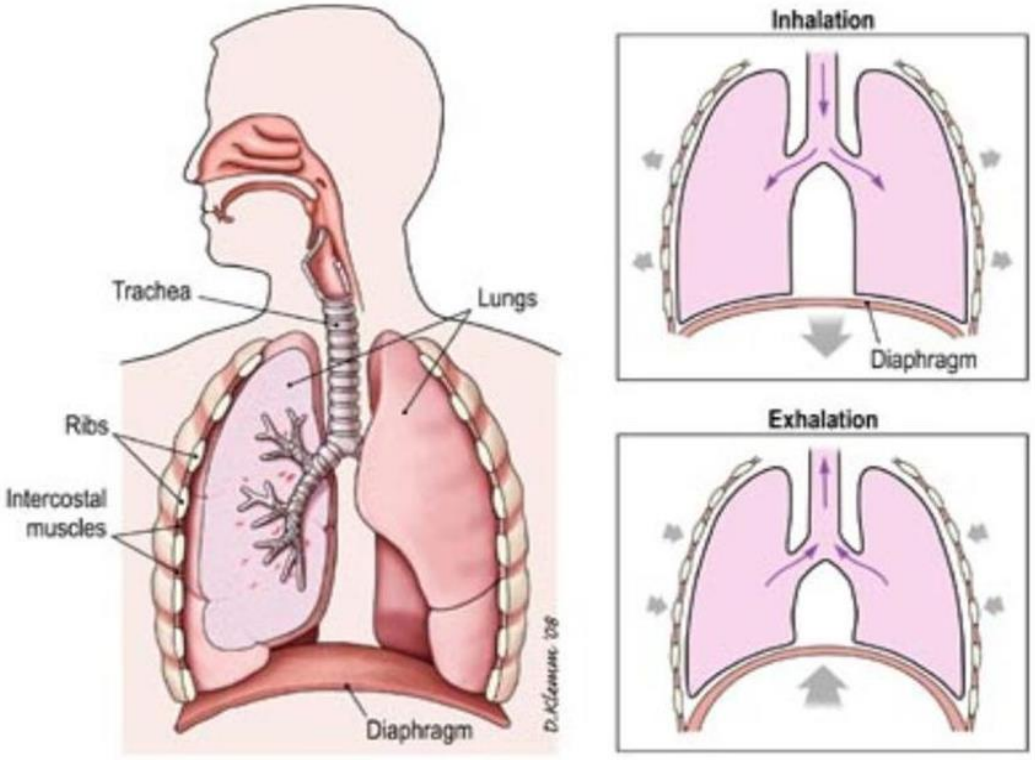

Figure 2.1: Lungs inhalation and exhalation mechanism (Marieb and Hoehn, 2007)

The base of the lungs located on the diaphragm on a side, while the top is located in the root of the neck. Each lung is covered by a membrane called pleura. The pleura is divided into two membranes, the one who is covering the external surface of the lungs known as the visceral pleura, and the lining membrane of the thoracic cavity and it is known as parietal pleura. The intrapleural fluid which acts as a lubricant to reduce the friction between layers during breathing fills the space between the two membrane layers. The lungs have a unique elasticity, whereas the lungs inflate during the inspiration and a reverse mechanism of exhalation deflate the lungs. This elastic features of the lungs due to the elastic fibres and collagen which form the lungs tissue (Marieb and Hoehn, 2007, Ward et al., 2010).

The contraction and the relaxation of the lung tissue, a diaphragm and chest rib during the inhalation and exhalation is shown in Figure 2.1. The mechanism of the inhalation is taken place due to subsequent contractions in the diaphragm, then it moves downward, and finally, the muscles between the ribs which are known as the inter-costal contract, which leads to the ribcage is being pulled outwards. The air is then drawn into the lungs by the negative intra-thoracic pressure as result of the thoracic cavity volume is increased. During the expiration mechanism, the reverse mechanism is taken place, whereas, the inter-costal muscle relax, the diaphragm return to its original anatomical position, therefore, pulling the ribcage down and leading to the lungs to be contracted. These actions subsequently reduce the volume of the thoracic cavity, and that enforce the air to be exhaled out of the lungs (Waldron, 2007, Rogers, 2010, West, 2012).

2.1.2 The respiratory airways

Respiratory organs and tissues are responsible for gaseous exchange; however, air from outside the body passes through those organs, and subsequently, oxygen and carbon dioxide exchange takes place (Figure 2.2). These organs and tissues are called the respiratory system to generate energy and perform metabolic functions; the human body requires oxygen, which can be breathed into the body during inspiration. Carbon dioxide, which is a waste gas produced due to chemical reactions within cells during the energy production process, can be removed or cleared from the body during expiration. CO_2 in high percentage is harmful to the cells (Bisgaard et al., 2001).

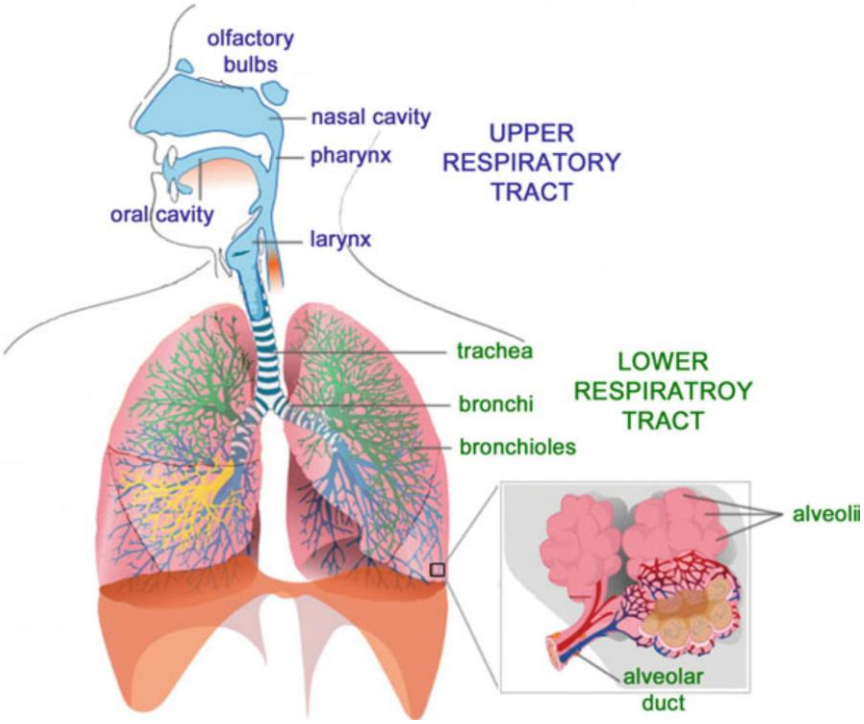

Figure 2.2: Schematic diagram of upper and lower respiratory tract (Bisgaard et al., 2001)

The respiratory system can be classified according to its function into the upper and lower respiratory tract. The upper respiratory tract includes nasal passages, pharynx and larynx, which are lined by respiratory ciliated epithelium, and secrete mucus, whereby cilia clear breath from unwanted particles or dust from airways and mucus secretion are propelled to the pharynx and can then be swallowed or coughed up.

The main functions of the upper respiratory tract are moistening, heating and filtering of the air entering the respiratory system. The condition of atmospheric air is normally 20 ^{o}C and ~ 50% moisture, when inspired the air is heated to the desired temperature of 37^{o}C and humidity of 99% in the upper respiratory tract (Waldron, 2007). The lower region of the respiratory tract starts from the trachea a rigid tube that consists of cartridge mainly to the alveoli is responsible for the gaseous exchange and respiration (Bisgaard et al., 2001, Hickey, 2003).

2.1.3 Conducting airways

It controls the exchange of atmospheric air in and out on a regular basis into the respiratory organs and tissues. The conducting airways are like an inverted tree in shape and form very complex tubes and branches. Moreover, these branches and tubes are narrower, thinner and more in number as they penetrate deeper into the lungs. The upper part of conducting airways, the trachea and larynx, carry air to and from the lower parts, whereas by the middle of the thorax it splits into right and left main bronchi, each of which controls the air feed to one of the lungs. Anatomically, the trachea (windpipe) is a muscular tube that is supported by a C-shaped cartilage ring that protects it and prevents it from collapse (Hickey A, 2005, Ward et al., 2010). A bronchial wall become thinner with the subdivision, and by level 16 of branching respiratory zone starts, and gaseous exchange takes place (Figure 2.3). On the other hand, airways from the trachea and the terminal bronchioles down to branch 16 are all known as a conducting zone of airways. It is called a conducting zone, as no gaseous exchange occurs within these branches due to lack of alveoli; this area is often anatomically known as dead space.

The conducting airways have two main functions; firstly, the delicate structure of the alveoli is protected by warming and humidifying inhaled air to avoid excessive exposure to dry cold

air, and secondly, to allow inhaled air to reach the more distal region where gas exchange occurs. Terminal bronchioles, which are down to distal parts of respiratory airways, branch into bronchioles, which in turn branch to tiny bronchioles. These ultimately become alveolar ducts, thin-walled sacs called alveoli, which are responsible for the respiratory gaseous exchange. Thus the zone from respiratory bronchioles down to alveoli is known as the respiratory zone (Weinberger et al., 2008). The lung airways branching system undergoes 23 bifurcations, whereas the airways surface area is less than the enormous surface area of the alveoli, which allows the efficient gas exchange to take place. In contrast, the surface area of human airways is approximately 2.5 m^2, while it is approximately 80 and 140 m^2 for the Alveoli and Alveolar wall respectively (Widdicombe and Wine, 2015).

Table 2-1: The respiratory airways' branching and dimensions (Weinberger et al., 2008)

Airways number and dimension			
Name of airway	Number	Diameter (mm)	Cross-sectional area (cm^2)
Trachea	1	25	5
Main bronchi	2	11-19	3.2
Lobar bronchi	5	4.5-13.5	2.7
Segmental bronchi	19	4.5-6.5	3.2
Subsegmental bronchi	38	3-6	6.6
Terminal bronchi	1000	1	7.9
Terminal bronchioles	35000	0.65	116
Terminal respiratory bronchioles	630.000	0.45	1000
Alveolar ducts and sacs	4×10^6	0.4	17.100
alveoli	300×10^6	0.25-0.30	700.000

The most popular model that describes the branching system of the respiratory airways (see Figure 2.3) is known as Weibel model (Weibel, 1963). It has been shown to help describe the branching and zones in the human respiratory system. It illustrates the branching of lungs and respiratory airways, which are divided into two regions or zones. Firstly, the conducting airways zone is down to branch 16 bifurcation, and the respiratory zone goes from branches 16 to 23. The transitional zone is the region that connects the two zones or regions; it consists of generation 17 through to 19 generation of the branching (Muttil et al., 2009). Muscles and receptors that are responsible for the bronchodilator are found in the conducting zone of airways; nevertheless, a respiratory airway inflammation is predominate in the conducting zone and is also present in parts of the respiratory zone.

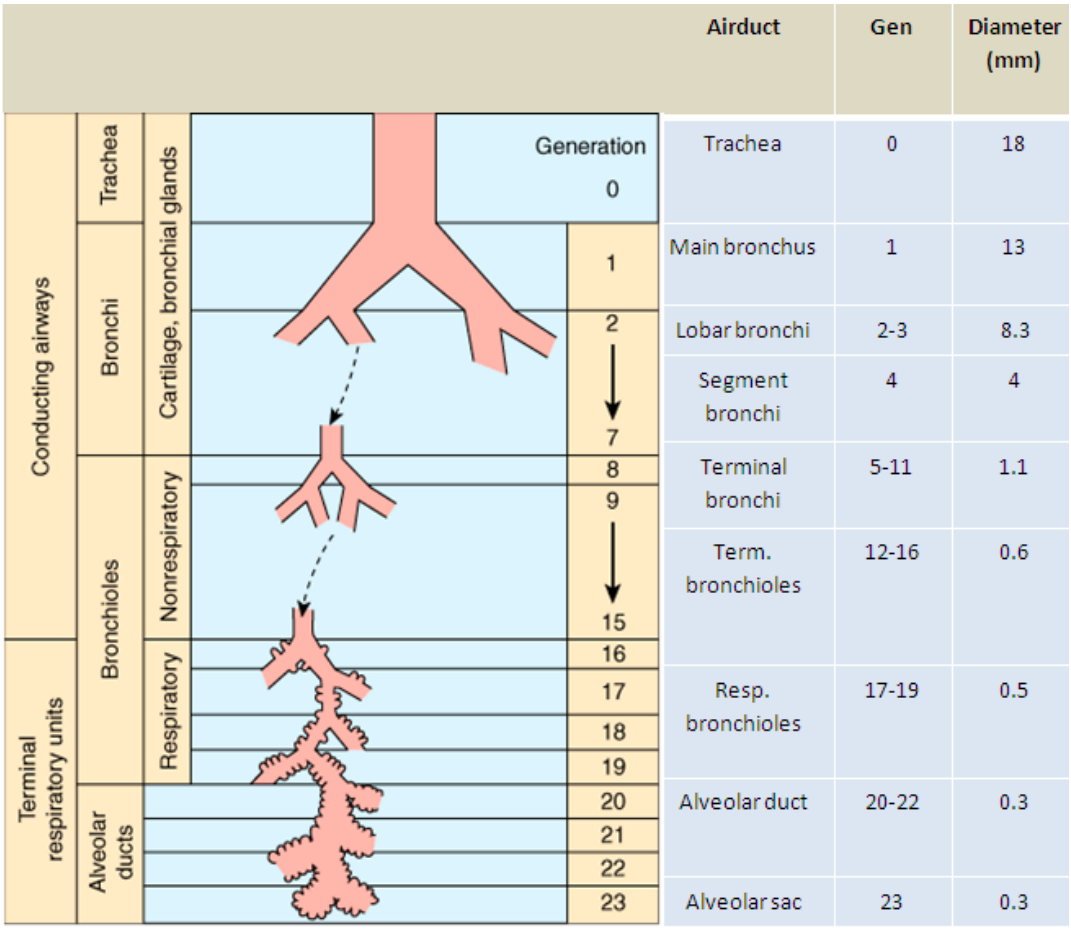

Figure 2.3**: Schematic diagram of airways Branching Model (Weibel, 1963)**

2.2 Lung function indices, volume and capacity

The data of the inspired and expired lung volume measured for the patients using a spirometry can be a useful tool to determine the severity of the pulmonary disorder the patients have, then it can also be characterised and quantified (Quanjer et al., 1993, Wanger et al., 2005, Hickey, 2006). The inhaled and exhaled volume is only forming part of the total lung volume during the normal breathing pattern.

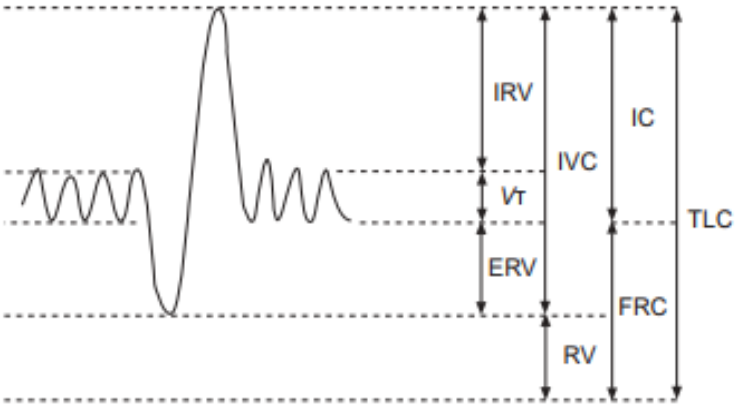

Figure 2.4: Static lung volumes and capacities based on volume – time spirogram (Wanger et al., 2005)

The pathophysiological status of the patient's lung can be determined using the lung volume and capacities information. The spirometry is used for the measurement of the air inhaled and exhaled which can be characterised by tidal volume (TV), inspiratory reserve volume (IRV), expiratory reserve volume (ERV), vital capacity (VC) and the inspiratory capacity (IC). The definition of these parameters is given below.

The volume of air remaining in the lungs, which are known as residual volume (RV) and functional residual volume (FRV) after expiration, can be estimated using both gas dilution methods and body plethysmography. The volume of the air that can be processed by the respiratory system of a healthy adult is approximately 10 to 20 m^3. The forceful vital capacity manoeuvre measurement provides important information and data. These data are the forced

vital capacity (FVC), forced expiratory volume in 1 second (FEV1) and the peak expiratory flow rate (PEFR). The area at which gaseous exchange in the lungs takes place is approximately 120 – 160 m^2; it is supplied with 2000 km of blood capillaries. For each breath at rest, a volume of 500 mL tidal air is inspired and expired from the lungs. However, this number will increase during heavy work. Also, the number of breaths of an adult during heavy work is a triplicate to the number of breath at rest which is 12 times every minute (Hickey, 1996, Hinds, 2012). The definition of the different lung volume parameter was deduced from the lung capacity measurement shown in Figure 2.4. These parameters are as follow:

- Tidal volume (TV): during a normal breath cycle, it is the volume of air that is inhaled and exhaled of the respiratory tract.
- Inspiratory reserve volume (IRV): after normal tidal inspiration, the IRV is the maximum volume that can be inspired at the end of normal inspiration or tidal inspiration.
- Expiratory reserve volume (ERV): is the additional maximum volume that can be exhaled out of the lungs at the end of tidal expiration.
- Inspiratory Capacity(IC): The maximum inspired volume of air after normal tidal expiration [IC= TV+IRV]
- Residual volume (RV): is defined as the remaining air in the lungs after a maximum expiratory effort
- Functional residual capacity (FRC): FRC is the air volume retained in the lungs after a normal tidal expiration [FRC= ERV +RV]
- Vital capacity (VC): is defined as the volume of the air that can be exhaled from the lungs after maximum inspiration [VC= IRV + TV +ERV]

- Total lung capacity (TLC): after an inspiratory effort, TLC is the air volume in the lungs, it can be calculated from the volumes as follow [TLC= IRV+TV+ERV+RV]

Pulmonary function tests (PFTs) are considered a useful tool to the clinicians to determine whether the obstructive or restrictive illness is present and identify the defect location. It comprises of three different tests including spirometry, lung volume and diffusion capacity. The spirometry is used to determine the presence and the obstructive severity defect in the airways. The scooped pattern flow volume loop is a main marker (Figure 2.5), then using numerical values FEV1 and FVC. The ratio of FEV1/ FVC < 80% indicate an obstructive defect, in addition, obstruction severity can be determined when compared to predicated lung volumes (Pellegrino et al., 2005).

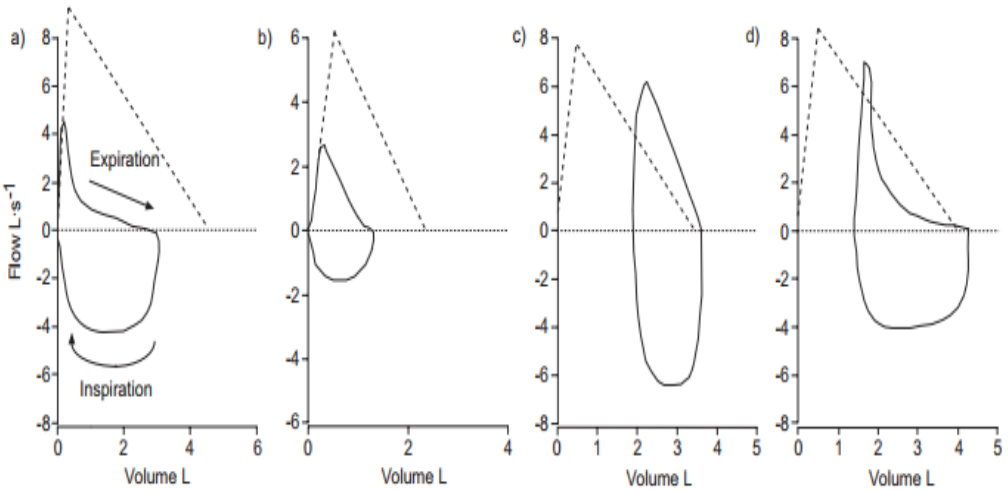

Figure 2.5: a, b). Examples of obstructive pulmonary defect characterised with low FEV1. c). typical restrictive defect characterised with low TLC. d). obstructive and restrictive defect low TLC and FEV1 (Pellegrino et al., 2005).

Diffusion capacity (DCLO) test is used to determine the gas transfer function of the lungs. It might be falsely decreased with patients have severe restrictive and obstructive diseases. It can be seen with intrinsic restrictive conditions such as Sarcoidosis and Amyloidosis, and extrinsic restrictive conditions such as obesity and neuromuscular disease. Factors such as obesity and race affect PFT parameters reading (Al Ghobain, 2012, Belacy et al., 2014).

2.3 Respiratory clearance mechanism, impact on dose absorption

The respiratory tract fundamental function is to filter the inhaled air before reaching the delicate compartments of the respiratory system. There are different mechanisms to clean the air inhaled. The clearance mechanism of the mucociliary layer is one of the important mechanisms to remove mucus and foreign particles deposited in the respiratory airways (Figure 2.6). Its mechanism involves the transporting of the mucus with deposited particles with the help of the ciliated cells in the pulmonary epithelium. The particles trapped in the mucus will be either swallowed or coughed up following the proximal transportation by the cilia (Stahlhofen et al., 1990, Olsson et al., 2011).

The goblet cells are responsible for the secretion of mucus, which consists of both watery, and gel phases (which contain mucin and glycoproteins). The production of mucus in healthy individuals is approximately 10 to 20 mL per day. However, in patients with chronic bronchitis, this volume can be increased up to 10 times, which thereby causes damage to the cilia lining of the airways (Toremalm, 1960).

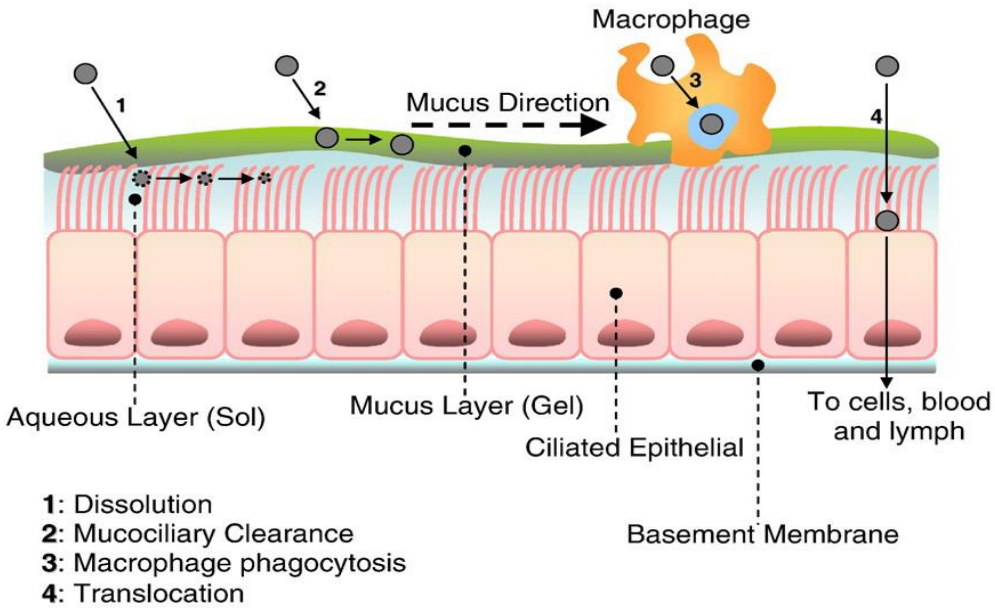

Figure 2.6: Schematic diagram shows the different clearance mechanism of the inhaled particles within the respiratory airways (Hussain et al., 2011)

The respiratory defence mechanism acts to prevent foreign particles entering the respiratory system. Similarly, inhaled aerosol particles are eliminated from the respiratory system by the defence mechanism to avoid the interaction with lung cells, that leading to poor respiratory disease control and therapy failure (Labiris and Dolovich, 2003, El-Sherbiny et al., 2015).

Inhaled particles clearance mechanism depends on the deposition site of the particles within the lungs. The mucociliary escalator acts to eliminate particles in the tracheobronchial tree, in contrast, particles in the lower alveolar region cleared by the mean of phagocytosis process of the macrophages (El-Sherbiny et al., 2011). It has been reported that particles in the range of 1.5 to 3μm reach the alveolar region are engulfed by the macrophages (El-Sherbiny et al., 2015). Furthermore, large porous particles will reach the alveolar region of the lungs due to their aerodynamic size; however, the clearance of these particles is reduced due to their large geometric diameter (Edwards et al., 1997, Olsson et al., 2011). Mucociliary clearance (MCC) rate found to be affected by different factors such as age, airways diseases (asthma and COPD) and smoking (Vastag et al., 1986, Messina et al., 1991, Olsson et al., 2011).

The inhaled drug particles (≤ 5μm) should be able to pass through the defence system of the airways to reach the airways and have a therapeutic effect (Tena and Clarà, 2012). Smaller particles will have more velocity to escape through the mucus and reach the peripheral region of the airways (Stahlhofen et al., 1990, El-Sherbiny et al., 2015). It is reasonable to assume that total lung deposition between healthy volunteer and patients with common asthma and COPD does not contrast to a clinically significant degree for any available product in the market. Nevertheless, the difference will be in the regional distribution of the total lung dose, whereas in a patient with the mentioned condition the cross-sectional area will be smaller and therefore, the inhaled drug particles will deposit more in proximal regions of the lungs (Thorsson et al., 1998).

2.4 Respiratory diseases

The respiratory system common diseases are asthma and the chronic obstructive pulmonary disease (COPD). The causes of these disorders vary from genetic factors, infections and irritating pollutants particles. The inhalation therapy is considered effective in the treatment of some local respiratory disorders; these treatments are very effective at low doses with less systemic side effects when compared to another route of drug administration (Nokhodchi and Martin, 2015).

2.4.1 Asthma

It is a chronic disorder occurs due to inflammation of the lungs airways, which causes the constriction of the pulmonary airways and becomes more sensitive to certain triggers such as dust, pollen and viral infections. This inflammation causes the lungs airways to spasm and narrowing, then excessive production of mucus, which results in the symptoms that accompanied asthma. Many cells and cellular element play a role, specifically, T-lymphocytes, macrophages, neutrophils and mast cells, *etc*. it is characterised by recurrent wheezing, breathing difficulties, chest tightness and coughing which normally happen in the early morning or at night. These symptoms are combined with a different degree of airflow obstruction which depends on the severity of the inflammation; this obstruction is reversible by using treatment and sometimes spontaneously (Masoli et al., 2004, Waldron, 2007, GINA, 2016). A chronic pathological condition affects people all over the world. It has been shown that 334 million people have asthma and in the next 20 years; this number is predicted to increase by 1/3 (~ 100 million), children are the most prevalence category (Lavorini and Corbetta, 2008, GINA, 2016). In the UK, asthma patients are around 5.4 million people, who are currently receiving treatment for the disease. Also, 1 in 11 children (1.1 million) in the UK has asthma, compared to 1 in 12 adults (4.3 million). In the recent data published by the NHS in 2014, 1216 people died from the complications of asthma. The National Health Service

(NHS) is spending a total of 1billion sterling pounds per year to cover the cost of emergency room visits, treatments and hospitalisation (AsthmaUK, 2016).

2.4.1.1 Causes of asthma

It can be inherited due to a genetic problem, or passed from the parents on their genes; some patients who have asthma have no family history of the disease. The fundamental causes that lead to asthma are not well known yet. However, the genetic disposition to atopy which stimulates the immune system to generate an immunoglobin IgE antibody to a certain element in the environment is considered as risk factor for the developing of asthma disorder (Schieken, 2002, Holgate, 2010).

In a patient with asthma, lungs airways are more sensitive to irritation than normal. The irritation of the airways can happen by exposure to triggers such as pollen, grass, mould, dust mites and trees, *etc*. This type of asthma is affecting both adult and children, but it is more common in children. These allergens can cause sneezing, wheezing, itchy eyes and a runny nose.

The viral infections and recurrent sinus inflammation can cause asthma. The well known rule of thumb is that environment where people live can determine whether they have asthma or not. The risk factors that are associated with the environment are:

- The exposure to fume from the exhaust system of cars, trucks, *etc*.
- Smoking during pregnancy or infancy
- Viral infections during infancy
- Exposure to inflammation triggers particles such as pollen, food preservatives and drugs, paint odour, cleaning product and temperature and weather changes, *etc*. (American Thoracic Society (American Thoracic Society, 2013).

2.4.1.2 Asthma pathogenesis

Asthma can be classified into two main types depending on the causes and the pathogenesis of each type. The main two types are the extrinsic (atopic) and intrinsic (non-atopic), whereas as atopic asthma occurs when the lungs are exposed to an external factor or allergen which trigger the inflammation in the lungs such as dust, pollen, *etc*. In non-atopic asthma, no external factors associated affecting the lungs (Holgate, 2008, Holgate, 2010, Nakagome and Nagata, 2011).

Atopic asthma normally associated with childhood and it is related to specific triggers that lead to an inflammation of the lung airways. Moreover, family history, eczema, and rhinitis are associated with this type of asthma. The pathogenesis of atopic asthma can be described as a sequence of pathways following the trigger of the lungs smooth muscles by an external factor such as an allergen, or known as environmental stimuli as shown in Figure 2.7. The release of immunoglobulin E (IgE) from the B-lymphocyte upon the exposure to a trigger, lead to series of inflammatory responses. The binding of the IgE to inflammatory cells to release chemical mediators. The main inflammatory cells are mast cells, esinophils and T-lymphocyte (Holgate, 2010).

The above mentioned chemical inflammatory mediators cause bronchoconstriction effect to the airways of the lungs, spams and mucus secretion, in response symptoms of asthma become more pronounced (Currie et al., 2005, Ward et al., 2010). Non-atopic asthma normally occurs due to viral respiratory infection or few triggers, which are not external. This type is associated with adulthood and IgE has no role in the pathogenesis of the disease. The symptoms of this type of asthma can be very severe and persistent (Lambrecht and Hammad, 2003, Ward et al., 2010)

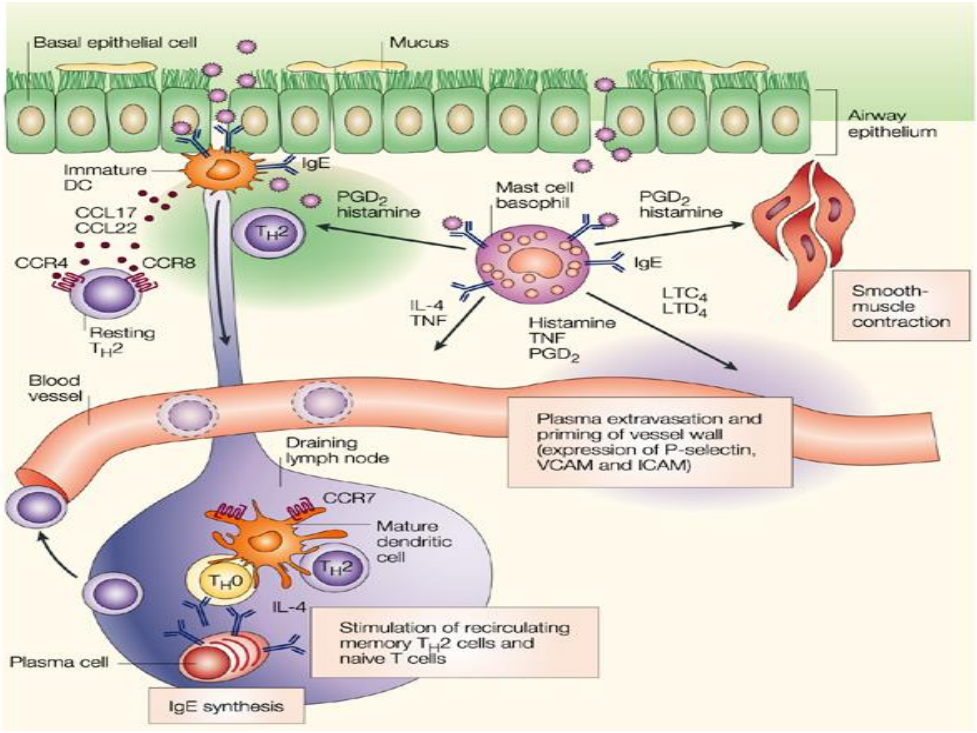

Figure 2.7: Demonstrate the inflammatory response of lungs smooth muscle in atopic asthma (Lambrecht and Hammad, 2003)

2.4.1.3 Symptoms of asthma

The symptoms of asthma differ between patients in term of severity and frequency. Moreover, the asthma symptoms may happen during the physical activity or at night . Some patients have an asthma attack symptoms more than one time a day, while others may have one in a week or so (Masoli et al., 2004, Holgate, 2010). The symptoms of the asthma characterisation can be used for the diagnosis purposes whether it is happened due to allergic or non-allergic environmental stimuli. The most common symptoms of asthma are as follow (GINA, 2016):

- Wheezing sound: During the inhalation, high wheezing sound can be heard.
- Breathlessness: inability to breath for seconds, or unable to do physical activity.
- Tightness: due to the lungs airways constriction patients feel chest tightness.
- Coughing: the inflammatory response to expel mucus for the airways.

In the asthmatic patients, different airways are affected such as trachea, bronchi and bronchioles. The symptoms of asthma can be worse during the exacerbations, whereas the

increase in the mucus secretion leads to a further constriction of the airways and some cases complete airways blockage. Asthma is associated with a decrease in both peak expiratory flow (PEF) and forced expiratory volume in one second (FEV1) (Barnes, 1996, Bradding et al., 2006, Holgate, 2010).

2.4.1.4 Classification of asthma

Asthma can be classified into four main groups depending on three main factors. Firstly, the prevalence of the symptoms during the daytime, symptoms during night time and the overall lung function as shown in Table 2.2.

Table 2-2: Classification of Asthma (Busse et al., 2015, GINA, 2016)

Severity level	Symptoms frequency		FEV1 or PEF (% predicted)	FEV1 or PEF (% variability)
	Diurnal	Nocturnal		
Intermittent	≤ 2 /week	≤ 2/month	≥ 80	< 20
Mild persistent	> 2/week but < 1/day	< 2/month	≥ 80	20-30
Moderate Persistent	Daily	> 1/week	> 60 - < 80	> 30
Severe Persistent	Continuous	Frequent	≤ 60	> 30

2.4.2 Chronic obstructive pulmonary disease (COPD)

It is a progressive pulmonary disease characterised by an obstruction in the airflow through the respiratory airways, the symptoms of the disease are not fully reversible. Upon the exposure to harmful and noxious gases and particles, the lungs airways progress an inflammatory response. COPD is considered as one of the common respiratory diseases; it affects 65 million people worldwide in the range of moderate to a severe state of COPD. It caused the death of 3 million people worldwide in 2010. In the UK an average of 1.2 million people are diagnosed with COPD in overall, and it is more prevalence in the age of 40 years and older. In 2012, the UK was in the top 20 countries with high mortality rate due to COPD. The disease is common in men than in women, but recent data showed that the number of women diagnosed with COPD increased significantly in the last ten years (British Lung Foundation, 2017). The main cause of the COPD condition is smoking which contributes to 90% of the cases according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD, 2016).

In COPD patients, two main conditions may comprise the disease; these conditions are chronic bronchitis and emphysema (Figure 2.8). Both can be characterised by different features as follow:

- Bronchitis is an inflammatory condition, which affects the bronchi causing an excess formation of mucus and thickening the lining of the bronchi walls. As a result of this condition the airways and the cilia are damaged which make it harder to breathe and slow the clearance of the mucus due to the lack of cilia.
- Emphysema is the other condition that comprises the COPD with bronchitis. It affects the small airways and the alveoli in the lungs. It will cause malformation of the air sacs as it becomes loose, which in turn leads to a bigger but fewer air sacs, which ultimately affect the gaseous exchange.

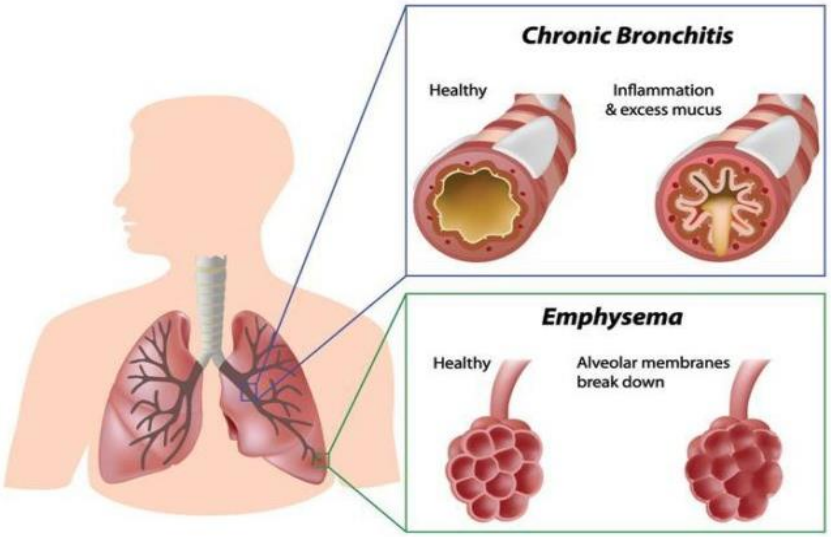

Figure 2.8: The difference in the airways obstruction between Emphysema and Bronchitis (American Thoracic Society, 2013)

2.4.2.1 Causes of COPD

The main causes of the COPD condition are cigarette smoke, infection of the bronchi and air pollution (Jensen et al., 2000, GOLD, 2016). The bronchitis is caused by the impairment in the ventilation because of the bronchi diameter decrease, which happens when the irritant persists to trigger the inflammatory cells. In some patients, chronic bronchitis can occur by the excess production of mucus in the patient lungs. In contrast, the emphysema causes the inability of exhaling the air out of the lungs as result of Alveolar wall damage. Air trapping is a common feature in patients with COPD (Morenz et al., 2012).

There are different causes and risk factors associated with the occurrence of COPD such as:

- Exposure to cigarette smoke: Long-term smoking is the most common cause of COPD
- Genetic: In some cases, the deficiency in Alpha-1 antitrypsin causes COPD.
- Nature of occupation: long-term exposure to dust or chemical irritant in the workplace is also a significant factor for COPD.
- Exposure to fume and fuel emission: the quality of life is an important factor. In developing countries, people are at high risk, due to the lack of good ventilated houses

- Dietary, Age and respiratory disease history (Mannino and Buist, 2007, GOLD, 2016).

The genetic factor between people affects the suitability disregard if the patient was a smoker or not. For some reasons, which are not identified yet, some smokers are not at risk, and others who are not smokers have COPD. The possible explanation is that patient who develops COPD has a deficiency in Alpha-1 antitrypsin which decreases the level of protease inhibitor (Barnes 2000, Barnes, 2004).

2.4.2.2 Pathogenesis of COPD

The pathophysiological process that causes the COPD is a complex process, which involves different inflammatory cells in the body. The main cells involved in the process are neutrophils and CD8+ Lymphocyte (Figure 2.9). These cells counteract each other, which results in various destructive changes in the epithelium of the smooth muscle in the lungs (Barnes 2000). The analysis of the inflammatory cells activity during the early stage can help the diagnosis of the disease. The increase in the inflammatory cells such as macrophage, T-lymphocytes, B- lymphocytes, and neutrophils which occur as a consequence of the destructive inflammation process in the small airways and the alveoli, this can be used when cell profile analysis is performed (Retamales et al., 2001, Barnes, 2004). Upon the exposure to the trigger factors such as noxious particles and harmful gaseous lead to an activation of these inflammatory cells and mediators. Furthermore, the oxidative stress as well as the imbalance between both protective and aggressive defence system in the lungs and respiratory airways considered as another possible process in the pathogenesis of the COPD (Barnes 2000). In people who smoke cigarettes, the increase in the oxidants compounds can result in serious damages to the protein and lipids and ultimately cause tissue damage. These oxidants also inhibit the anti-proteases enzymes (α1 antitrypsin, AAT) that lead to further damage in the balance between the protease and the anti-proteases (Carr et al., 2016).

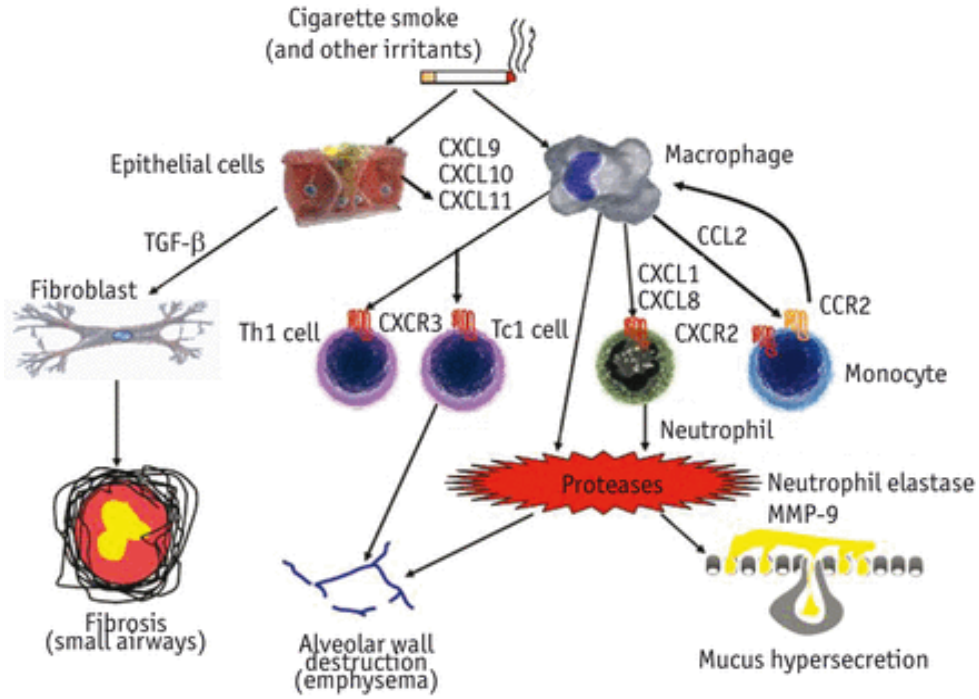

Figure 2.9: Pathophysiology and the inflammatory process in COPD (Barnes, 2010)

2.4.2.3 Symptoms of COPD

The signs and symptoms accompanied by COPD disorder can vary from mild to very severe symptoms. Normally, the symptoms start mild in the early stages. The early signs are persistent or a phlegmy cough, and in other cases, the symptoms start with short of breath in the early stages (American Thoracic Society, 2013, GOLD, 2016). The common signs and symptoms of COPD vary between patients and include:

- Phlegmy or a persistent cough , dyspnoea, breathlessness
- Wheezing and nocturnal breathlessness
- Weight loss and Tiredness

According to (American Thoracic Society, 2013)‘‘Many people with COPD develop most, if not all, of these signs and symptoms."

2.4.2.4 Classification of COPD

In COPD, both the peak expiratory flow (PEF) and forced expiratory volume in one second (FEV1) decreased in all patients. It has been classified into four groups (mild, moderate, severe and very severe) depending on the degree of the airflow obstruction or in other words, the disease severity (BTS, 2016, GOLD, 2016). These criteria can be measured using spirometry, hence, help in the diagnosis of the COPD as shown in Table 2.3.

Table 2-3: Classification of COPD according to the disease severity (GOLD, 2016).

COPD stage	Spirometric criteria	Signs and symptoms
Mild	• FEV1/FVC < 0.7 • FEV1≥ 80% predicated	Smoker's cough, no sign of abnormal lung function.
Moderate	• FEV1/FVC < 0.7 • 50% ≤ FEV1< 80% predicated	Progressive stage, symptoms are more pronounced, i.e., shortness of breath.
Severe	• FEV1/FVC < 0.7 • 30% ≤ FEV1< 50% predicated	Exacerbation stage limits physical activity, shortness of breath become worse at this stage
Very severe	• FEV1/FVC < 0.7 • FEV1< 30% predicated/ < 50% with chronic respiratory failure	Impairment in the life quality, life threatening exacerbation attacks more common at this stage

Despite the fact that COPD is an irreversible disorder, some test can be carried out such as reversibility testing to assess the COPD prognosis and therefore, determine the appropriate treatment to help manage the symptoms (BTS/NICE, 2016).

2.4.3 Asthma and COPD differences

Asthma and COPD have some similar characteristics in the signs and the symptoms such as the coughing and wheezing. However, they are two different respiratory conditions in different features such as the disease onset, symptoms frequency and the reversibility of the obstructive airways (Table 2.4).

- The disease onset: In asthma, the disease onset is common in the childhood, while in COPD, more common in the age category of the mid-50s in smokers and ex-smokers (Hogg et al., 2004, BTS, 2016).
- Exacerbations: asthma is characterised by chest tightness, shortness of breath and cough, which normally occur due to an allergen, exercise and viral infection. In contrast, the respiratory tract infections are considered as the main cause of COPD exacerbation (Pauwels, 2001)
- Prognosis: The treatment of asthma aims to reverse the symptoms and are almost symptoms free between the exacerbation attacks. In COPD, airways obstruction reverse partially, but symptoms are persistent almost every day (BTS/NICE, 2016)
- Pathogenesis: The number of eosinophils and neutrophils in the inflammatory response vary between COPD and asthma. Whereas, the percentage of eosinophils is more in asthmatic patients when compared to COPD patients. The percentage of neutrophils is much higher in COPD patient than asthma patient (Carr et al., 2016).
- Treatment and maintenance therapy: Inhaled corticosteroids are effective in relieving eosinophils mediated inflammation in Asthmatic patients, while neutrophils mediated inflammation is more resistant to these agents (George and Brightling, 2016). In COPD smoking cessation is helpful to reduce the progression of lung function as well as shortness of breath, then bronchodilators can be used (BTS, 2016).

The difference in the maintenance therapy can be seen from the usage of corticosteroids and bronchodilators, whereas, the first line treatment in the asthmatic patient is corticosteroid to control the inflammation caused by the trigger factors, then bronchodilator can be used to control the symptoms. In COPD, the opposite is true for the treatment of COPD conditions. Where Bronchodilator is the first line of the drug of choice in the maintenance treatment and corticosteroid can be used only in some cases where bronchodilators are not effective to control the symptoms in a patient with moderate to severe conditions along with frequent exacerbation (Barnes 2000, BTS/NICE, 2016).

Table 2-4: Common differences between Asthma and COPD, including common differential diagnostic symptoms (Yawn, 2009).

Patient history	Asthma	COPD
Smoker / non-Smoker	Possibly	Nearly all
Symptoms < 45 years	Frequent	Less frequent
Chronic cough symptoms	Infrequent	Common
Nocturnal wheezing and breathlessness	Common	Uncommon
Extent breathlessness	Variable	Persistent
Variability in symptoms day to day	Common	Uncommon

2.4.4 Treatment and Management of Asthma

Asthma is known as a multi-factorial disorder. Therefore, the complete cure of the disease is difficult. The first treatment of the asthmatic patient can be done by avoiding the trigger or stimulus factor that cause inflammation and the airways obstruction, reduce the usage of the drug therapy, tackle the cause of possible exacerbation and ultimately achieve the normal lungs function on daily activity (GINA, 2016).

The main components of asthma disorder are the inflammation and the airways constriction. Thereby, treatments that minimise these two components are considered as the effective asthma treatment regimens. Inhaled corticosteroids (ICSs) such as fluticasone, budesonide and beclomethasone, leukotriene inhibitors, xanthines (theophylline) muscarinic antagonist (*e.g.,* long-acting tiotropium and short-acting ipratropium) and mast cell stabiliser, which inhibits the release of chemical mediators, act with medication mentioned above to reduce the airways inflammation. Bronchodilator, which can be classified into short-acting β-2 agonist (SABA) such as salbutamol and long-acting β-2 agonists (LABA) such as formoterol and salmeterol, can be used to dilate the lung airways and reduce bronchoconstrictions. The combinations of LABA with ICS have been found to have a synergistic effect and help asthma control (Barnes and Godfrey, 1998). Another agent can be used for the management of the asthma symptoms such as anti-allergic non-steroidal agents (cromoglycate). The guidelines in the management of asthma have illustrated that ICS are the standard of asthma control (Suissa et al., 2000, Murphy, 2007). The management of asthma can be summarised in 5-steps approach designed by the British Thoracic Society (BTS, 2016). These steps were based on the use of SABA (*e.g.,* salbutamol and terbutaline) as first line relievers, then followed by the use of ICS (*e.g.,* budesonide and fluticasone) as preventers. The final step involves the use of LABA (*e.g.,* salmeterol) and an oral corticosteroid for short courses in the very severe cases and the exacerbation.

Step 1: SABA such as salbutamol used as a reliever, it has a quick onset of action (peak at approximately 15mins) which ensure a quick relief from the symptoms. This medication can be inhaled as required by the patient. However, the use of more usage might indicate the poor control of asthma such as using 2 or more canisters per month or more than ten puffs per day. SABA is a recommended bronchodilator therapy on daily basis use.

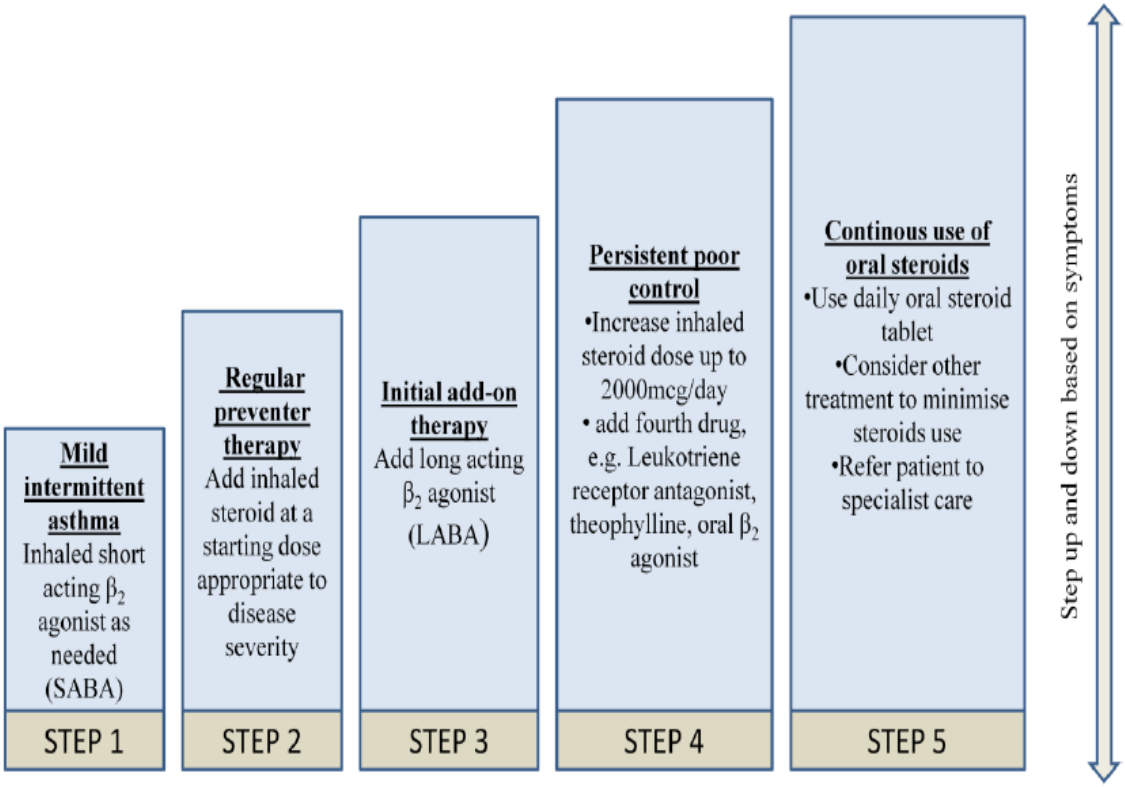

Figure 2.10: Stepwise plan for the management of asthma symptoms (BTS, 2016)

Step 2: In this step, the use of inhaled corticosteroid becomes an option, depending on how the symptoms have improved using the SABA. The dose use should be within an acceptable safety range to improve lung function and prevent exacerbation. Early use of ICS lead to an improvement in the asthma management and less use of other medication (O'byrne et al., 2001, Bernstein, 2008, Corrigan et al., 2009).

Step 3: In patients with severe symptoms, the increase of ICS use may lead to unwanted side effects. Therefore, BTS, 2007 recommended before increasing the dose of ICS, a combination of LABA such as salmeterol, formoterol with ICS should be used to improve the lungs function. Thereby reduce the probability of exacerbation of symptoms (Kips and Pauwels, 2001a, Kips and Pauwels, 2001b, Pauwels et al., 2003, Shepherd et al., 2008).

Step 4: If a moderate dose of ICS is not effective, and there is a response to the use of LABA, but the asthma control is still poor, in this case, the dose of ICS should be increased, while LABA remains constant. If this does not improve the lungs function, then the use of leukotriene receptor antagonist, theophylline or slow release tablets of β-2 agonist is recommended. LABA should not be given alone; it always has to be given in combination with ICS for asthma treatment (Currie et al., 2005, Tee et al., 2007).

Step 5: In this step, the use of oral steroids is recommended in the lowest dose possible that help control asthma symptoms. At this stage, the patient will experience severe symptoms and exacerbation. Therefore they should be referred to specialist care (Waldron, 2008). These receptors have to be available for the medication to activate the β-2 adrenergic receptors. The epithelium of the airways starting from the large bronchi downward to the terminal bronchioles have a high density of these receptors (Carstairs et al., 1985).The use of bronchodilators in an asthmatic patient were found to enhance the clearance mechanism in the pulmonary airways (Hasani et al., 2005).

The dose of ICS should be used in the minimum dose possible to relief symptoms and reduce the unwanted side effects such as cataracts and increased risk of infections. The use of ICS reduces the hospitalisation and death cases due to asthma (Kips and Pauwels, 2001b).

2.4.5 Treatment and management of COPD

Patient with suspected symptoms of COPD should be examined by undergoing different pulmonary function capability testing such as post-bronchodilator spirometry and pulmonary function test. These procedures are aimed to examine the degree of airways obstruction, the extent of condition severity and the probability of reversibility. A chest radiograph can be used to confirm the condition and exclude any thought of other overlapping respiratory condition. The diagnostic tests that should be done are forced expiratory volume in a second (FEV1) in addition to the volume of air exhaled using fast and forceful exhalation (FVC).In patient with COPD, these two parameters reduce as well as the ratio FEV1/ FVC which is considered as a diagnostic marker for the obstruction in the pulmonary airways. An FEV1/ FVC ≥ 0.7 suggests no obstruction in the airways (Pauwels et al., 2003, GOLD, 2016).

The aim of COPD management can be summarised into main points such as:

- To ensure the diagnosis of the disease in early stages accurately.
- It helps control the common symptoms associated with the condition.
- Prevent complications by reducing the number of exacerbation incidence.
- Improve the patient's quality of life.

The treatment of COPD is required to improve the breathing pattern of the patient, and that can be achieved by improving the patient FEV1 profile. The drugs used for the treatment of COPD have been investigated to which extent this medication capable of retrieving or improve the FEV1 (López-Encuentra et al., 2005).

COPD Management involves two main strategies to control the disease and help the patient on a daily basis. These strategies are non-pharmacological management and pharmacological management (Figure 2.11).

2.4.5.1 Non-pharmacological management

- Smoking cessation: In the early stages of COPD, stopping smoking is considered a key factor. The patient history of smoking can help during the diagnosis as well as the management of the disease. Therefore, these data should be obtained from the patients to know the number of year's patient have been smoking cigarettes for as well as the number of packs per day/week *etc*.
- Vaccination: pneumococcal and influenza vaccine annually.
- Pulmonary Rehabilitation: Patient with recurrent exacerbation, it includes physiotherapy, disease education, exercise and nutritional support.
- Travel advice: patient with low FEV1 % and having a long-term oxygen therapy should be informed that air travel might increase the risk of pneumothorax (Achilleos and Powrie, 2011)

2.4.5.2 Pharmacological management

The pharmacological management of COPD comprises of different lines and each of this medication has to be given under certain conditions to improve the lung function and help the patient with daily life (Hasani et al., 2005). This medication can be classified as follow:

- **Bronchodilators**

The patient with COPD condition should take an inhaled bronchodilator medication to help with the symptoms. If the inhalation of bronchodilators showed signs of improvement in the lung function and improve exercise tolerance, it is preferable to continue prescribing the treatment (Celli et al., 2004, Hasani et al., 2005, Achilleos and Powrie, 2011). Cazzola and Page (2014) stated "Bronchodilators are central to the treatment of chronic obstructive pulmonary disease (COPD) because they alleviate bronchial obstruction and airflow limitation, reduce hyperinflation and improve emptying of the lung and exercise

performance". For this reason, ''all guidelines highlight that inhaled bronchodilators are the mainstay of the current management of all stages of COPD'' (Cazzola and Page, 2014).

The first choice is using the short acting β-2 agonist SABA (salbutamol and terbutaline), these treatments described due to their rapid onset of action, in addition to the relief the symptoms (BTS/NICE, 2016). Inhaled long-acting muscarinic antagonist LAMA, e.g., ipratropium bromide (Atrovent®) is as effective as SABA as it targets M1 and M3 receptors; the only disadvantage is that in some cases, the slower acting mechanism of this treatment(Achilleos and Powrie, 2011). The long-acting β-2 agonist LABA such as salmeterol and formoterol should be considered if the SABA treatment (four times daily) did not improve the symptoms and enhance the lung function. These treatments have a long acting mechanism approximately 12-14 hours. Additionally, some studies have shown the importance of using LABA over the use of SABA as the first line in the management of stable COPD (Van Noord et al., 2000, Cazzola et al., 2004). The use of LABA in COPD patients inhibit the mast cells mediator release and thereby reduce the nerve activation (Nials et al., 1994). The combination treatment of LABA and LAMA are found to provide a synergistic effect, which will provide an improvement in the lung function and maintain the effect overnight, compared to daytime improvement when single treatment administered. The reason for that is LAMA will decrease the vagal tone that happens in COPD, therefore, airways relaxation when the receptor activated by LABA, *e.g.*, a combination of formoterol and tiotropium (Duova Rotacap®) (Cazzola and Tashkin, 2009).

- **Inhaled corticosteroid (ICS)**

The use of ICS in the treatment of COPD has not understood the reason is that during the COPD the inflammation is caused by neutrophils. The use of ICS is controversial in the management of stable COPD (Keatings et al., 1996, Barnes, 2010). In patients with FEV1 < 50% experiencing an exacerbation twice a year despite the use of LABA, the use of ICS with

high dose should be considered. The combinations of ICS with LABA such as Beclomethasone dipropionate / Formoterol fumarate was found to decrease the chance of exacerbation by approximately 25% when compared to the effect of LABA alone in reducing the risk of exacerbation (Nannini et al., 2007, Barnes et al., 2013).

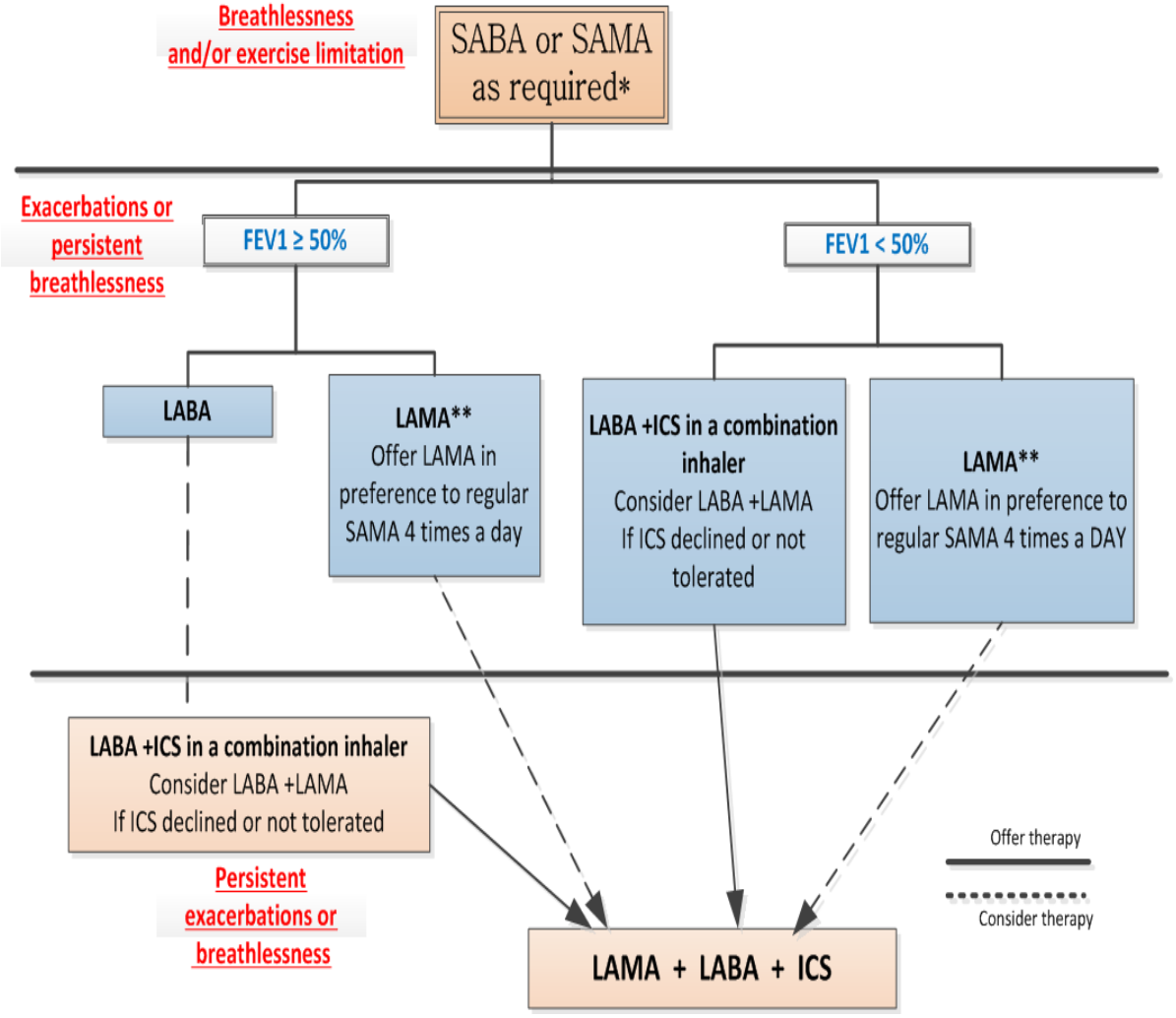

Figure 2.11: Strategies for the management of stable COPD (Achilleos and Powrie, 2011)

Guidelines have been changed and patients with FEV1 less than 60% were approved to use a combination of ICs and LABA (Seretide Accuhaler®) (Calverley et al., 2003, Roche et al., 2014). The short course use of oral corticosteroid is considered during an exacerbation. However, patient must not use this treatment for maintenance therapy due to their serious unwanted side effects (Achilleos and Powrie, 2011).

The study showed that formoterol has an effect in the treatment of COPD, due to its ciliary enhancement mechanism and bronchodilation, whereas the mucociliary clearance significantly improved when formoterol used for a week (Melloni and Germouty, 1992). Formoterol lipophilicity properties give it the ability to entrain within the membrane, which means activation last for a longer period (Anderson, 1993). The mucolytic agent usages in the management of COPD for a patient with a chronic cough, nonetheless, no evidence shown improving symptoms in a patient with exacerbation or reduce the frequency of the exacerbation. When these treatment used for more than two months, meta-analysis data showed that the exacerbation reduced by 29% when compared to placebo (Poole and Black, 2001).

- **Theophylline for COPD patients**

The use of theophylline in COPD, even though it has side effects when used in bronchodilator doses, however, there is increasing evidence showing that it has a role in controlling the inflammation in COPD patients. It's anti-inflammatory response occur at low plasma concentration, the role that theophylline plays is pronounced at low concentration by activating the histone deacetylases, and thereby improving the anti-inflammatory effect of ICS (Barnes, 2006). Theophylline should be used in patients who are not able to use the inhaled therapy, or it can be used after bronchodilators SABA and LABA (Achilleos and Powrie, 2011).The mechanism of theophylline was found to be enhanced when combined with oxidative stress (Barnes, 2005). In addition to the use of theophylline as bronchodilator, it has a significant cardiovascular effect in COPD patients, whereas study by Matthay, 1987 state that ''Administered to COPD patients orally as a sustained-action preparation or intravenously as aminophylline, theophylline enhances both right and left heart systolic pump function and lowers both pulmonary artery pressure and pulmonary vascular resistance'' (Matthay, 1987).

- **Supplemental long-term oxygen therapy (LTOT)**

The use of LTOT in COPD patients should be assessed if the patient shows the following criteria (BTS/NICE, 2016):

- Severe status of airways obstruction
- Patient show signs of Cor pulmonale
- Patient with hypoxaemia (Sa02≤ 90%, threshold of ≤ 88%)
- Cyanosis
- Polycythaemia

This step is introduced to improve the patient survival, daily exercise, sleep and cognitive performance in a patient with hypoxaemia (GOLD, 2016). The oxygen determination in patients is needed; arterial oxygen saturation can be measured using the pulse oximetry (SpO_2), in addition to the physiological indication for oxygen supply such as oxygen tension (PaO_2) < 7.2 kPa (54mmHg). The target is to maintain the SpO_2 level above 90% during the patient's rest and sleep to prevent of tissue hypoxia. In some cases, when CO_2 retention occurs, signs of patient acidemia should be monitored, if acidemia occurred, then mechanical ventilation is essential for patient survival (BTS, 2016).

2.4.6 Summary of COPD and asthma treatment

The guidelines data produced by the (BTS, 2016, GOLD, 2016) demonstrated that inhaled bronchodilators, as well as inhaled corticosteroid, are the main choice in the treatment of asthma and COPD. Therefore, it is crucial to ensure successful dose emission from the marketed inhaler devices, also, to ensure that the emitted dose from the device has the tendency for distal respiratory airways deposition during the inhalation manoeuvre.

2.5 Pulmonary route of drug administration

The pulmonary route of administration was used over many years. The history of the origin of inhaled therapies was established 4000 years ago in India by smoking the leaves of the *Atropa belladonna* plant to treat coughs. During the 19th and early 20th centuries, asthmatic patients smoked cigarettes that contained *stramonium* powder mixed with tobacco to treat their disease symptoms (Bisgaard et al., 2001, O'Callaghan et al., 2001, Ashish et al., 2012). Inhalation devices that have been developed recently can be classified into three main categories - the improvement of nebulisers and the production of two types of compact inhalation devices: pMDIs and DPIs (Grossman, 1994, Labiris and Dolovich, 2003).

Pulmonary drug delivery is considered as one of the most efficient routes for drug administration. The inhalation route of administration was used to deliver the drug to the site of action in different forms such as vapour of aromatic plants and myrrh, whereas by the 19th-century liquid nebulisers were coming into use for treatment via the pulmonary drug delivery route. It has been used mainly to treat respiratory tract diseases such as asthma and chronic obstructive pulmonary disease (COPD) over the last few decades (Bisgaard et al., 2001, O'Callaghan et al., 2001, Ashish et al., 2012). Recently, it has been used for other purposes than treating lung disease, whereas it is used in the treatment of diabetes, for vaccination and also gene therapy (Laube, 2014). The use of pulmonary route to deliver medication to the lungs epithelium directly is more efficient and reduce the chance of potential side effects that caused by the use of another route of drug administration. It has some significant advantage, despite the fact that the bioavailability of drug through inhalation route depends on the proper technique of device use and the particle size following the formulation dispersion inside the device (Chege and Chrystyn, 1994, Chrystyn, 2007, Vincken et al., 2010).

The common advantages of using inhalation route are listed below (Chrystyn, 2006).:

- High local concentration and targeting
- The low doses of drug used (for instance, salbutamol is 200µg, when compared to an oral dose of 4 mg of salbutamol).
- Low local and systemic concentration, thus reducing the toxicity
- Efficacy and rapid onset of action
- Bypass the first pass metabolism
- It is needle-free pulmonary route delivery
- Low enzymatic activity when compared to the liver
- Huge specific surface area, making this route suitable for protein and peptides.

Therefore, the inhalation route of drug administration is the favoured route for the treatment of different diseases especially respiratory conditions such as asthma and COPD. The local and systematic efficiency of drug delivery to the lungs depends on three major parameters including the patient inhalation manouvere, the inhaler device and the formulation (Chrystyn, 2006, Ibrahim et al., 2015).

2.5.1 Mechanism of inhaled particle deposition

Particles deposition in the respiratory tract can be defined as particles with a mass median aerodynamic diameter of ≤ 5µm and are more likely to enter the lungs and make deposits in different regions of the airways according to their particle size (Chrystyn, 1999). The deposition of the particles to the lung airways occurs *via* three major mechanisms: inertial impaction, gravitational sedimentation and Brownian diffusion (Figure 2.12). Velocity as well as the size of particles, affects the particle deposition. Particles can also be deposited on the surface of the pulmonary tract by other mechanisms such as interception and electrostatic precipitation (Heyder et al., 1986, Gonda, 1990, Heyder, 2004, Lindström, 2004).

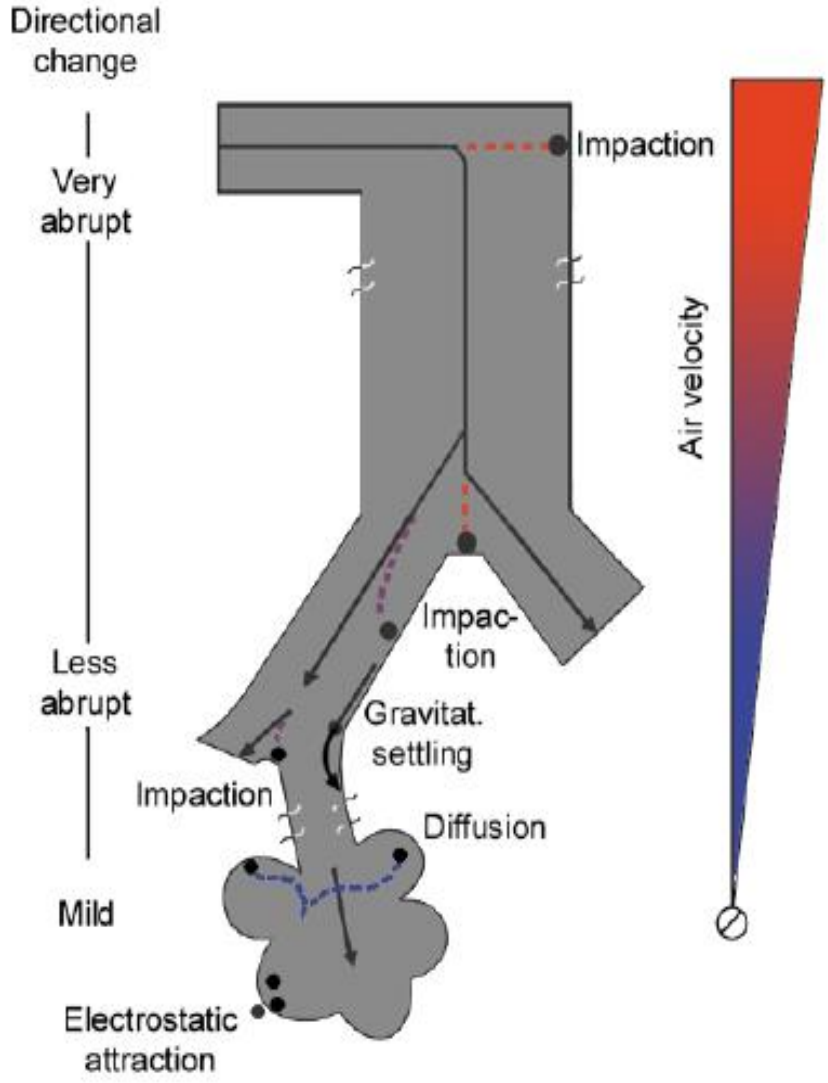

Figure 2.12: Mechanisms that control inhaled particles deposition in the respiratory airways (Hussain et al., 2011)

2.5.1.1 Inertial impaction

Particles with a large diameter and high intensity tend to follow the impaction mechanism when it is travelling at high velocity in the air stream. The change of airways direction makes it difficult for the large particles to follow the airstream. The deposition of the particles by the inertial impaction will take place at the bifurcation of the large airways, larynx, trachea and mouth. Particles with diameter > 10μm are deposited by the impaction mechanism (Hickey A, 2005, Tena and Clarà, 2012)

2.5.1.2 Gravitational sedimentation

This mechanism occurs with smaller particles (0.5-5μm), whereas particles that move down the airways will be affected by the slow flow of the air velocity due to the opposing resistive forces of the air and tends to be deposited due to the gravitational force. The gravitational sedimentation mechanism will take place as a result of breath holding after the inhalation manoeuvre. Uniform particles distribution throughout the respiratory airways can be achieved by sedimentation, which in turn could be enhanced by slow, deep breaths and holding the breath after inhalation (Tena and Clarà, 2012).

2.5.1.3 Brownian diffusion

The diffusion mechanism occurs with particles size smaller than 0.5μm; furthermore, the diffusion mechanism of particle deposition increases as the particles size decreases. Particles tend to diffuse to the surrounding surface due to the residence time of particles being long in addition to the short travelling distance to reach the airways. The diffusion mechanism is enhanced by holding the breath after inhalation. The diffusion mechanism is inversely proportional to particle size and relates directly to the residence time of the particles in the airways (Gonda, 1990).

2.5.1.4 Other mechanisms Electrostatic charge effect and interception

Electrostatic precipitation occurs when charges of opposite sign are induced by charged particles onto the walls of respiratory airways that electrically conduct when normally uncharged. Consequently, the electrostatic force attracts the charged particles to the surface of the airways. For that reason, charged particle deposition will be greater than the neutral particles. The interception mechanism of the deposition takes place when the particles dimensions are similar to that of the airways the particles are passing through; for example, the small airways and alveoli (Stuart, 1984, Hillery et al., 2002).

2.6 Inhaler devices for pulmonary drug delivery

The successful management of lung disease can be affected by the adequate use of the inhalers and thereby the delivery of the drug to the lungs. The availability of the inhaler that is easy to use, able to deliver the medication to the lungs in a reproducible and cost-effective manner is crucial for successfully inhaled treatment (Selroos et al., 1996, Pedersen et al., 2010, Vincken et al., 2010). There are different criteria that the ideal inhaler device should include:

- Firstly generating an aerosol bolus with an optimum particle size in the range of 0.5 to 5μm
- Dose delivery uniformity over the course of treatment
- Easy to use
- Patient preference and robustness
- Finally, portable and inexpensive (O'Connor, 2004, Brand, 2005, Chrystyn, 2007, Ibrahim et al., 2015). The DPIs and MDIs are the most commonly prescribed inhaler devices, nebulisers are used in certain situation depending on the patient's status.

The criteria of the ideal inhalers (Figure 2.13) do not exist in inhaler devices in the market nowadays, nonetheless, prescribing the right inhaler for the right patient can maximise the benefits of the inhaler use.The effectiveness of the inhaler device is found to be affected by the correct choice of the device and the proper inhalation technique (Barnes and Godfrey, 1998, Haughney et al., 2010, Scichilone, 2015). Different parameters should be taken into consideration to choose the right inhalers such as tolerability, ease of use by the patient and preference (Newman, 2005).

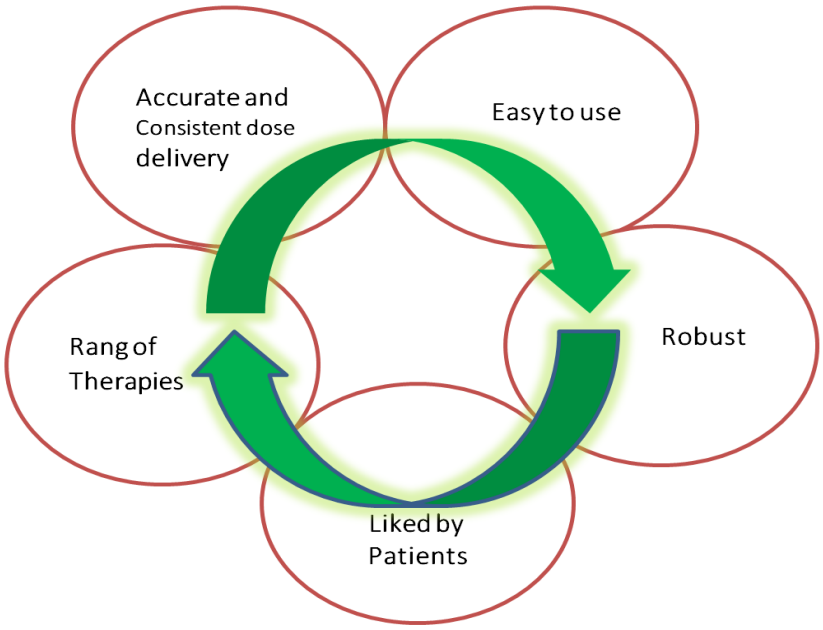

Figure 2.13: Criteria of an ideal inhaler device (Chrystyn, 2007)

2.6.1 Nebulisers

The nebulisers are the first inhalation devices to be developed and used for the treatment of different respiratory disorders. They have been developed over years, however the first nebuliser was only invented in1849, followed by portable nebuliser in 1858, after that the glass bulb nebuliser was introduced in the 1930s and the plastic bulb nebuliser in 1940 (Grossman, 1994, Anderson, 2005). The first compressed nebuliser was designed and introduced in Germany in 1930s, which was known as Pneumostat®. Both solutions and suspension formulated active pharmaceutical ingredients (API) can be aerosolised from this device. The solvents used are usually either water alone or water in combination with co-solvents such as ethanol and propylene glycol (Clarke and Newman, 1984).

The minimal inspiratory effort required for aerosol delivery from a nebuliser is 6 to 8 L/min (Newhouse et al., 1976, Lavorini, 2013). The patient data suggest the effectiveness range of the nebuliser vary from 10 - 50% of the delivered dose (Clark, 1995). The nebulisers operate by converting the drug solution or suspension into fine aerosol droplets for inhalation. It can be used for patients who cannot use the pMDIs or DPIs correctly, including patients with an

acute exacerbation who require a high therapeutic dose such as antibiotics (*e.g.,* Tobrmycin Inhalation solution 300mg/ 5mL, TOBI®) such a high dose can not be delivered using pMDIs or DPIs. The nebulised medication can be breathed in using a mouth piece or face mask (Fink, 2000, Boe et al., 2001, VanDevanter and Geller, 2011).

There is medication wastage up to 50% of the medication nominal dose retained in the dead space of the chamber or exhaled during the exhalation manoeuvre using nebulisers. In addition to the oropharyngeal deposition caused by large droplets size which are unable to reach the peripheral airways (Smaldone and LeSouef, 2001). The nebulisers can be classified depending on the design and the mechanism of dose release into three main types: Jet nebuliser, Mesh and Ultrasonic nebulisers as shown in Figure 2.14.

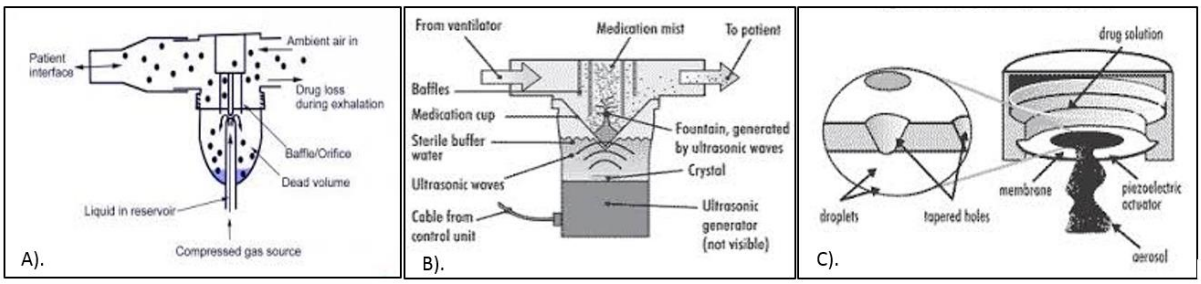

Figure 2.14: Schematic diagrams of a). Air- jet nebuliser b) Ultrasonic nebuliser and c) Mesh nebuliser (Ghazanfari et al., 2007, Ari, 2014)

Jet nebuliser converts the drug solution into a fine mist for inhalation using compressed gas, oxygen cylinder or electrical compressor (Dhand, 2003, Ari, 2014). Ultrasonic nebuliser has a piezoelectric crystal vibrating at high frequency (1-3 MHz). It is unsuitable for high viscous solutions/ or suspension, heat sensitive material (*e.g.,* proteins) and large residual volume post inhalation (Ari and Fink, 2011, Ari, 2014). Mesh nebuliser uses multiple apertures in a mesh or aperture plate; the medication solution is forced by the mean of micro-pump through this aperture to generate the aerosol (Ari, 2014). The nebulisers are used for some patients, but they are not as popular as the MDIs and DPIs for the delivery of

medication into the lungs (Clark, 1995). There are different reasons for that such as their bulky nature although there are some portable patient nebulisers, difficult to clean, time - consuming (Dolovich et al., 2005).

2.6.2 Metered dose inhalers (MDIs)

MDIs were firstly introduced in 1956. They remain the most prescibed inhalers (Vaswani and Creticos, 1998). They represent about 80% of the inhaler products on the market (O'Connor, 2004). There are different reasons for the popularity of these devices as they are inexpensive, simple to manufacture and available with different drug formulations (Chrystyn, 2007). pMDIs are used in the delivery of a wide range of active pharmaceutical ingredients (APIs) including bronchodilators, anti-cholinergic, anti-inflammatory and corticosteroids. APIs form 1% or upper limit of 2% w/w of the formulation to reduce the chance of valve clogging (Smyth, 2003). When used effectively pMDIs are as effective as other pulmonary drug delivery systems (Fink, 2000). There are four main functional components of pMDIs: The canister, metering valve, actuator and mouthpiece as shown in Figure 2.15. The propellants are the driving force to expel the drug formulation from the canister. The propellant forms at least 80% w/w of the formulation, reducing the amount of the propellant has been shown to affect the spray characteristics of the inhaled aerosol (Fink, 2000, Ju et al., 2010). In addition to the drug substance, pMDIs may contain other substances such as surfactant and co-solvent mixture to improve the stability of the formulation and spray characteristics of the inhaled aerosol.

The pMDIs are either suspension or solution based formulation of drug in propellants. For decades, chlorofluorocarbons (CFC) have been used as propellant in pMDIs formulation. In 1987 the Montreal Protocol banned the use of the CFC propellants due to their depletion effect on the ozone layer. The hydroflouroalkanes (HFAs) such as HFA134a and HFA227 are

used as alternative in the formulation of pMDIs (Hou et al., 2015). The solution pMDIs have been shown to be more efficient than suspension pMDIs in maximising the drug delivery to the lungs (Clay and Clarke, 1987, Hickey, 2006, Gibaldi, 2007). The deposition of the aerosol in the lungs improved with HFA- pMDIs due to the reduction in the oropharyngeal deposition, which occurs due to forcful blast of CFCs propellant (Dolovich, 1999, Gibaldi, 2007, Usmani, 2015).

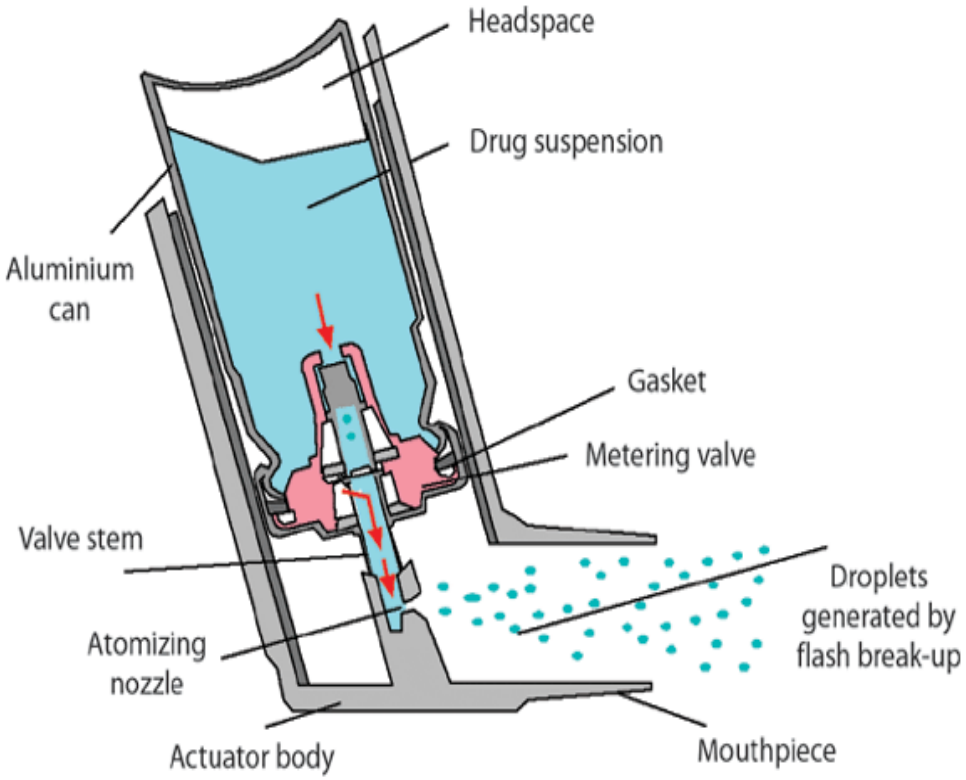

Figure 2.15: Schematic diagram showing the components of pMDIs

Many problems have been identified with MDIs in term of the effectiveness of the devices and the usability. The patient inhalation technique is one of the most concerns, some studies reported that up to 94% of the patients have a poor inhalation technique (Brocklebank et al., 2001, Crompton et al., 2006, Lavorini et al., 2008, Roche et al., 2013). Patient training on how to use the device correctly did not completely solve the problem of the coordination

between patient inhalation and dose actuation. Many patients were unable to use the device correctly after training (Crompton, 2004, Al-Showair et al., 2007a, Hardwell et al., 2011, Gowrishankar, 2013).The problem with poor coordination is that the medication is either emitted too early or too late in the inhalation manoeuvre cycle (Crompton, 1981, Donnell, 2001). The patient failure in achieving a slow inspiration technique was one of the main problem associated with the use of pMDIs. Furthermore, studies showed that fast inhalation could lead to high oropharyngeal deposition which is not required for good inhalation therapy. Therefore slow inhalation followed by breath-holding duration was found to improve lung deposition (Newman et al., 1981a, Newman et al., 1981b, Newman et al., 1982, Everard et al., 1995, Al-Showair et al., 2007a, Chrystyn and Price, 2009, Haughney et al., 2010). A minimum inspiratory flow rate of 20 L/min was reported to be sufficient for pMDIs (Newhouse et al., 1976) and holding the breath for 10 seconds found to provide better drug deposition than 4 seconds (Newman et al., 1981c). Several studies reported that lung deposition and clinical outcome were improved when correct inhalation technique was achieved by the patients (Al-Showair et al., 2007a, Lavorini et al., 2010). High velocity aerosol bolus was also associated with pMDIs poor lung delivery about 10 - 15% of the emitted dose reaches the lungs (Newman et al., 1981c).

2.6.2.1 pMDIs with spacer and valve holding chamber

Over the years, a wide range of add-on devices such as spacer tubes, Valve holding chambers and mouthpiece extensions has been introduced to solve the coordination problem with pMDIs Figure 2.16. These add-on devices, if used properly, will help reduce the throat deposition and lead to a subsequent increase in the peripheral deposition in the lungs airways. The spacers were introduced to help to reduce the effect of the cold Freon effect, while valve holding chamber showed to reduce the medication dose waste especially in the patient with poor inhalation technique (Vaswani and Creticos, 1998, Fink, 2000, Aswania and Chrystyn,

2001). The dose of non-respirable particles that impact on the wall of the spacer tubes has only marginal effect on the dose of the respirable particle (particles with a diameter of > 5µm) (O'Callaghan et al., 1994, Terzano, 1999).

The use of a spacer with pMDIs allow the patient to inhale at a distance from the inhaler mouthpiece to the patient mouth, thus allowing more time for the aerosol to slow and the propellant to evaporate and thereby reduction in the particle size ensuring more benefits from each dose by increasing the fraction of the inhaled aerosol (Broeders et al., 2009). The decrease in the oropharyngeal deposition with the use of spacer tubes attributed to the decrease in the aerosol particle velocity (Newman and Newhouse, 1996). The use of low volume spacer can lead to up to 60% decrease in the respirable dose. Therefore, it is recommended to use a spacer with a volume of > 100mL (Fink, 2000).

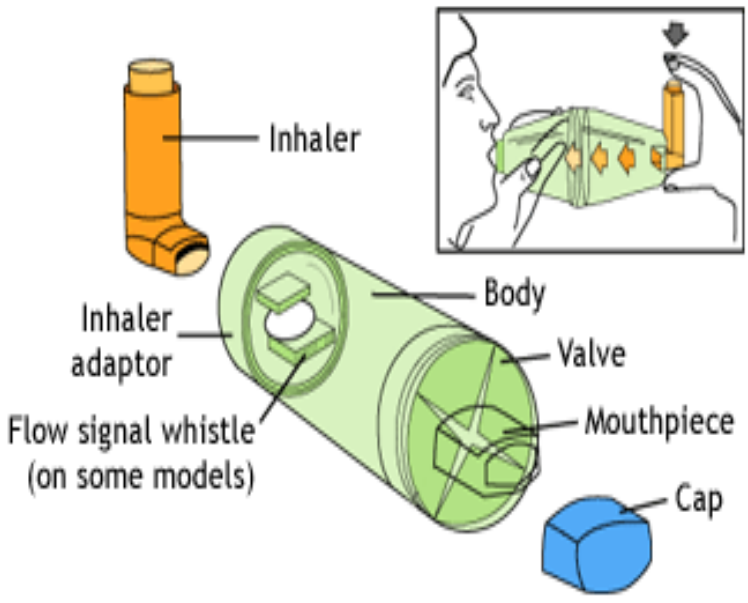

Figure 2.16: pMDIs with spacer and valve holding chamber

The pMDIs with the spacer can be as effective as nebulisers in the management of acute asthma attacks (Cates et al., 2006). Spacers are considered as an efficient method in adult and children patients who cannot use the pMDIs properly (Pedersen, 1996). Although, spacers

dose delivery inconsistency was reported to be due to the electrostatic charges of the aerosol causing drug particles attachment to the spacer plastic and holding chamber walls (Barry and O'callaghan, 1995). This effect of electrostatic charges can be significantly reduced by washing the spacer with 1: 5000 household detergent and left to dry, the spacers' performance improved by using anti-static linings (Barry et al., 1993, Wildhaber et al., 2000).

2.6.2.2 Breath actuated Pressurised metered dose inhalers

The breath-actuated MDIs have been introduced, due to the coordination difficulties that patients have experienced when using the pMDIs between the dose actuation and the patient inhalation manoeuvre. About 50% of the pMDIs users have a poor inhalation technique (Donnell, 2001, Pothirat et al., 2015). Breathe actuated pMDIs contain a flow-trigger system that driven by a spring, the latter automatically release the dose from the canister of the MDIs upon the start of the patient inhalation manoeuvre which means coordination is automatic as shown in Figure 2.17. The examples of breath-actuated MDIs are the device developed by Teva Pharmaceuticals Ltd, UK such as low inhalation flow rates Autohaler® (30L/min) and Easibreathe® (20 L/min) (Newman et al., 1991b, Broeders et al., 2009, Laube et al., 2011).

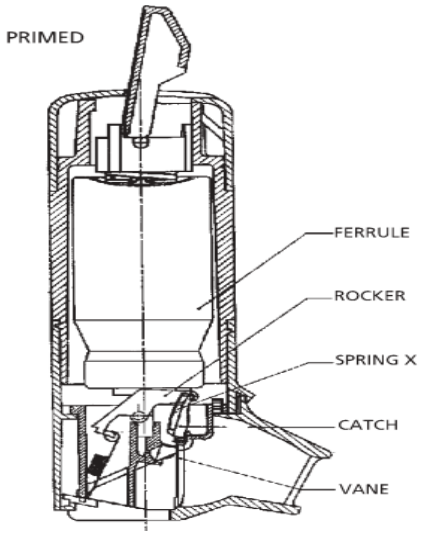

Figure 2.17: Breath actuated metered dose inhaler

The inhalation flow requirement for these devices is achievable by most of the patients including patients with severe airways blocking, furthermore the delivered dose, and dose consistency was not affected by any increase in the inspiration effort (Terzano, 2001). The breath-actuated MDIs have criteria that increase patient compliance and confidence such as the clicking sound upon actuation and the taste of the propellant in the dose which assure the patient that the dose has been delivered (Newman et al., 1991b, Donnell, 2001).

Although, these devices have brought no improvement in a patient with good coordination technique. Several studies demonstrate that this device improves lung deposition and decrease the throat deposition in a patient with poor coordination technique (Newman et al., 1991b, Schecker et al., 1993, Soria et al., 2002). Breath-actuated MDIs can be a good substitute to the pMDIs because it is easier to use, teach, patient preference, complaince and improved lung depsotion (Chapman et al., 1993, Price et al., 2003, Giraud and Allaert, 2009).

Nonetheless, when compared to pMDIs the breath actuated devices are requiring a higher flow rate of 20 L/min and 30 L/min respectively, in addition, it does not solve the cold Freon issue and limited to only salbutamol and beclomethasone dipropionate compared to a wide range of medications with pMDIs (Newman et al., 1991b). The pMDIs remain popular in the delivering of the medication for the management of asthma and other respiratory obstructive disease, notwithstanding the difficulties patient face when using this device. The advantages and disadvantages of drug delivery systems for the delivery of medications to the lungs are illustrated in Table 2.5.

Table 2-5: Advantages and disadvantages of different aerosol delivery systems (Dekhuijzen et al., 2016)

Inhaler devices	Advantages	Disadvantages
Pressurised Metered dose Inhalers (pMDIs)	Portable, convenient and compact Multi-dose device Relatively cheap Available for most inhaled medication Cannot contaminate content Dose indicator	Contains propellant Required coordination Patient cannot use it correctly High throat deposition
pMDIs + Spacer	Low flow rate dependency Easier to coordinate More convenient for large doses Less throat deposition Higher lung deposition than pMDIs	Bulky inconvenient Static charge for plastics spacers Additional cost to a pMDIs
Breath actuated-MDIs	Convenient, compact Multi-dose device No coordination required Cannot contaminate contents	Cold Freon effect Required moderate flow to operate
Dry powder inhalers (DPIs)	Portable, convenient Breath actuated No propellant Dose indicator Locking system when empty	Require a inspiratory flow Not appropriate in Emergency Many patient cannot it use properly Most types are moisture sensitive
Soft mist inhalers (SMIs)	Portable, compact Multi-dose device Low flow rate dependency High fine particles High lung deposition No propellants Dose indicator Locking mechanism when empty	Not breath actuated device
Nebulizer	Can be used at any age No technique required Vibrating mesh is portable and does not require an outside energy May dispense inhaled medications not available with pMDIs or DPIs	Jet and Ultrasonic types required an outside energy source Long treatment time Inconsistent performance between different types Risk of bacterial contamination Newer generation nebulizers are expensive

2.6.3 Soft mist inhalers (SMIs)

Soft mist inhalers are new generation inhalers, which are neither pMDIs nor DPIs. Respimat® Soft mist™ is the only available SMI in the market, introduced by Boehringer Ingelheim GmbH (Dalby et al., 2004, Anderson, 2006).

Respimat® is a handheld device similar in size to both pMDIs and DPIs. The mechanism of aerosol cloud generation from the drug solution require the metered dose of liquid to be converted into a small droplets with a high tendency for lung deposition without the need of additional force (*e.g.*, propellants). In Respimat®, mechanical energy generated by the spring is responsible for aerosolisation mechanism. The spring can be easily compressed by the patient when using the device, therefore, using mechanical force ensure reproducible and reliable energy source, and also independent of the variable patient's flow. It is a ‘‘press and breathes’’ device (Zierenberg et al., 1996, Anderson, 2006, Dekhuijzen et al., 2016). Also the respimat® has a relatively long generation time of the aerosol cloud comparing to pMDIs (approximately 1.5 sec) that allow more time for the patient and enhance the coordination between the patient inhalation and the dose actuation. The patients should use a longer inhalation time > 2second after dose actuation, to meet the long aerosol output by the device. Notwithstanding, the difference in the aerosol cloud generation time, the correct inhalation technique is similar to that used with pMDIs (Dalby et al., 2004). The drug contained can be defined by the colour code and the internal mechanism of the respimat® illustrating the medication solution stored in the cartridge (Figure 2.18).

Respimat® device is designed to avoid the use of a spacer, battery or external power source. Furthermore, the fine particle fraction (FPF) in the aerosol cloud that is generated by the

respimat® is higher than that generated by pMDIs and DPIs 70% FPF, consequently reducing the throat deposition and enhancing the lower airways drug deposition. In clinical trials, respimat was equally effective in a patient with COPD, the bronchodilator at half of the dose delivered from a pMDIs,. The device was more preferred by the patient with COPD and other obstructive pulmonary diseases over the pMDIs (Dalby et al., 2004, Anderson, 2006). Studies on the performance of Respimat® showed that delivery of flunisolide ethanolic solution and an aqueous solution of fenoterol have a fine particle fraction of 66% and 81% of the nominal dose respectively (Zierenberg et al., 1996, Eicher and Zierenberg, 2002). The SMI device was shown to offer the same level of therapeutic efficacy and safety with less dose of combination bronchodilator when compared to pMDIs.

Figure 2.18: Demonstrate structural components of Respimat®

2.6.4 Dry powder inhalers (DPIs)

DPI were introduced into the market to overcome the CFC-propellant and poor patient coordination technique experienced with the pMDIs (Vidgren et al., 1988, Tarsin et al., 2006, Lavorini, 2013). Aerohaler® and Spinhaler® were the first DPIs introduced to the market in 1950s (Bell et al., 1971, Clark, 1995, Sanders, 2007). These devices were followed by other devices such as blister (*e.g.*, Accuhaler® and Turbuhaler®) and reservoir DPIs (e.g. Clickhaler®, Easyhaler®). These DPIs differ in their internal design, resistance and thereby dose emission characteristics (Chrystyn, 2006, De Boer et al., 2017).

DPIs are breath actuated devices and have some advantages over the pMDIs such as it is more stable, propellant free and large doses can be introduced (Ashurst et al., 2000). Over the last decades, several DPIs devices have been introduced into the market to deliver inhaled bronchodilators and corticosteroids. They have shown a higher lung deposition and dose consistency with less technique error when compared to the use of pMDIs (Borgstrom et al., 1994, Lenney et al., 2000, Molimard et al., 2003, Chrystyn et al., 2016). DPIs devices were classified depending on the design of the inhalers into single dose and multi-dose DPIs as shown in Table 2.6 (Srichana et al., 1998, Chrystyn, 2007).

The formulation of DPIs is usually drug particles in combination with a carrier, which plays an important role in the formulation stability, mixing and uniformity. The most commonly used carrier is lactose. The principle of operation for most DPIs depend on the turbulent energy generated upon the patient inhalation manoeuvre inside the inhaler, this energy lead to dose de-aggregation and dispersion of the drug formulation. Several studies showed that patient inability to achieve the threshold inspiratory flow lead to incomplete dose de-aggregation and consequently low lungs deposition (Palander et al., 2000, Weuthen et al., 2002).

Nonetheless, new generation DPIs have recently been introduced which have shown to require a low inspiratory flow rate (approximately 30 L/min) and produce higher fine particle fraction with a tendency to lower airways deposition (> 40 % of the nominal dose) (Colthorpe et al., 2013).

Table 2-6: Types of DPIs available in the market (Chrystyn, 2007)

Inhaler Device (Manufacturer)	Dosage forms
Single Dose Inhalers	
Spinhaler® (Aventis)	Spincaps
Rotahaler® (GlaxoSmithKline)	Rotacaps
Aerolizer® (Novartis Pharma)	Capsules
Breezhaler® (Novartis, Pharma)	Capsules
Handihaler® (Boehringer Ingelheim)	Capsules
Multi-dose inhalers	
Multiple Unit-Dose Inhaler	
Diskhaler® (GlaxoSmithKline)	Rotadisk (4-8 doses)
Elipta® (GlaxoSmithKline)	Powder on strip
Accuhaler® (GlaxoSmithKline)	Powder on strip
Reservoir Systems	
Turbuhaler® (AstraZeneca)	Reservoir
Clickhaler® (Innovata Biomed)	Reservoir
Easyhaler® (Orion Pharma)	Reservoir
Novolizer® (Meda)	Reservoir
Genuair® (AstraZeneca)	Reservoir
Twisthaler® (Merk)	Reservoir
NEXThaler® (Cheisi)	Reservoir

The DPIs can be classified depending on the functional element composition into two main groups: single dose inhalers and multi-dose inhalers as described in the Figure 2.19.

Where, in single dose inhalers the dose separate in the form of capsules and has to be filled into the device and pierced by the patient prior to inhalation to allow dose aerosolisation (Chrystyn, 2007).

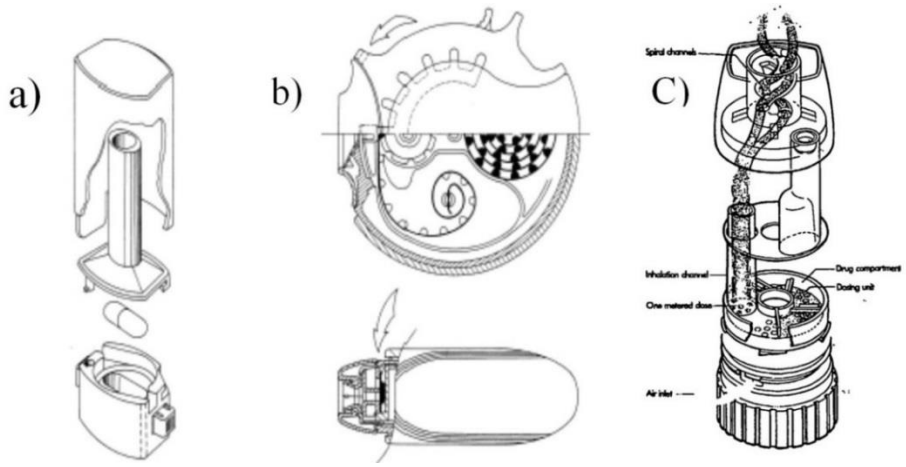

Figure 2.19: Schematic diagram of the marketed DPIs (a: Aerolizer®; b: Accuhaler®; c: Turbuhaler®)

The fact that DPIs require a minimum PIF to activate the device and release the dose, therefore, forceful inhalation manoeuvre is required (Borgstrom et al., 1994, Haidl et al., 2016). It is considerd as the main drawback of the DPIs, as each DPI needs a defined inspiratory flow rate to produce the respirable dose after dispersion. Several studies have shown that not all the patients are capable of producing the required inspiratory flow, furthermore, pre-school asthmatic children, patient with severe COPD and patients with acute exacerbation have a problem to achieve these conditions (Pedersen et al., 1990, Bentur et al., 2004, Al-Showair et al., 2007b). The dose wastage in the use of the DPIs can be reduced by coating the device and capsule with force control agent. Coating has shown to reduce drug retention in the device and increase the fine particle dose (Heng et al., 2013). In fact, there is no ideal inhaler and not all the inhalers are the same. Therefroe, patients using DPIs are recommended to achieve a strong, forceful, deep inhalation maintained for as long as possible (Laube et al., 2011, Scichilone, 2015).

2.6.4.1 Breezhaler®

It is a single dose, capsule based DPI that was introduced by Novartis (Basel, Switzerland) as shown in Figure 2.20. it is being approved for the delivery of both LABA indacaterol and anticholinergic glycopyrronium bromide for the treatment of COPD (Lavorini et al., 2017).

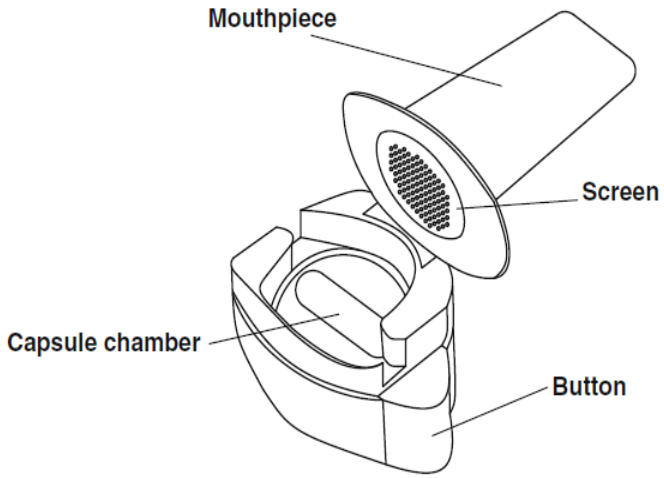

Figure 2.20: Schematic diagram of Breezhaler® Capsule based inhaler

Before an inhalation manoeuvre, the patient has to open the mouth piece of the device, put the capsule in the device chamber, and pierce it by pushing the buttons on the both side of the inhaler device. The audible click sound upon piercing the capsule means that the device is primed, during the inhalation manoeuvre the patient should hear a whirring noise to reassure dose emitted from the capsule. The patients are advised to check if the inhalation manoeuvre was correct by checking amount left in the see-through capsule (Buhl and Banerji, 2012, Berkenfeld et al., 2015). It is a low resistance with an airflow resistance of 0.017 $kPa^{0.5}$ min/L an inhalation flow of approxiemtly 111L/min was required to achieve 4kPa thorugh the inhaler (Pavkov et al., 2010, Dal Negro, 2015). It has been reported that Breezhaler® is more preffered by patients with COPD when compared to Handihaler® (Colthorpe et al., 2013).

2.6.4.2 Aerolizer®

It is a single dose capsule based DPI was introduced mainly to deliver formoterol (Foradil Aerolizer, Novartis, Pharma AG) (Criée et al., 2006). Figure 2.21 shows a schematic diagram of the main functional element of the device. It should be used as instructed in the patient information leaflet (PIL) similar to that illustrated for Breezaler®

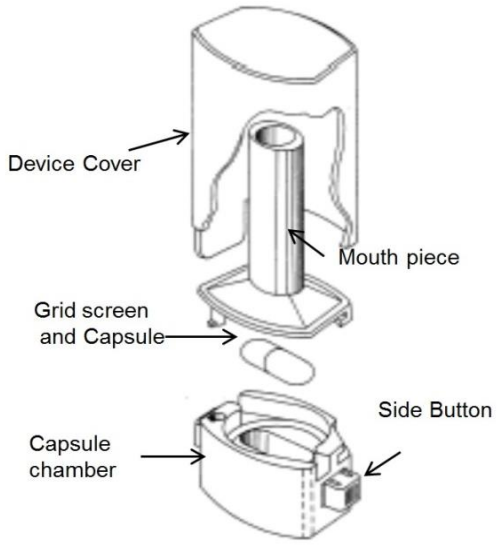

Figure 2.21: Schematic diagram of parts Foradil Aerolizer®

The Foradil Aerolizer is used for the delivery of single dose 12µg doses of LABA formoterol with rapid onset of bronchodilation. It has been shown to effectively provide a long-term control in patients with a different grade of asthma and COPD severity (Bensch et al., 2001, Rossi et al., 2002). It is a low resistance device with an airflow resistance of 0.019 $kPa^{0.5}$min/L and high inhalation flow rate is achievable through this device (Dal Negro, 2015). It has been reported that dose emission from Aerolizer® is flow dependent (Nielsen et al., 1997). Furthermore, high inhalation flow through Aerolizer® may result in unwanted oropharyngeal deposition, therefore, reduction in the amount of drug particles reaching the lower airways (Nielsen et al., 1997, Broeders et al., 2001, Usmani et al., 2005).

2.6.4.3 Easyhaler®

It is a multiple dose inhaler device, which contains 200 doses of medication. It is a high resistance device 0.050 $kPa^{0.5}$ min/L and an inhalation flow of approximately 41 L/min was required to achieve 4 kPa through the device (Dal Negro, 2015). The mixture of APIs and carrier (formoterol and lactose) is stored in a hopper at the bottom of the device. The drug powder is filled on a rotating drum using dosing cup, whereas, shaking the inhaler vertically is a good practice to ensure even dispersion. Upon use, the device is held upright and by pressing the top of the Easyhaler® dosing cup deposit the drug formulation into the inhalation channel and it becomes available for inhalation (Chrystyn, 2006).

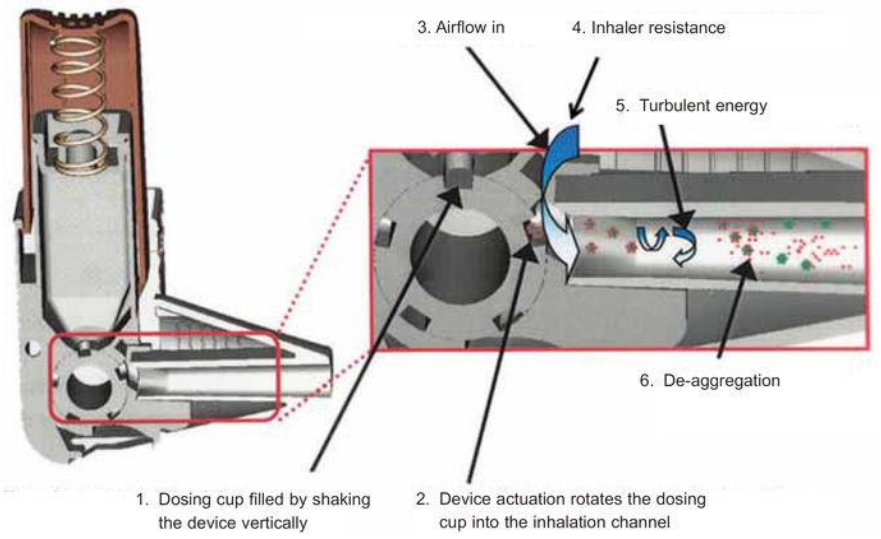

Figure 2.22: Schematic diagram of Easyhaler®

The turbulent energy generated by the patient de-aggregates the dose into fine respirable particle (≤ 5µm) which have high tendency to reach lower airways. The Easyhaler® was designed to overcome the problem of double dosing, with the help of counter mechanism, the patient can see if the dose was loaded or not and prevent the use of an empty device (Azouz and Chrystyn, 2012).

2.7 Mechanism of dose de-aggregation from DPIs

Drug particles for inhalation are generally small ≤ 5μm. Drug partricles are high cohesive and exhibit poor flow rate for this reason they are either formulated as an adhesive mixture using a large carrier such as lactose or formulated as soft spherical pellets of drug alone (De Koning et al., 2002, Chrystyn, 2003, Hoppentocht et al., 2014).

DPIs are breath actuated and an adequate force of inhalation is required to disintegrate the soft pellets or detach drug particles from the surface of the carrier to produce an aerosol cloud for lung deposition. The design of the inhaler device plays an important role in disintegration, de-aggregation and dispersion of DPIs formulation (De Koning et al., 2002, Telko and Hickey, 2005). There are different mechanisms of disintegration varying from the simple screen mechanism such as Rotahaler® and Diskhaler® to a cyclone and twisted powder channels mechanism in Spiromax® and Turbuhaler® respectively. The disintegration systems are divided into two types: - non-specific and specific disintegration (Figures 2.23 and 2. 24). Non-specific system is related to devices with low resistance to inspiratory airflow. These devices are characterised by their large variation to the respiratory flow, inconsistency in dose emission and low fine particle dose (De Boer et al., 1996). In contrast, the specific system is related to devices where the resistance to airflow is high resulting in a reduction in the velocity of the inhaled particles and hence minimising the unwanted oropharyngeal deposition. Therefore, the amount of the inhaled particles reaching the lungs will be maximised (De Koning et al., 2002).

Patient inhalation manoeuvre generates turbulent energy inside the device (Pressure drop), the latter is the product of inspiratory flows and the resistance of the inhaler device (Clark and Hollingworth, 1993) as shown in the mathematical equation highlighted below:

$$\sqrt{\Delta P} = R \times Q \qquad \text{Eq.2.1}$$

Where ΔP; is the turbulent force, Q; inhalation flow, R; the device resistance

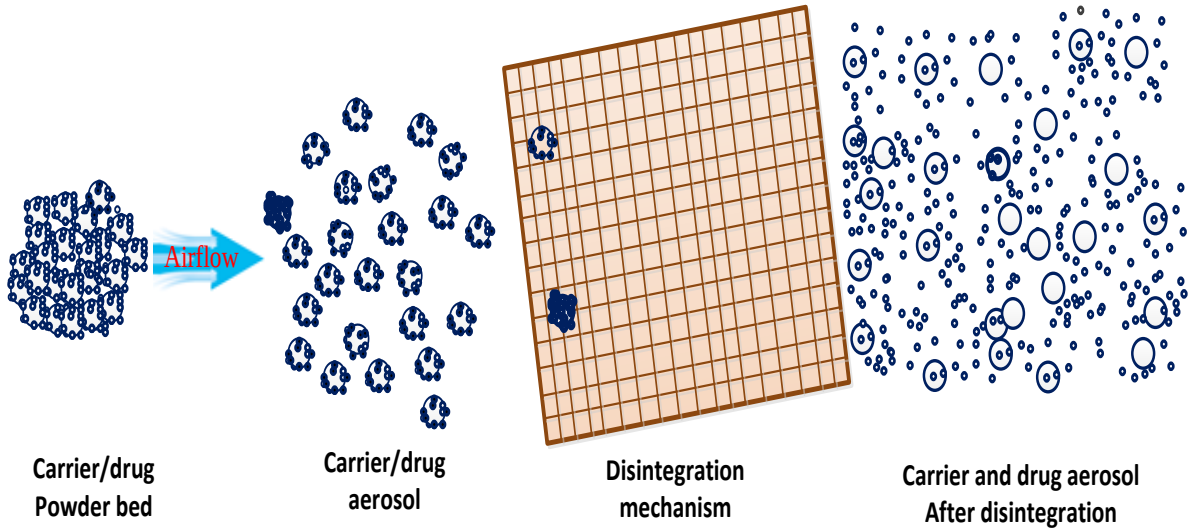

Figure 2.23: Schematic diagram of the dose de-aggregation mechanism of drug particle lactose crystals carrier through non-specific system (De Koning et al., 2002).

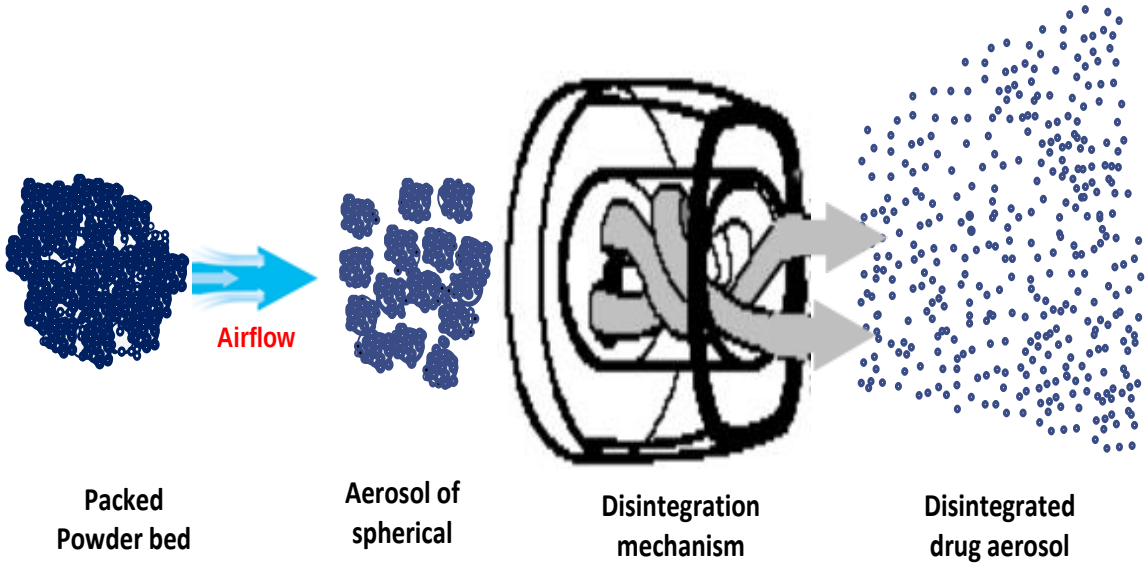

Figure 2.24: Schematic diagram of the dose de-aggregation mechanism of soft spherical pellets through a specific system (De Koning et al., 2002).

The pressure drop that occurs inside the DPIs represent the turbulent energy; it can be measured by different related units either kilopascals (kPa) or cmH_2O [1kPa equal 10.1972 cmH_2O]. This energy force disintegrates the formulation into emitted dose that contains particles with the likelihood for lung deposition (Chrystyn, 2003, Chrystyn, 2009). Passive DPIs require an inspiratory flow which is an important factor to de-aggregate the formulation and produce the respirable dose, whereas large lactose carrier particles will hit the back of throat and which are then swallowed (Pitcairn et al., 1994, Srichana et al., 1998, Virchow et al., 2008).

The successful dose de-aggregation will result in a more lung deposition and low oropharyngeal deposition. DPIs development focuses on achieving maximum fine particle fraction (FPF) which represent particles with ≤ 5 μm, these particles have the tendency for lower airways deposition (Chrystyn, 1999, Bisgaard et al., 2001). Furthermore, particles in the range 2–5 μm are also important for performance effectiveness. Generally, FPFs form approximately 20 -70 % of the emitted dose for which a lung deposition vary between 5 -30% for conventional DPIs (Newman and Busse, 2002, Islam and Cleary, 2012). The device performance and the fine particle dose are dependent on the formulation particle size, strength of cohesive and adhesive forces between drug particles and carriers and finally the patient inspiratory flow to overcome these forces (Telko and Hickey, 2005).

2.8 Factors affecting inhaled drug particles deposition in the lungs

There are different factors that govern inhaled drug particles into the lower airways such as

- The physical characteristics of aerosol inhaled particles (particle velocity, size, density, shape, and porosity)
- Patients' characteristics (disease state, degree of airways obstruction, BMI and age)
- Breathing pattern and the inhalation technique (including MIF, Vin and ACIM) (Heyder, 2004, Tena and Clarà, 2012)

2.8.1 Physical characteristics of inhaled aerosol particles

The particle size of the inhaled aerosol particles plays an important role in the deposition of the inhaled particles. Aerosol's particle size affects both the extent and the site of particles deposition (Chrystyn, 1997, Usmani et al., 2003). The fraction of the fine particles in the dose determines the lung distribution, the dose with high fraction of particles in the range of 2-5μm produce good lung distribution (Chrystyn, 1997). The size of the inhaled particles is characterised by both the mass median aerodynamic diameter (MMAD) and the geometric standard deviation (GSD). The MMAD obtained from the particle size distribution of the emitted dose that divides the cumulative weight into half in which 50% of the particles are larger than the median and 50% of the particles are smaller than the median of the particles. The GSD is representing the dispersion of the inhaled particles whereas particles with ≤ 2.1 are monodisperse and particle with GSD of ≥ 2.1 are polydisperse. The fine particle size dose (FPD) is representing the particles that $\leq 5\mu m$ aerodynamic diameter which is related to a therapeutic dose that reaches lower airways (Pritchard et al., 1986, Schulz, 1998, Musante et al., 2002). The respiratory airways Anatomy has been evolved in a very sophisticated manner and act as a filter to prevent the airborne particle to be inhaled into the lungs.

The particles with MMAD of ≥ 5μm tend to deposit in the oropharyngeal region, while particle with smaller MMAD ≤ 0.5μm penetrates deeper into the airways. However, it can be exhaled before reaching the surface of the airways and produce no therapeutic effect. The FPD divided into three categories depending on particle size 3-5 μm, 1-3 μm and <1 μm (Morén, 1987).

Several studies have been conducted to investigate the effect of holding the breath post inhalation to increase the deposition of the particle with very small MMAD. The exhalation of these particles had been dramatically decreased by holding the breath after the inhalation manoeuvre (Suarez and Hickey, 2000, Usmani et al., 2003, Haughney et al., 2010, Carvalho et al., 2011). The lung deposition is enhanced when inhaled particles are within 2 to 5 μm aerodynamic diameter especially in a patient with obstructive lung diseases as the airways become narrower and aerosol is unlikely to penetrate deeper (Chrystyn, 1999). In contrast particles with an aerodynamic diameter of 2 μm are likely to deposit in the lower airways and alveoli (Usmani et al., 2003, Pedersen et al., 2010). The velocity of inhaled particle can influence the deposition location of the inhaled particles. The impaction of the inhaled particles is increased by increasing the flow rate leading to an increase in the throat deposition (Kleinstreuer et al., 2007). Study conducted on 10 asthmatic patients using salbutamol consisting of particles with MMAD of 1.2, 2.8 and 5μm showed that particles with 2.8μm diameter provided the best bronchodilator response (Zanen et al., 1996). The peripheral airways deposition found to increase with smaller aerosol particles. However, because of no smooth muscle in the alveoli area, dilatation response is less. Suggesting that the particle size of β-2 agonist should be approximately 2.8μm, and for a patient with COPD particles should be within the range between 2-3 μm (Terzano, 2001). Targeting the desired site for inhaled particles for deposition is crucial for effective treatment (Asgharian et al., 2006). The role of the small airways is significant in the treatment of asthma

and COPD, whereas inflammation have been found to be more in the respiratory zone. For an instant, a patient with frequent exacerbation has a higher obstruction in the small airways due to the high air trapping in these airways (Hamid et al., 1997, Battaglia et al., 2005). The submicron particle sizes (≤ 1μm) are not suitable for inhalation, because these particles are normally exhaled from the lungs. Particles in the range of 1-3 μm are preferable for deeper lung deposition. When these particles are inhaled with an adequate flow rate, holding the breath (ideally 10s) will enhance the distal airways deposition (Usmani et al., 2005, De Boer et al., 2015). The particles with an aerodynamic diameter of ≤ 3μm have 80% chance of deposition in lower airways of which 60% being deposited in the alveoli (Patton, 1996, Laube et al., 1998). The particle size of inhaled aerosol with an MMAD ≤ 2μm should be used to enhance the lung deposition of jet nebulisers, thus reducing the dose used for providing therapeutic effect.

Furthermore, the safety and efficacy of the inhaled aerosol is dependent on the particle size as it affects both extent and site of deposition (Clay and Clarke, 1987). Another study showed that less amount of terbutaline was produced from formulation containing drug with particle size ≥ 5μm diameter, resulting in most of the dose deposited in the oropharyngeal region (Rees et al., 1981). Particles diameter, particles shape of drug and carrier are other important parameters to consider when formulating DPIs. Elongated drug particles and carrier have been shown to enhance *in-vivo* salbutamol deposition (Larhrib et al., 2003). The optimum lung deposition site varies between different inhaled medications. Furthemore, Usmani et al., 2005 highlighted that for bronchodilators (β-2 agonists and anticholinergic) smaller particles 1.5 μm showed no therapeutic effect notwithstanding reaching the lower airways. Therefore, proximal deposition for particles with 3-6 μm diameter have been found to provide more bronchodilatation effect (Usmani et al., 2005, Virchow et al., 2008, Haughney et al., 2010)

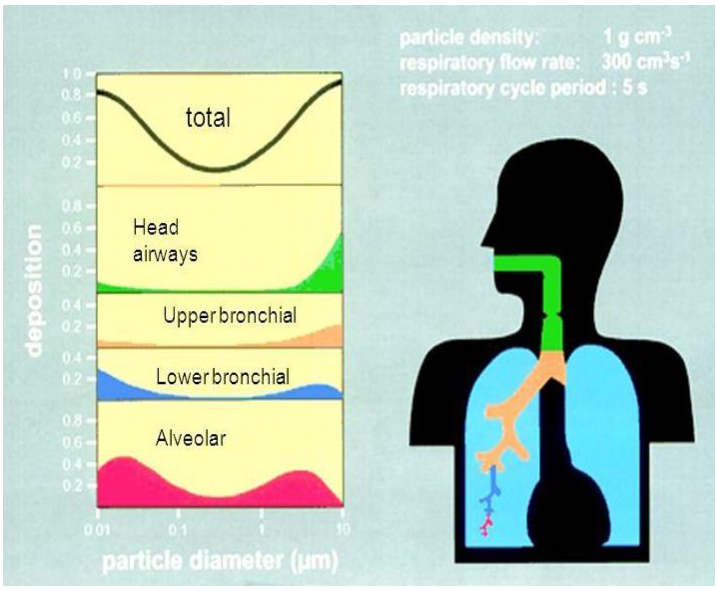

Figure 2.25: Total and regional deposition of unit density spheres in the human respiratory tract predicted by the ICRP deposition model for oral inhalation at rest (Heyder, 2004).

Studies have shown that most currently prescribed corticosteroids are deposited mostly in the central airways (Esmailpour et al., 1997), uniform central and periphral distribution is more preferred for inhaled corticosteroid (Hamid and Tulic, 2007). Mitchel et al., 1987 showed that no difference in the particle size and therapeutic effect of two aerosol particle sizes of salbutamol (1.4 and 5.5 µm) diameter in a patient with stable severe asthma (Mitchell et al., 1987). The drug particles in the formulation of DPIs are normally hygroscopic. Therefore, particles grow in size when they entering the lungs. The relative humidity of the lungs are approximately 99.5% which may cause change in size distribution of inhaled particles (Labiris and Dolovich, 2003).

The initial particle size is an important factor that determines the rate of particles growth, whereas particles with aerodynamic diameter of $\leq$ 1µm can be increased up to 5 folds in size in contrast to only three folds rate of growth for particles with aerodynamic diameter of $\geq$ 2µm (Labiris and Dolovich, 2003). The change in the particle size as result of the growth that takes place in the respiratory airways can alter the deposition site of the inhaled aerosol particles, and

thereby result in more proximal or central deposition (Barnes and Godfrey, 1998). The particles emitted from pMDIs have shown to be less affected by the hygroscopicity when compared to particles emitted from DPIs (Terzano, 1999). The particles deposition in lower airways affected by the density and porosity of the particles in the formulation. The deposition of particles with high density and a small GSD was higher when compared to particles with similar aerodynamic characteristics but had lower density and larger geometric size (Heyder, 2004). It has been reported, "Polydisperse aerosols containing a significant proportion of submicron particles will deposit in the pulmonary airways with greater efficiency than aerodynamically similar aerosols comprised of geometrically larger porous particles" (Musante et al., 2002).

2.8.2 Patient's inhalation variables and characteristic

2.8.2.1 Airways obstruction

The patient's characteristics can influence the particle deposition in the respiratory airways for example COPD and asthma lead to narrowing and obstruction in the respiratory airways. The airways geometry has been shown to have an effect on the deposition of the particles especially that $\geq$ 6μm (Inthavong et al., 2010). The air stream is diverted from the obstructed to unobstructed airways resulting in an increase in the airstream velocity and hence deposition in the central airways by the high impaction force, and thereby fewer particles in the obstructed airways (Dolovich and Labiris, 2004, Luo et al., 2007). Several studies have shown the correlation between the patients FEV1 and the particle deposition in the lungs. COPD patients have shown less total lungs deposition compared to healthy volunteers. This difference was attributed to lower FEV1 in the COPD patients in addition to high obstruction in the airways (Pavia et al., 1977). The increase in the peripheral lungs deposition can be achieved when the FEV1 increase following bronchodilation (Sanchis et al., 1973, Zainudin et al., 1990, Usmani et al., 2005).

2.8.2.2 Age and Gender

The age and gender also affect the inhaled particles deposition (Figure 2.26), the decrease in the lung volume as well as the anatomical changes to the respiratory airways with age lead to decrease in the overall particles deposition and more central deposition (Agnew, 1984, Hussain et al., 2011). The gender, studies found that women have a high amount of inhaled particles deposited in the upper airways due to the changes in the geometry of the respiratory tract (Pritchard et al., 1986). Kim and Hu, (1998) study shown that ‘‘Total lung deposition was comparable between men and women for fine particles (0.1 to 2.5μm particles diameter) but drug particles deposition was consistently greater in women than men for coarse particles regardless of flow rates used’’(Kim and Hu, 1998).

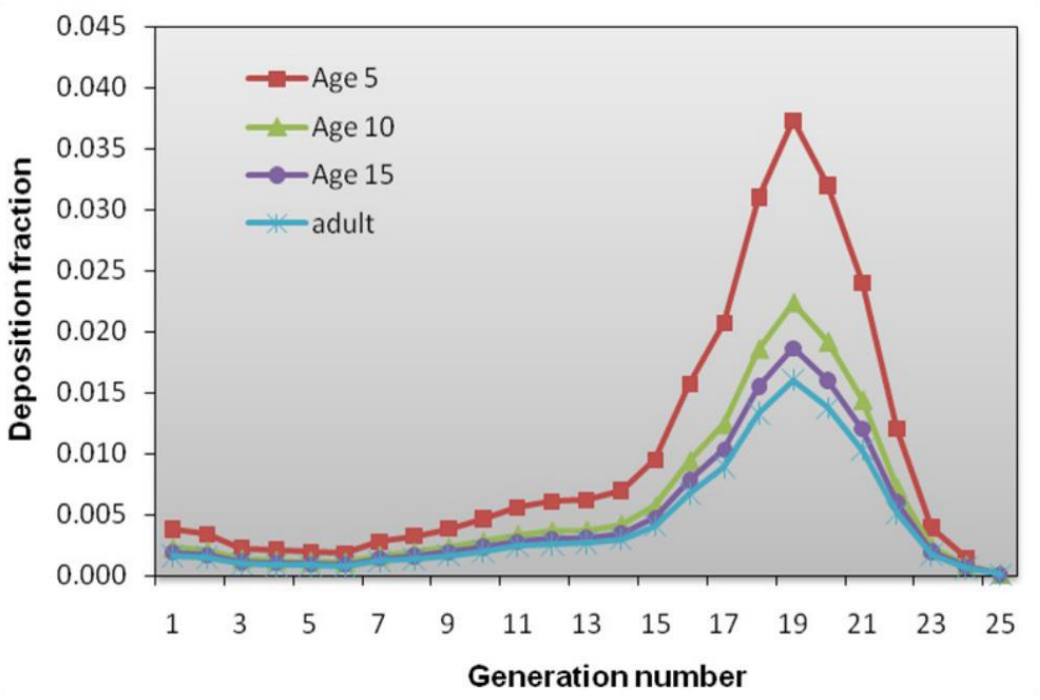

Figure 2.26: The difference in the geometry of the lungs and the total lung deposition for 5, 10 and 15 children and adult for 3μm particle size (Hussain et al., 2011)

2.8.2.3 Breathing pattern and inhalation technique

The regional deposition of inhaled aerosol particles is affected by patients' inspiratory flow rate. Increasing the tidal volume at constant flow can result in an increase in the residence time of particles, so more particles tend to get deposited in the peripheral airways by the gravitational mechanism (Gehr and Heyder, 2000, Musante et al., 2002). The technique of breath holding after each inhalation when using pMDIs for 10s was found to increase inhaled aerosol particle deposition (Newman et al., 1982), whilst allowing monodisperse particle deposition in the peripheral airways (Heyder, 2004). Furthermore, inhalation speed is correlated with the particle size of MDIs, which ranges from 3 to 6 μm; MDIs were found to have a greater bronchodilation effect when inhaled slowly (Usmani et al., 2005, Virchow et al., 2008). The deposition in the lungs generally increases with the duration of the breathing cycle (Adcock and Chung, 2008). Slow flow rate was found to increase the deposition in the small airways by up to 70% (Svartengren et al., 1996). In the case of DPIs, forceful inhalation is required to de-aggregate the formulation into respirable particles, given the low rate of deposition (Everard, 2001, Everard, 2003, Chrystyn, 2007, Haughney et al., 2010).

Patients' inhalation techniques as well as the correct use of the inhaler device are important factors that affect the deposition of inhaled aerosols in the lungs. In fact, maximising drug deposition involves a complex inter-relationship between the formulation, patient's inhalation manoeuvre (including the inhaled volume, inspiratory flow, breath holding after inhalation), and inhaler device (Timsina et al., 1994, Azouz and Chrystyn, 2012). Several studies have shown that training patients on how to use the inhaler devices can help enhance the overall therapy outcome and increase deposition in the lungs (Giraud and Allaert, 2009, Bonini and Usmani, 2015). Patient preference for specific inhaler devices should be taken into consideration, as the

appropriate device can enhance patient adherence and therapy outcome (Lavorini and Fontana, 2014). Dal Negro and Povero (2016) stated, "Substantial differences exist in patient's preference and acceptability of inhalation devices, mainly related to the handling and the understanding of the different devices" (Dal Negro and Povero, 2016). Choosing the right inhaler device for the right patient is as important as the medication itself owing to the differences in patient preference and the inhalation capability. It is also important to increase patient adherence to the inhaler device (Al-Showair et al., 2007b, Chrystyn and Price, 2009, Haughney et al., 2010, Scichilone, 2015). Some patients with asthma and COPD find it difficult to achieve the required inspiratory flow rate for effective dose emission and training them on how to use their inhaler devices was found to be useful in improving inhalation flow through DPIs. In contrast to DPIs, patients have shown good practice of slow inhalation through pMDIs (Al-Showair et al., 2007b). Therefore, educating the patients and providing training support can be helpful in improving poor inhalation technique and the use of inhaler devices (Lavorini et al., 2014, Canonica et al., 2015).

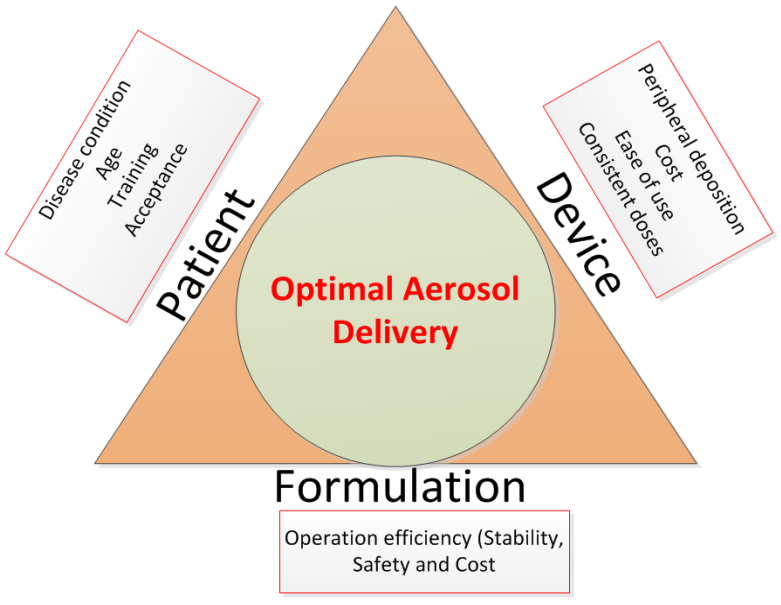

Figure 2.27: Factors affecting the optimal aerosol dose delivery (Ibrahim et al., 2015)

2.9 Patient inhalation profiles

The difference in the patient's COPD and asthma results in a difference in the patient lungs capabilities, whereas the inhalation profile (IP) of the patient can be characterised by different parameters, starting from the flow patient use to inhale and exhale, lungs volume and finally the initial acceleration rate of inhalation manoeuvre.

The IP of the patient as shown in (Figure 2.28) characterised by following main parameters:

- Peak inspiratory flow rate (PIF) expressed in unit of [L/min or mL/s]
- Inhalation volume (Vin) [L or mL]
- Initial acceleration rate (ACIM) [L/s^2]
- Time to the peak inhalation flow (Tp) and the inhalation time (Ti)

The pressure profile that occurs as result of the patient inspiratory flow and inhaler resistance can be transferred into flow profile (2.28) if the device resistance is known (Clark and Hollingworth, 1993, Olsson and Asking, 1994, De Boer et al., 1996, Olsson et al., 2013).

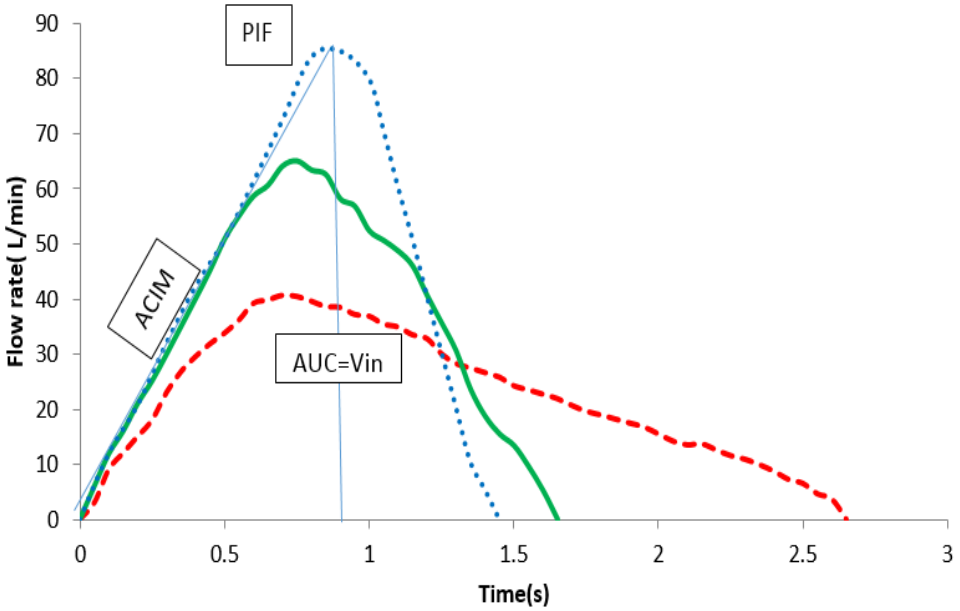

Figure 2.28: Schematic diagram showing different inhalation profiles among patient with COPD [Red; Low profile, Green; Medium profile, Blue; high profile]

Breath-actuated DPIs depend on the PIF, which is one the most important parameters of the IP to achieve adequate dose de-aggregation. The minimum PIF for each DPI should be achieved to generate the required turbulent energy for effective release of the dose (Clark and Hollingworth, 1993, Laube et al., 2011, Price and Chrystyn, 2015). For instance, Easyhaler® requires a PIF of approximately 28 L/min (Koskela et al., 2000) whereas Accuhaler® requires of 30 L/min (Nielsen et al., 1998) and Aerolizer® requires forceful inhalation to release the dose (Nielsen et al., 1997). An inhalation flow rate greater than 30 L/min was required for effective dose emission from Turbuhaler® (Pedersen et al., 1990, Nadarassan et al., 2010). Several studies demonstrated that during the routine use of DPIs, some patients found it difficult to achieve a fast flow rate (Pedersen, 1986, Pedersen and Steffensen, 1986, Chrystyn, 2003, Azouz et al., 2015b). Failure to achieve the minimum flow rate might result in the patient receiving no medication. Patients who cannot achieve the desired inhalation flow, such as children and patients with severe asthma and COPD, through the DPIs should be prescribed low-resistance DPIs or pMDIs (Pedersen, 1986, Chrystyn et al., 2002, Price et al., 2013).

Chrystyn and Price (2009) discussed the relationship between the dose emissions from different DPIs (capsule, blister, or reservoir-based DPIs) and patient IP (Figure 2.29). As compared to other devices, dose emission from the capsule-based DPIs does not occur immediately after the start of the inhalation manoeuvre. Capsule-based DPIs require more inhaled volume than blister and reservoir devices to empty the dose. For this reason, in the PIL, the manufacturers recommend the patients inhale twice for each capsule to ensure dose emptying (Chrystyn and Price, 2009, Laube et al., 2011).

The PIF achieved through DPIs differs depending on the intrinsic resistance of the device; for instance, fast inhalation flow through low-resistance devices (*e.g.*, Aerolizer® and Breezhaler®) can lead to undesired oropharyngeal deposition (Borgström, 2001, Haughney et al., 2010). Low-resistance DPIs show less flow rate dependency than other types of DPIs (Palander et al., 2000, Weuthen et al., 2002).

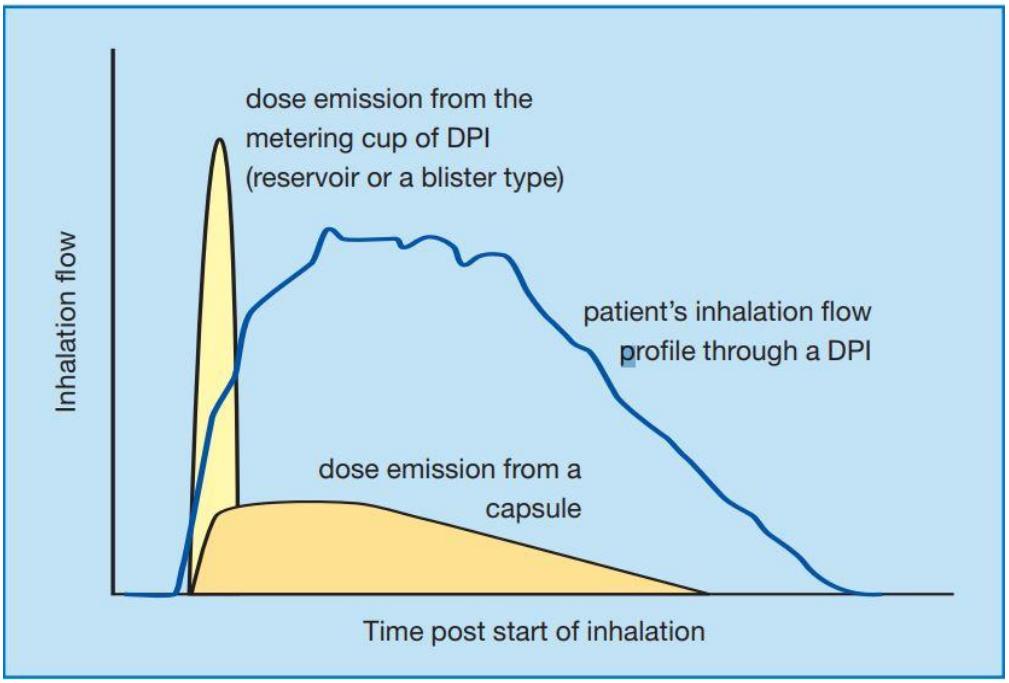

Figure 2.29: Schematic design of patient inhalation profile through different type of DPIs formulation (Chrystyn and Price, 2009)

In contrast, high resistance DPIs devices showed a high lung deposition and less oropharyngeal deposition, which might be as result of the high-pressure change in these type of the DPIs (Borgström, 2001). Figure 2.29 also highlights the difference in the ACIM (Fast and slow ACIM) for two profiles despite the fact both profiles have the same PIF. Several studies have shown the importance of fast inhalation from the start of the inhalation manoeuvre to effectively de-aggregation the dose formulation (Everard et al., 1997). Fast acceleration rate was found to increase the total emitted dose (TED), Fine particle dose (FPD) and reduce the MMAD (Kamin et

al., 2002, Laube et al., 2011). The efficiency of the DPIs depending on the PIF achieved by the patient and this must be generated from the start of the inhalation (De Boer et al., 1997). It has also been shown that PIF and fast ACIM are directly related and playing an important role in appropriate dose de-aggregation from DPIs (Broeders et al., 2001).

Vin is another important factor in the dose emission from the DPIs (Kamin et al., 2002, Alaboud et al., 2010). The Vin was found to be different between DPI devices; some devices require more inhaled volume than others do. For example, the Diskus® requires a Vin as low as 150 mL in contrast to the Turbuhaler® which require a minimum of 1 Litre inhaled volume to empty the dose (Azouz et al., 2015b). The difference in the inhaled volume between DPIs was attributed to their internal design and the intrinsic resistance. For example, a significant increase in fine particle fraction was observed with the Aerolizer® when the air inlet size was reduced. These results were explained by the increase in the air velocity and turbulent inside the inhaler device (Zhou et al., 2013, Shur et al., 2015).

The difference in the Vin between the Diskus® and Turbuhaler® was attributed to the length of the inhalation channel. The inhalation channel in the Turbuhaler® is relatively long and includes a cyclone, whereas the inhalation channels of the Diskus® is very short, thus dose is expected to be released rapidly from the Diskus® compared to Turbuhaler®. The low resistance devices have also been reported to require larger inhaled volume when compared to the higher resistance devices (Azouz and Chrystyn, 2012). The Vin is an important factor for dose emptying and delivers the particles deeper into the respiratory airways especially for capsule-based DPIs (Laube et al., 2011).

Pavia et al., 1977 showed that inhaled drug particle could travel deeper into the lungs by increasing the Vin during the inhalation manoeuvre (Pavia et al., 1977). Focusing on the

minimum force to de-aggregate the dose from the DPIs should be considered for successful dose emission. The patients cannot inhale in the same manner, therefore prescribing the right inhaler for the right patient and evaluating whether the patient can achieve the minimum inhalation flow to actively operate the device is critical for successful therapy outcome (Chrystyn, 2003, Al-Showair et al., 2007b, Chrystyn, 2009).

2.10 DPIs internal resistance to airflow

The dose emission from DPIs depends on the effect of different inspiratory parameters such as MIF, Vin and ACIM. In addition, dose emission from the device relies on its resistance, which differs from one device to another (Clark and Hollingworth, 1993). The equation (2.1) relates the peak inhalation flows, the resistance of the inhaler device and Clark and Hollingsworth (1993) describe the turbulent energy as follows:

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

Where ΔP; is the turbulent force, Q; inhalation flow, R; the device resistance

The DPIs resistance to airflow can be defined as following: $R = \sqrt{\Delta P}/Q$. The change in the pressure across the device is directly related to the inhaler resistance to airflow (R) and the inspiratory flow rate (Q) (Clark and Hollingworth, 1993). Therefore, the inhalation flow for a set inspiratory effort will be lower when the resistance inside the device is high, generally the inspiratory flow rate through high resistance device is lower than low resistance device (Assi and Chrystyn, 2000, Azouz and Chrystyn, 2012). The intrinsic resistance is usually deduced from the slop of the line of the $\sqrt{\Delta p}$ vs inhalation flow, examples of intrinsic resistance of some marketed DPIs are reported in Table 2.7

Table 2-7: The resistance of some marketed DPIs (Frijlink and De Boer, 2004)

Inhaler	Resistance($KPa^{0.5}$.min/l)
Spinhaler® (Aventis)	0.016
ISF Inhaler Cyclohaler	0.019
Novolizer® (Viatris)	0.028
Diskhaler (GlaxoSmithKline)	0.032
Diskus® (GlaxoSmithKline)	0.034
Ratiopharm® Jethaler (Ratiopharm)	0.036
Handihaler® (Boehringer Ingelheim)	0.042
Turbuhaler® (AstraZeneca)	0.0367
Inhalator® (Boehringer-Ingelheim)	0.0540

Drug deposition in the oropharyngeal cavities can be reduced by using a mouthpiece, which controls the resistance of the inhaler to the airflow and the direction of the aerosol cloud in the mouth and throat (De Boer et al., 1997). Each DPI is unique characterised by its intrinsic resistance, which results in a difference in the PIF generated during the inhalation manoeuvre and the dose emission released from the inhaler device. The relationship between PIFs and pressure drop through the inhaler device (Chrystyn, 2009). The relation between the $\sqrt{\Delta \mathbf{P}}$ and inhalation flow is linear for all the inhaler devices. The slope of the line represents the intrinsic resistance of the device. The slope of the line is higher for high resistance devices and Vice versa. To achieve for example a pressure drop of 6 Cm $H20^{1/2}$ requires an inhalation flow of 100 L/min from an Aerolizer® (Low resistance device) and about 45 L/min from an Easyhaler® (High resistance device). The Figure 2.30 shows that all DPIs are not the same and prescribing the right inhaler is important to ensure that patient is comfortable using the device and more importantly can receive the required dose (Chrystyn, 2007, Chrystyn, 2009).

The difference in the DPIs devices design, geometry and dimension result in an intrinsic resistance to airflow through the inhalers. The intrinsic resistance of DPIs differ between devices due to the difference in the design (Dal Negro, 2015). There are different elements that determine the intrinsic resistance of DPIs including air inlet, inhalation channel and the mouthpiece of the inhaler. These elements lead to an increase in the kinetics energy of the airflow during the inhalation manoeuvre.

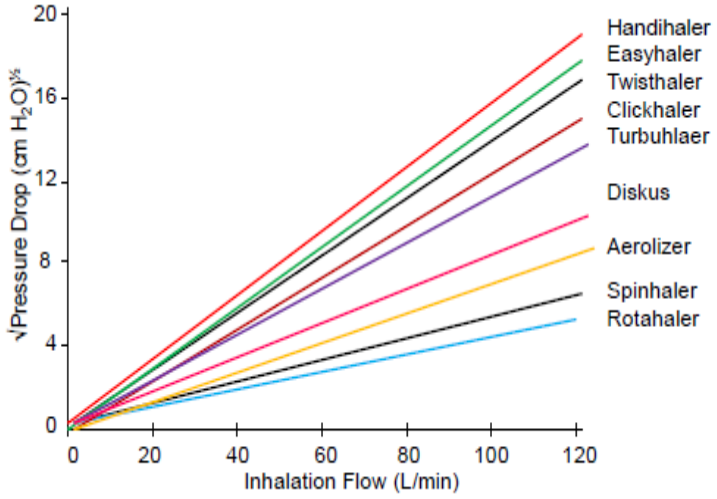

Figure 2.30: The resistance of different DPIs (Chrystyn, 2009)

The quality of the emitted dose from DPIs are controlled by different parameters such as inspiratory flow rate generated by the patient, intrinsic resistance and type of the inhaler device and the powder formulation (Clark and Hollingworth, 1993, Chrystyn, 2003, Chrystyn, 2007, Ibrahim et al., 2015). The resistance of the DPIs is an important factor in air flow restriction and therefore in minimising the oropharyngeal deposition of the aerosol particles (Dolovich, 1995, Price and Chrystyn, 2015). The inhaler devices were classified into 4 categories according to their inhalation flow requirement to achieve 4kPa pressure drop across the inhaler device as follow: low, medium, medium-high and high resistance inhaler (Dal Negro, 2015).

Lung deposition was found to be greater with a high resistance DPIs (Svartengren et al., 1995, Chrystyn, 2009). Thus inhaling against a high resistance device has been shown to reduce the oropharyngeal deposition and enhance the peripheral deposition of the drug particles due to reduction of inhaled particles momentum (De Boer et al., 1996, Borgström, 2001). The DPIs with high resistance along with greater patient airways obstruction will result in low inspiratory flow rate achieved by the patient (Chrystyn, 2009, Azouz et al., 2015b).

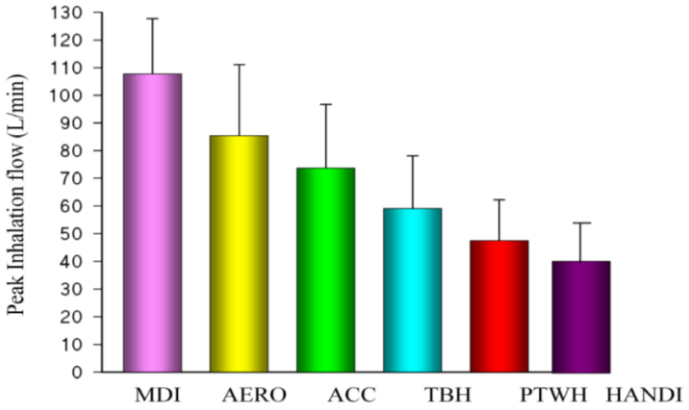

Figure 2.31: Mean (SD) peak inhalation flows of asthmatic patients inhaling through different marketed inhalation devices MDI and DPIs (Azouz et al., 2015b)

Since DPIs are flow rate dependent and achieving the desired flow rate is crucial for overall management of airway disease. Therefore the efficacy of the medication can be affected when a patient of acute wheeze or low lungs function fail to meet inhalation manoeuvre requirements to release the dose from the inhaler device (Pedersen, 1987). Not all the patient inhale through DPIs in the same manner due to the difference in the inhaler resistance, then DPI design and formulation should be suitability tailored for a wide range of patients of all age and disease severity. It is widely accepted that an inhalation flow rate of 30L/min through a DPI is the minimum threshold energy for dose emission as demonstrated with the Turbuhaler® device (Newman et al., 1991a, Bisgaard et al., 1998).

2.11 In-vitro dose emission from DPIs

The DPIs are known to operate due to the turbulent energy that created inside the inhaler by the inhalation manoeuvre. The interaction between the patient inspiratory flow rate and the specific resistance of the device determine the quality of the emitted dose (Clark and Hollingworth, 1993, Chrystyn, 2003, Al-Showair et al., 2007b). The turbulent energy increase with the inhalation flow rate therefore forceful and fast inhalation from the start of inhalation manoeuvre through DPI will lead to a much better dose de-aggregation (Laube et al., 2011, Dal Negro, 2015). Several studies have been conducted to show the flow dependent dose emission of different DPIs.The size distribution and dispersions pattern of aerosol from DPIs are dependent on the flow rate through the device. Formoterol emitted from an Aerolizer® was examined within the flow range of asthmatic school children aged 3-10 years (Nielsen et al., 1997). The results of the study show the flow dependency of Foradil Aerolizer® within the PIF obtained from the schoolchildren.

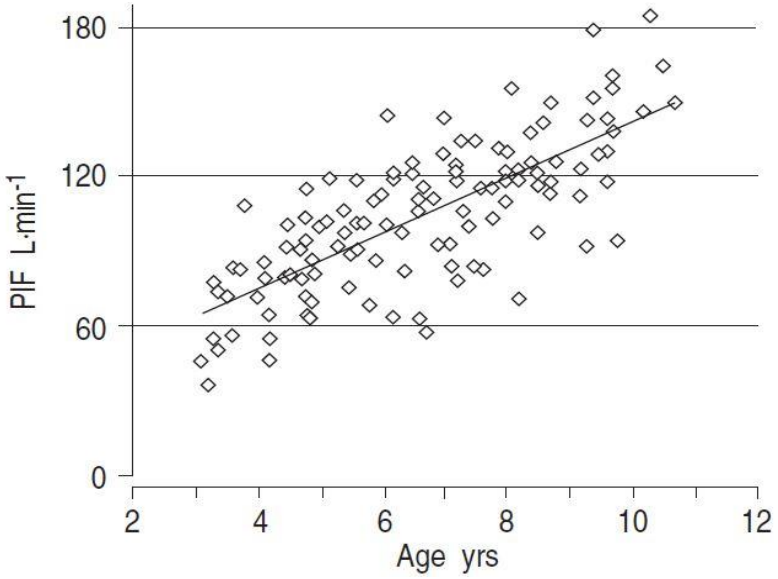

Figure 2.32: Relationship between the Peak inspiratory flow (PIF) through Aerolizer® and age in 126 asymptomatic, asthmatic children aged 3-10 years

Ross and Schultz (1996) have compared the dose delivery of three different inhaler devices Diskhaler®, Rotahaler® and Turbuhaler® at two MIF values of 30 and 60 L/min, the study results have shown that DPI dose emission was less than the labelled dose and more dependent on the inhalation flow (Ross and Schultz, 1996). The performance of two inhaler device MDI and DPI containing the same drug powder and strength was studied at different MIF values of 30, 60 and 90 L/min. the results showed that DPI was more flow rate dependent than MDI (Feddah et al., 2000).

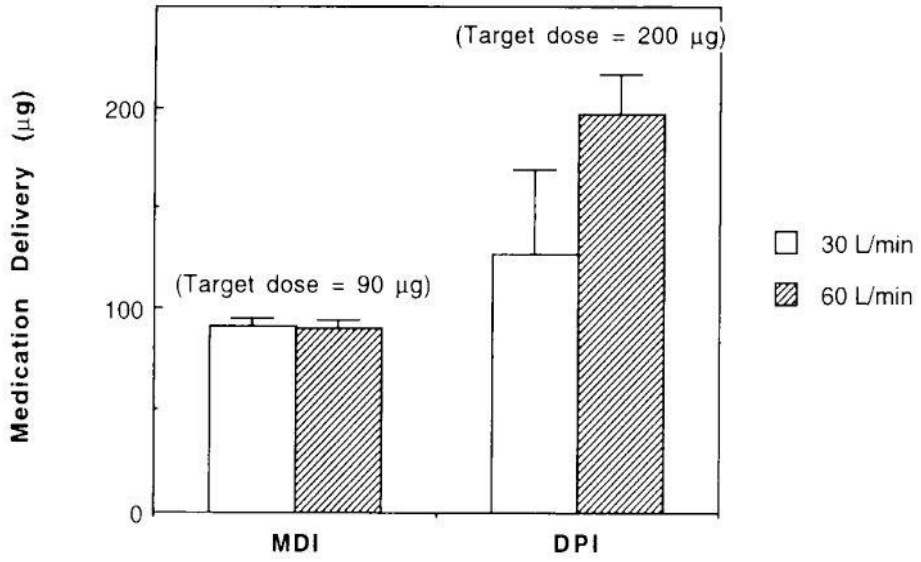

Figure 2.33: dose emission from a salbutamol pMDIs and Diskhaler® at different inhalation flows (Ross and Schultz, 1996)

The dose de-aggregation of three DPIs namely (Turbuhaler®, Spinhaler® and Diskhaler®) have been shown to increase with PIF. Hence fine particle dose as a percentage of the nominal dose has been shown to be high 35-40% with Turbuhaler®. In contrast, the percentage of fine particle dose was only 10 and 23% for Spinhaler and Diskhaler respectively at 60L/min flow rate (De Boer et al., 1996). Furthermore, dose emission from the Accuhaler® has been shown to be independent to flow rate (Ashurst et al., 1998).

The effect of PIF and IFR on different DPIs was studied, results showed that Turbuhaler® is more sensitive to IFR and producing higher fine particle dose when compared to Accuhaler® and Cyclohaler® (De Koning, 2001). The de-aggregation of the inhaled aerosol (formoterol) was dependent on the PIF for both Aerolizer® and Turbuhaler®. The amount of formoterol reaching the alveolar is significantly increased for PIF > 28.3 L/min (Hill and Slater, 1998) suggesting breakage of drug aggregates to produce fine aerosol for deep lung penetration. The impaction of the PIF was further confirmed using gamma scintigraphy (Dolovich et al., 1988, Newman et al., 1991a, Borgstrom et al., 1994) showing an increase of the total lung deposition with increasing the PIF for both terbutaline and budesonide from Turbuhaler®. The inhaled particles sizes have shown to be affected by the PIF, both Aerolizer® and Turbuhaler® found to have the same fine particle (Figure 2.34). However, Turbuhaler® shows more dose emission dependency than Aerolizer® (Weuthen et al., 2002).

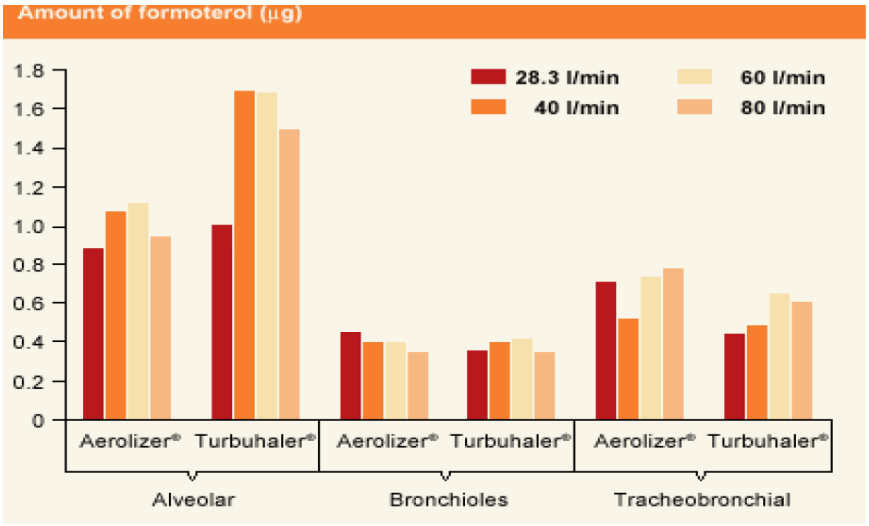

Figure 2.34: In-vitro dose emission comparison of two DPIs (Turbuhaler® and Aerolizer®) at different PIFRs (Weuthen et al., 2002)

There is a required threshold for each DPIs for adequate dose dispersion and more respirable particle with high lung deposition (≤ 5µm) (Chrystyn, 2003, Haughney et al., 2010). Sumby et

al., (1992) demonstrate that low resistance device such as Diskhaler® requires an inspiratory flow which is half that required for high resistance device such as Turbuhaler®. The study determines that inspiratory effort which is a function of pressure drop and the inhaled volume is more insightful than inspiratory flow when assessing patient usage of DPIs (Sumby et al., 1992). The performance of the Foradil Aerolizer® and Oxis Turbuhaler® at high and low inhalation flow rates were investigated using ten doses out of the entire content. Results showed that the fine particle dose was equivalent at high PIF but not at low; this might be due to the difference in the formulation or aerosolisation mechanism (Chew and Chan, 2001).

The flow dependent dose of combination (Formoterol and Budesonide) emitted from Turbuhaler® has been studied. The results showed (Figure 2.35) the effect of increasing PIF was more noticeable in the fine particle dose. Furthermore, dose emission was ≥ 100% of the nominal dose at 90 L/min, comparing to less dose emitted at a flow rate of 30 and 60 L/min (Tarsin et al., 2004).

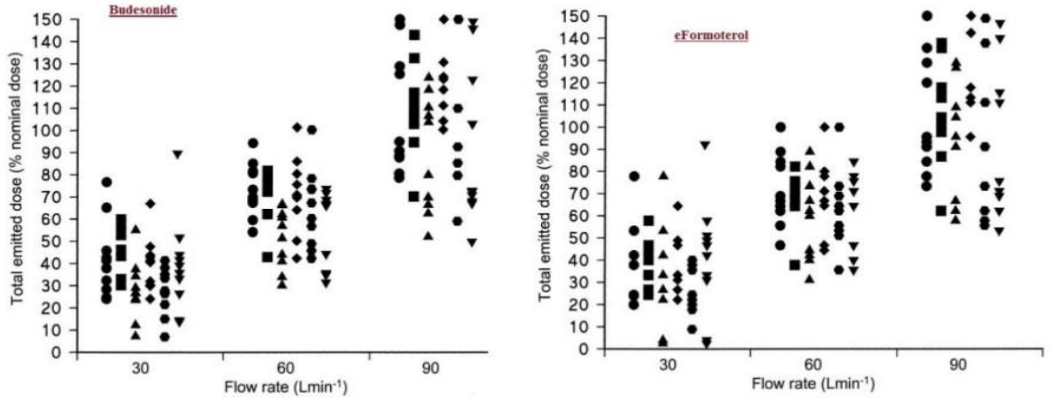

Figure 2.35: The amount of budesonide and formoterol (% of labelled dose) from six inhalers tested using in-vitro inhalation flow rates 30, 60 and 90L/min (Tarsin et al., 2004)

In a different study, within the clinical range of inhalation flows (15 to 60 L/min), the Clickhaler showed inhalation flows independence for adults or children above six years (Newhouse et al.,

1999). The fast inhalation flow rate showed significant dose emission compared to slow flow rate from Bricanyl Turbuhaler® (500µg), suggesting Patients with low inhalation flow rate and those who cannot achieve a high Vin should inhale twice and as deep and hard as possible (Abdelrahim, 2010).

The flow dependent dose emission property of three different DPIs was studied, as shown in Figure 2.36. When inhalation flows increased a significant difference in the emitted dose, and fine particle fraction was shown with Turbuhaler® (Palander et al., 2000). On the other hand, increasing the inhalation flows from 30 to 90 L/min produced less difference in the emitted dose and the fine particle fraction from an Easyhaler® and Accuhaler® suggesting that DPI performance is also inhalation device dependent (Palander et al., 2000).

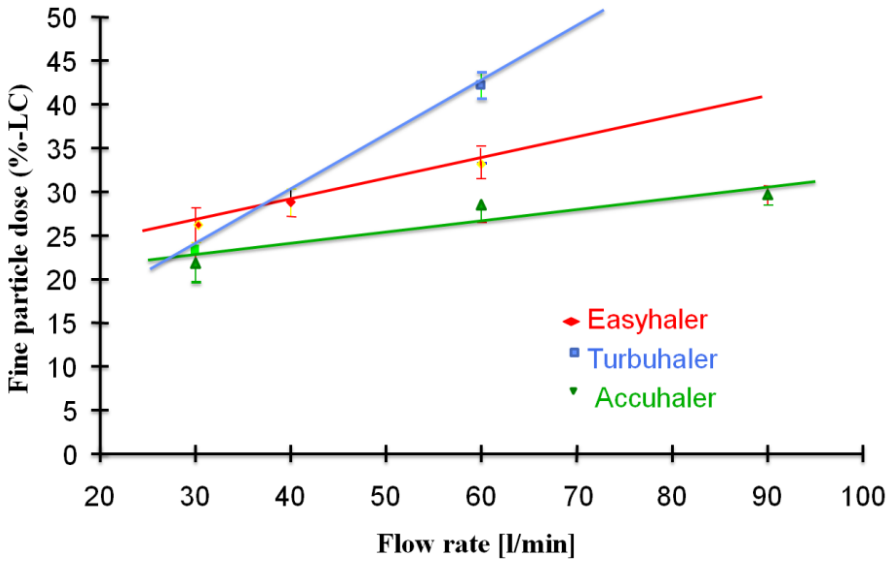

Figure 2.36: Fine particle dose (% of labelled dose) for three types of salbutamol containing DPIs (Palander et al., 2000)

The *in-vitro* study was conducted to assess the dose consistency from Salbutamol Diskus® and Terbutaline Turbuhaler®; the results showed that dose emitted from Diskus® was more consistent

throughout the device life when compared to Turbuhaler® at flow rates of 30, 60 and 90 L/min (Malton et al., 1995). The changes up to 6 folds in the inhalation flow rate showed no changes in the total emitted dose and fine particle fraction for Diskus® inhaler. Suggesting that Turbuhaler® is more dependent on the inhalation flow rate (Prime et al., 1995).

In another study, most of the young patients including three years old children were able to achieve PIF of ≥ 30 L/min through Accuhaler®, which means 100% labelled dose would be received by the patients (Gunawardena et al., 1995). The Turbuhaler® and Diskus® should be used with caution in young children, the reason for that is the children are unable to achieve the required flow rate through Turbuhaler® resulting in low and variable dose emission (Bisgaard et al., 1994). Several studies investigated the inhalation flow through different DPIs; in some devices, 30L/min was not sufficient to de-aggregate the dose such as Turbuhaler® (Nadarassan et al., 2010). However, 30L/min was found to be sufficient to operate the Easyhaler® (Koskela et al., 2000) and provided a similar result to that achieved with pMDIs attached to a spacer which requires a flow rate < 30L/min (Newman et al., 2001).

Newman et al., (2001) have reported that MMAD decreased with an increase in the PIF. Therefore, the central lung deposition can be avoided with the smaller MMAD during the fast inhalation flow. Novolizer® showed an increase in the lung deposition with the flow rate, but no improvement in the penetration index. Abdelrahim et al., (2013) showed Turbuhaler® flow rate dependency, whereas TED and FPD have improved significantly with the increase in the flow rate over the range of (10-60 L/min). The more effect was seen at a flow rate of 30 L/min suggesting that pharmacopoeia should consider more flow rates than the one corresponds to 4kPa pressure drop through the inhaler (Abdelrahim et al., 2013).

The effect of the Vin on the dose emitted from three DPIs Autohaler®, Accuhaler® and Turbuhaler® have been investigated using Andersen cascade impactor with different inhaled volumes (Vins) 0.15, 0.5 and 1L (Kamin et al., 2002). The results showed that the change in the Vin showed no effect on the MMAD of the particles that were emitted from the Turbuhaler®; nevertheless, changing the Vin had a significant effect on the mass output. Furthermore, the Turbuhaler® showed a high variation in the fine particle fraction, with 3.4% to 22.1% of the labelled dose, but less effect was observed with Accuhaler® 10.6% to 18.5 % of the labelled dose. The Autohaler® showed more constant output and a high fine particle fraction of 43.1% to 56.6% of the labelled dose.

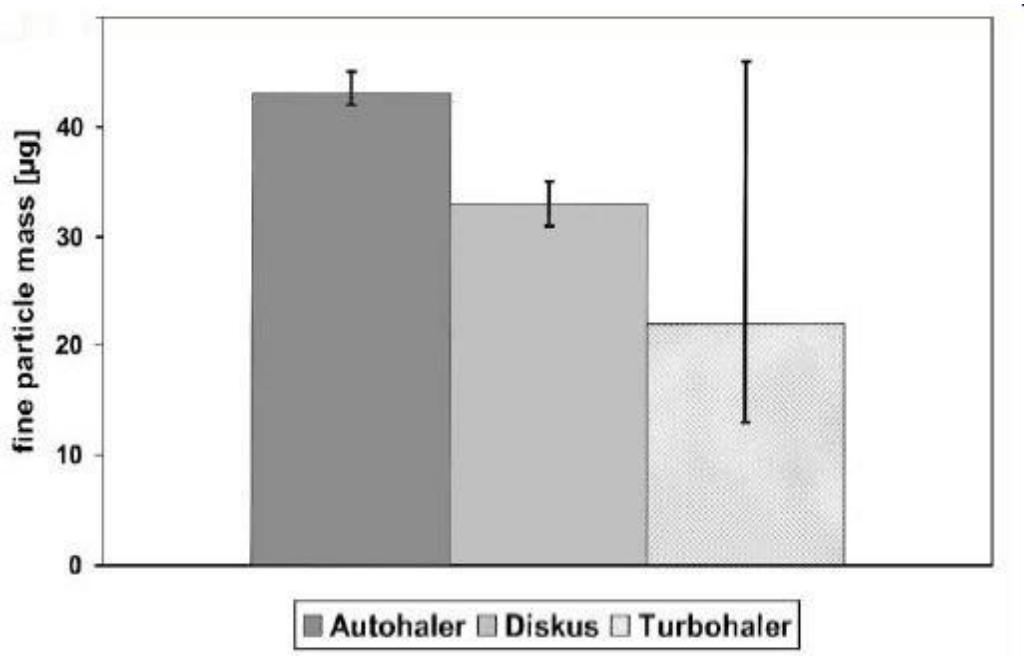

Figure 2.37: Fine particle mass for three different types of DPIs (Kamin et al., 2002)

The study showed that not only does inhalation flow affect the mass output and particle size distribution of the aerosol emitted dose, but there are other factors that have an impact on the dose emission from the DPIs such as ACIM and Vin (Kamin et al., 2002).

Recently, modifications of the *in-vitro* and *in-vivo* methodologies were introduced to obtain more accurate results that represent the real life use of the patients. In these methods, various inhalation flow rates and volumes were used instead of using pharmacopoeia recommendations for DPIs performance testing. For example, the use of *in-vivo* recorded profiles in the laboratory to determine the fine particle dose using breath simulator to mimic patient real life use. Patients were unable to replicate vacuum pump square wave profile (Azouz et al., 2015b).

The technique was used to record the IPs of asthmatic children aged 4-8 years when these patients inhaled through two DPIs namely Accuhaler® and Turbuhaler®. The fine particle mass of fluticasone propionate from the Diskus® was compared with Turbuhaler® in delivering budesonide fine particles in children with asthma (Bisgaard et al., 1998).

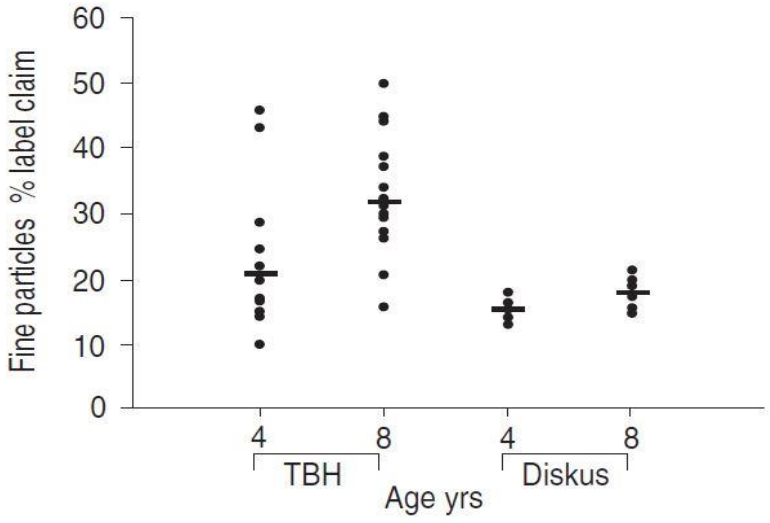

Figure 2.38: Fine particles mass (% of labelled dose) obtained from Turbuhaler®; the Diskus® using inhalation profile for four and eight year's children (Bisgaard et al., 1998)

The results showed that the emitted dose was 87-89% and 56-62% of the labelled dose for Diskus® and Turbuhaler®, respectively. Conversely, a lower fine particle dose was emitted from the Diskus® ranging from 15-18%, whilst, a fine particle dose emitted from the Turbuhaler®

found to be 21-23%. The Diskus® has shown more dose consistency, and both devices showed a flow rate dependent dose emission. Surprisingly the study found that aerosol cloud was released before even achieving the peak inspiratory flow.

Tarsin et al., (2006) have also studied the performance of Accuhaler® Seretide (Fluticasone Propionate and Salmeterol) and Turbuhaler® (Budesonide and Formoterol) dose emission characteristics using recorded IPs of twenty severe asthmatic patients using an Electronic Lung™. The study demonstrated the less flow rate effect on the dose emission from Diskus® compared to more pronounced flow dependent dose emission from Turbuhaler®. In addition, a reverse trend was observed between the flow rate and the MMAD of the inhaled particles from both devices (Tarsin et al., 2006).

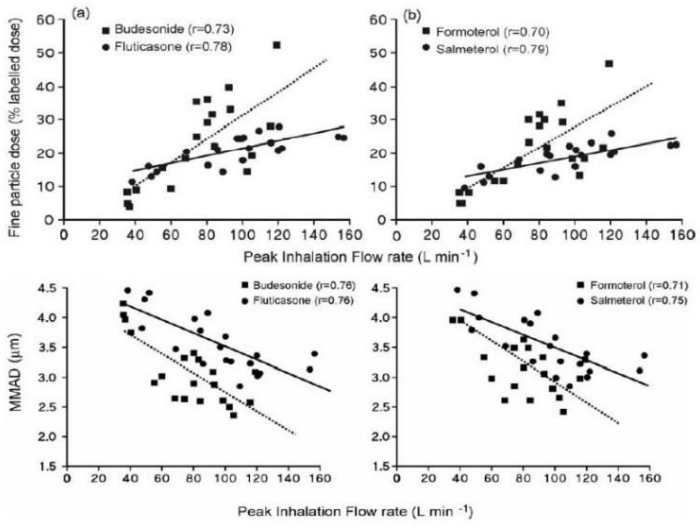

Figure 2.39: The FPD (% nominal dose) and MMAD (µm) of (a). Turbuhaler® and (b) Accuhaler® versus the peak inhalation flow rate (Tarsin et al., 2006)

Nadarassan et al., (2010), adapted the traditional method for in-vitro dose emission characteristics. The introduction of mixing inlet allowed dose emission characteristics to be measured at flow rates below 28.3L/min as shown in Figure 2.40. The mixing inlet was aimed to

extrapolate the required flow rate from the ACI constant flow. The flow rate set at the mouthpiece was the desired flow, while constant flow rate as recommended by the ACI manufacturer was pass through the stages. The compressed air role was to introduce the supplementary air from the mixing inlet side port. They have used this method to study the dose emission characteristics of Turbuhaler® using a range of inhalation flow rate ranging from 5-60 L/min at the mouthpiece and 4L Vin; the ACI assembled to be operated at 60 L/min. The results showed that dose emission from Turbuhaler® is more flow rate dependent with flow rate above 30 L/min, suggesting flow rate below 30L/min was not enough to efficiently de-aggregate the dose. Furthermore, the result of MMAD showed the steepest decrease with flow rates above 30 L/min (Nadarassan et al., 2010).

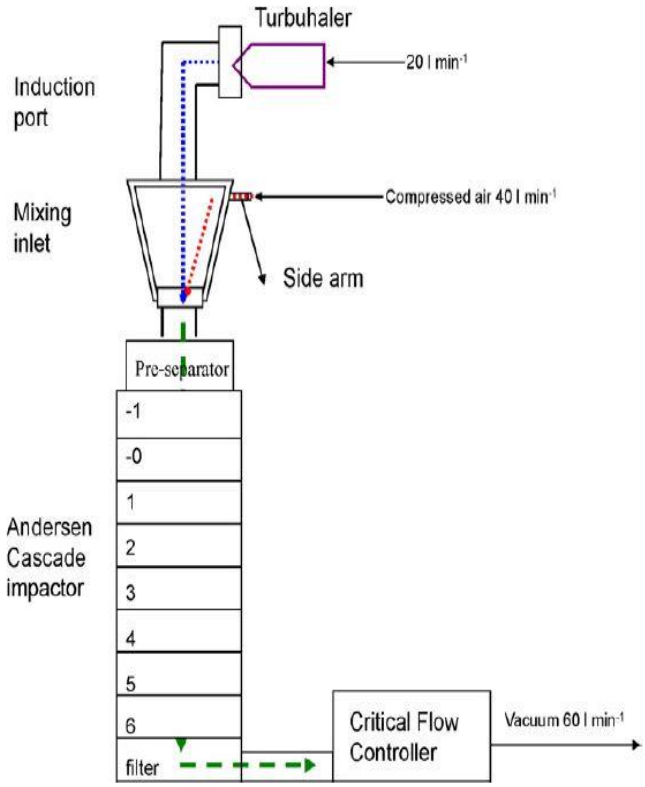

Figure 2.40: Schematic diagram of the ACI-mixing inlet method (Nadarassan et al., 2010)

Yakubu et al., (2013) have used the method mentioned above to assess dose emission aerodynamic characteristics from four inhalers (Accuhaler®, Easyhaler®, Clickhaler® and the Turbuhaler®) aerodynamic dose emission characteristics at different inhalation flow rates (10-60 L/min) and two Vins 2 and 4 L. The outcome of this study showed that flow rate dose emission dependency for all assessed inhalers (Yakubu et al., 2013). However, no significant difference in the dose emission characteristics was observed when inhaled volume increased from 2 to 4 L. Suggesting that the use of flow rate equivalent to 4 kPa pressure drop should be revised, and also 4 L Vin required by the pharmacopoeia should be replaced by the use of 2 L Vin since no difference in dose emission was shown by increasing the inhaled volume from 2 to 4 L.

The method developed by Nadarassan et al., (2010) was modified afterwards to compensate the flow from the vacuum pump with a supplementary air flow introduced by the mean of mixing inlet (Olsson et al., 2013, Chrystyn et al., 2015). This method allows airflow of 0 L/min to be achieved at the mouthpiece and a constant flow through the ACI (*e.g.*, 60 or 90 L/min). The dose emission from the device as an inhalation profile is replayed by using the breath simulator (Copley scientific Ltd, UK). This method is a more realistic approach to represent the dose patient would receive in real life use.

Olsson et al., (2013) have used aforementioned *ex-vivo* methodology by replacing the compendial square waved profiles by patients' IP. Figure 2.41 demonstrates the experiment set-up, where the impactor was set 80L/min flow rate the complementary air was then opened, and flow was adjusted to 0 L/min at the mouthpiece. After that, the breath program generated (BPG) is being connected to the piston of the electronic lungs. Flow through the inhaler that is equivalent to the IP occurs as a result of piston movement. The compressed air was used to compensate the remaining airflow to keep the flow constant.

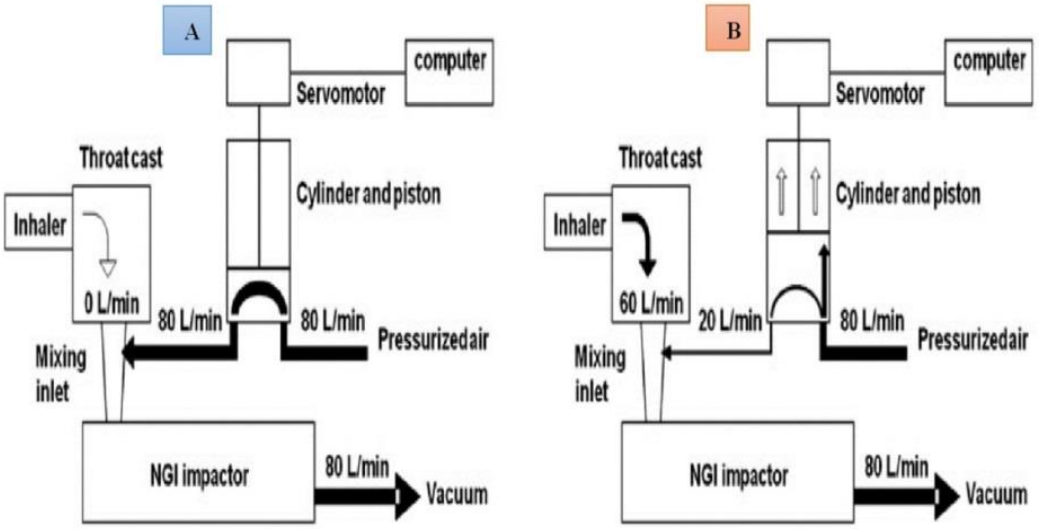

Figure 2.41: Demonstrate the method set-up by Olsson et al., 2013; whereas (A) inhaler with no flow (B) flow generated through the inhaler using BPG system (Olsson et al., 2013)

Chapman et al. (2011) and Colthorpe et al. (2013) have used the similar *ex-vivo* methodology, but instead of using electronic lungs the Next Generation Impactor (NGI) was coupled with flow volume simulator as shown in Figure 2.42. Supplementary air flow of 100 L/min was provided through the side arm of the mixing inlet in order to equalise the constant vacuum pump flow through the impactor. Therefore, during the experiment, a flow of 0 L/min was kept at the mouthpiece of the impactor. Patient profiles were reproduced at the mouthpiece by modulating the air flow using flow –volume simulator(Chapman et al., 2011, Colthorpe et al., 2013).

Chapman et al. (2011) assessed the aerodynamic dose emission characteristics of Breezhaler® (Indacaterol) and Handihaler® (tiotropium). The results showed that fine particle fraction (FPF) was higher with Breezhaler® than Handihaler® 27% and 10% respectively. The Handihaler® showed a higher oropharyngeal deposition than Breezhaler®. Furthermore, the patients were able to use both devices correctly after seven days and showed more preference using the Breezhaler®.

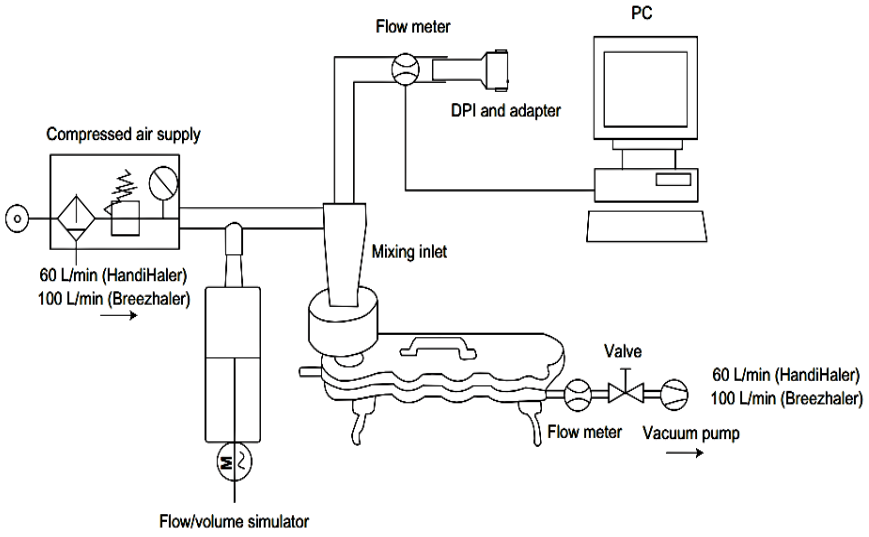

Figure 2.42: Schematic diagram of experimental set-up with flow-volume simulator

Colthorpe et al. (2013) used the same methodology to investigate the performance of Breezhaler® (Glycopyrronium) and Handihaler® (tiotropium) capsule-based devices. The results showed that low resistance device Breezhaler® provided higher FPF and emitted dose with greater consistency compared to the Handihaler®. Therefore, it might be useful for COPD patient with different severity to use Breezhaler®. Furthermore, Zanker et al., (2013) have used the COPD patients' IP to assess the performance of Breezhaler in delivering dose glycopyrronium dose. The study results demonstrate that over the range of the IPs used dose emission showed high consistency, additionally inter and intra-thoracic dose deposition consistency(Zanker et al., 2013).

A similar principle was adapted by Chrystyn et al. (2015) to investigate the effect of COPD patients' IPs and throat geometry on the dose emitted from Spiromax® and Turbuhaler®. Three categories of patient profiles were selected (weak, medium and strong) based on the inspiratory flow achieved by the patients. Furthermore, three sizes of throat were used. The results showed that Spiromax® produces higher dose consistency and higher fine particle dose (FPD) than Turbuhaler for all the profiles used.

Buttini et al. (2016) have also adapted the method to study the effect of patients' IPs and the throat change on the *in-vitro* inhalation performance of two DPIs (NEXThaler®, Cheisi Pharmaceutics S.p.A., Parma, Italy) and (RS01, Plastiape, Lecco, Italy). The results showed that NEXThaler® produced high fine particle dose of the formulation containing Formoterol fumarate and Budesonide dipropionate for all the IPs used despite the change in the parameters (PIF, Vin and ACIM). Contrarily, capsule based inhaler required a higher inspiratory volume, whereas the fine particle mass of the two drugs was decreased significantly when low volume IP used. The study was analysed using multivariate analysis to predict the system performance ((Buttini et al., 2016a).

2.12 Summary

The DPIs flow rate dose emission dependency *in-vitro* have been studied over the years using the inhalation flow produced by the vacuum pump which does not reflect the routine use of the inhaler devices. The square wave profile achieved by the pump is not realistic, and patients cannot replicate this profile in real life. Several *in-vivo* studies showed that most of the patient with asthma and COPD were unable to achieve flow (4 kPa) and volume (4 L) suggested by for *in-vitro* dose emission testing of DPIs. Instead, using a range of the MIFs and Vins to test the DPIs will be useful to determine the optimum condition of using each DPI. The square profile is not reflecting the inhalation manoeuvre parameters correctly (MIF, Vin and ACIM) especially acceleration rate ACIM]. Therefore the IPs use is so important to determine the effect of each parameter on the dose emission from DPIs. Additionally, to determine the limit of inhalation manoeuvre condition below which dose de-aggregation is not complete and less dose of medication to be released from the inhaler device. Recently, breath simulator and other instruments were developed in order to enable the use of IPs instead of square wave profiles. The breath simulator (BRS) introduction brought a new prospect to study the dose emission uniformity as well as aerodynamic dose emission characteristics. It can be coupled with either Andersen cascade impactor (ACI) or next generation impactor (NGI) using mixing inlet side arm. Mixing inlet enables supplementary air to be introduced and therefore flow rate of 0 L/min at the impactor mouthpiece is achieved. Several studies have used this *ex-vivo* methodology to determine the dose emission characteristics from different DPIs.

Chapter 3. Materials and High Performance Liquid Chromatography (HPLC)

3.1 Material

3.1.1 General apparatus and materials:

- Analytical weight balance (Mettler Toledo, Leicester, UK)
- pH meter (Jenway, UK)
- Ultrawave U300 benchtop bath (Ultrawave Scientific Laboratory Supplies Ltd, UK)
- Glass Pasteur pipettes (Fisher Scientific, UK)
- Parafilm^{M} (Fisher Scientific Ltd, UK)
- Hot plate and Magnetic stirrer (Cole-Parmer®, UK)
- Luer-slip plastic syringes 1 ml (Thermo Scientific, UK)
- 17 mm syringe filter Nylon 0.45 µm (Thermo Scientific, UK)
- HPLC 2mL amber vials (Thermo Scientific, UK)
- Glass volumetric flasks different volumes (Fisher Scientific Ltd, UK)
- Petri dishes (Fisher Scientific Ltd, UK)

3.1.2 Dose emission measurement Apparatus and material:

- Dose unit sampling apparatus for DPIs (DUSA; Copley Scientific Ltd, UK)
- 47mm glass fibre filter (Whatman, UK)
- High capacity pump HCP5 (Copley Scientific Ltd, UK)
- Critical flow controller TPK2000 (Copley Scientific Ltd, UK)
- Digital flow meter 2000 (Copley Scientific Ltd, UK)
- Inhaler devices (Foradil Aerolizer®, Onbrez Breezhaler® and Easyhaler®)
- Mouthpiece Adapter for (Aerolizer® , Breezhaler® and Easyhaler®)

3.1.3 Aerodynamic dose emission characteristics material and apparatus

- Onbrez Breezhaler® Mouthpiece Adapter for Alberta's throat
- Inhaler device (Onbrez Breezhaler®)

- Idealised Alberta throat (IAT) (Copley Scientific, UK)
- Mixing Inlet (MI) (Copley Scientific, UK)
- Andersen Cascade Impactor (ACI) (Copley Scientific, UK)
- High capacity vacuum pump (HCP) (Copley Scientific, UK)
- Critical Flow controller, TPK 2000 (Copley Scientific, UK)
- Breathing Simulator, BRS3000 (Copley Scientific, UK)
- Compressed air cylinder (Thermo Scientific, UK)
- Glass microfiber filter papers 82.6 mm (Whatman™, UK)
- Pro-power silicone lubricant (Premier Farnell plc, UK)
- Digital flow meter 2000 (Copley Scientific Ltd, UK)
- Copley Inhaler Testing Data Analysis Software (CITDAS) version 2.00 (Copley Scientific, UK)

3.1.4 Laboratory apparatus

3.1.4.1 Dose unit sampling apparatus (DUSA)

DUSA for dose emission testing of DPIs is shown in Figure 3.1. It consists of the collection tube, two rinsing caps, one filter support base and metal ports to connect the DUSA to the critical flow controller during the experimental set-up at one end, while the other end forms a mouthpiece adapter to attach the DPIs. The collection tube was fitted with 25mm glass fibre filter to increase the aerosol retention upon aerosolisation, which enhances particle retention down to 0.3 microns. The volume of the collection tube is 500 mL which approximates the volume of the human oropharynx (Copley, 2015).

Over the years, DUSA is used to measure the dose emission uniformity as well as the consistency of the dose emitted from both MDI and DPIs. Additionally, it is widely used to

determine the intra and inter-device variability (Hindle and Byron, 1993, Byron, 1994, Hindle and Byron, 1995).

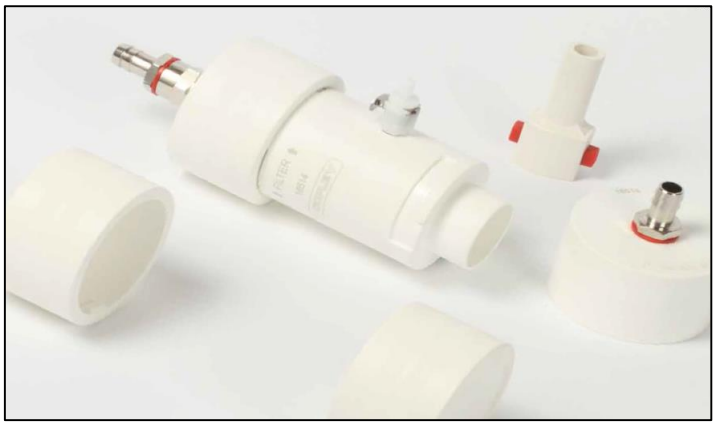

Figure 3.1: Dose unit sampling apparatus (DUSA) components for DPIs testing

The DUSA collection tube has a pressure tap (P1) which is connected to the critical flow controller and allow device resistance measurements during the experiment. The filter attached to the collecting tube allows dose collection with high precision at a high flow rate (100 L/min). Figure 3.2 show procedure of achieving stable high flow rate (120L/min) when two HCP-5 connected in series (Copley, 2015).

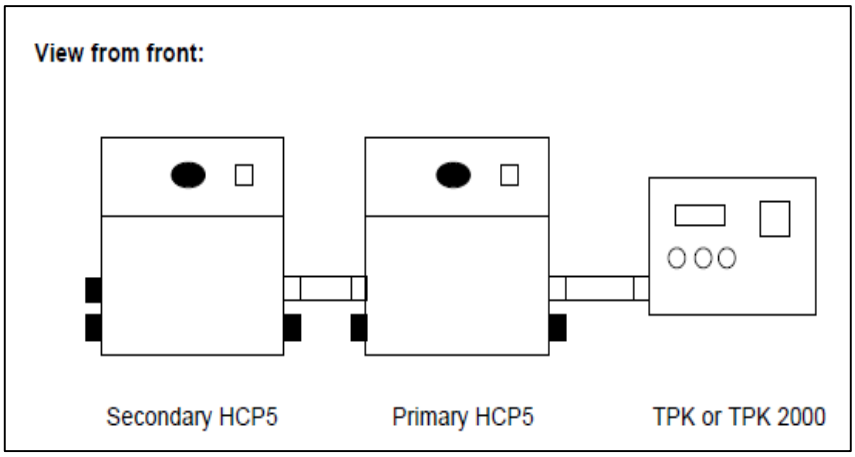

Figure 3.2: Schematic diagram of series connection of vacuum pumps (Copley, 2015)

3.1.4.2 Andersen Cascade Impactor (ACI)

Andersen cascade impactor (ACI) consists of an induction port, pre-separator and eight stages stacked in series. Each stage has different number and size of holes drilled precisely, underneath each stage is a collecting plate designed to collect the aerosolised dose corresponding to a different aerodynamic particle size of the inhaled particles. The last stage is known as the filter stage which collects the very fine particles as shown in Figure 3.3 (EP, 2013, USP, 2014, Copley, 2015). The ACI is widely used to determine the aerodynamic characteristics of the aerosol dose emitted from different inhaler devices (Copley, 2008). It provides useful data about the emitted dose, fine particle dose and mass fraction to assess the performance of inhaled aerosol. The aerodynamic characteristics of the emitted dose determined by ACI will provide detailed information about both the dose emitted from the device as well as the dose that has the potential to reach the lungs (Chrystyn, 2003, Mitchell et al., 2007).

Figure 3.3: Andersen cascade impactor (ACI) set-up for (A) MDI (B) DPI (Copley, 2015)

The particles less than 10 µm in size aerosolised from the device into the ACI will be measured accurately. Each stage of the ACI is specified with a cut-off diameter corresponding

to certain particle size, particle within this size will deposit into the collection plates, while particles below that size will pass through to the next stage. After the aerosolisation of the dose, deposited particles on each of the collection plates are recovered, and the quantity of the drug deposited is analysed using an appropriate validated analysing method (Copley, 2008). The stages hole diameter is decreased in descending order. Consequently, the air velocity increases as the particles travel through the ACI. Initially, ACI was calibrated to operate at a flow rate of 28.3L/min, and then modification was introduced to enable the ACI to work at high flow rates such as 60 and 90 L/min. The stages cut-off diameter (µm) at different flow rates are shown in Table 3.1. At 60 L/min, two stages 0 and 7 are replaced with stages -1 and -0, while at 90 L/min three stages 0, 6 and 7 are replaced with stages -2, -1 and -0. The changes are also made to collection plates (plates with a centre hole) (Copley, 2015).

Table 3-1: The cut-off diameter (µm) and stages configuration of ACI at different MIFs

Cut-off diameter (µm)	28.3 L/min	60 L/min	90 L/min
*Stage -2	……	…….	8.0
*Stage -1	……..	8.6	6.5
*Stage -0		6.5	5.2
*Stage 0	9.0	…….	……..
*Stage 1	5.8	4.4	3.5
Stage 2	4.7	3.2	2.6
Stage 3	3.3	1.9	1.7
Stage 4	2.1	1.2	1.0
Stage 5	1.1	0.55	0.22
Stage 6	0.7	0.26	…….
Stage 7	0.4	……	…….

*= Stages with collection plate that has a centre hole (Holo plates).

The ACI configuration can be adjusted to work at flow rates below 28.3L/min and above 90 L/min using the equation Eq. 3.1 (Van Oort, 1995):

$$\mathbf{ECDF2 = ECD\ 28.3\ (28.3/F2)^{0.5}} \qquad \text{Eq. 3.1}$$

Where;

ECDF2 = is the effective cut-off diameter at another flow rate,

ECD28.3 = is the effective cut-off diameter at standard flow rate of 28.3 L/min

F2 = is the other flow rate in L/min

Although laser diffraction was introduced to provide a quick assessment of the inhaled aerosol, this technique is limited to the measurement of the geometric of the inhaled aerosol but not the aerodynamic diameter. Inertial impaction techniques such as Twin stage impinger (TSI) and multi-stage liquid impactor (MSLI) provide an assessment of the aerodynamic characteristics of the inhaled aerosol with less detailed information due to the fewer stages (Zeng et al., 2006).

ACI is widely used as it provides more detailed information regarding the quality of inhaled product by simulating the aerodynamic particle distribution in different regions of the respiratory tract (Figure 3.4), in addition to a forecasting on the dose the patient will receive when using the inhaler device (Dunbar and Mitchell, 2005, Mitchell et al., 2007). The multi stages composition of the ACI enable the differentiation between the API and other components in the formulation and it measures the whole dose and not only part of it which allows complete characterisation (Copley, 2008).

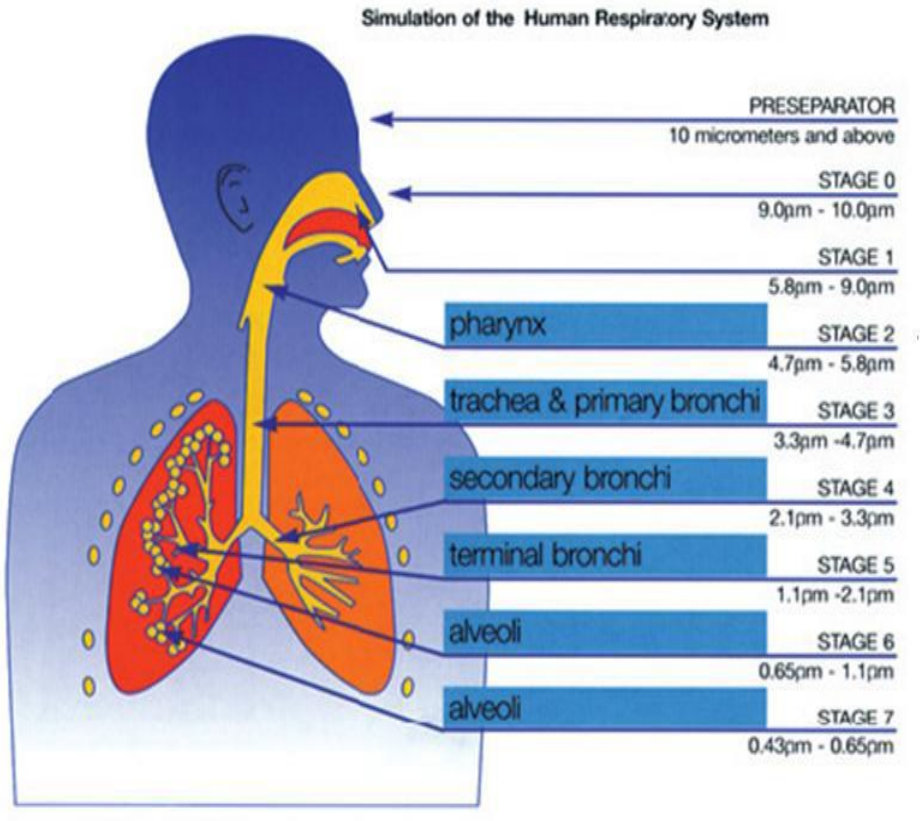

Figure 3.4: Description of regional lung deposition (Dunbar and Mitchell, 2005)

The experimental procedure of using ACI can be explained as follow: The inhaler under test is connected to the ACI by the means of a mouthpiece adapter; the latter plays an important role ensuring no air leaking between the device and the ACI during the test. Then, the stages of the ACI are stacked accordingly in descending order according to the stages hole size. The ACI is operated by the impaction mechanism, upon the aerosolisation; the dose will be drawn through the impactor by the mean of a high capacity vacuum pump (HCP) which is connected to the outlet of the ACI. Therefore, the particles deposition on each stage is governed by their inertia. The nozzles or jets play a role in directing the air as well as the airborne particles into the collection plate (Copley, 2008, Copley, 2015). According to the particles inertia, a particle with sufficient inertia will influence the specific stage plate, while particles which are smaller or having insufficient inertia will carry on with the air stream and travel to the next stage. This mechanism is continuous until the very fine particles reach the filter stage of the impactor the

mechanism is illustrated in Figure 3.5. The ACI measurement accuracy of emitted dose from either DPIs or MDIs can be affected by the particle bouncing back into the air stream when they hit the collection plates and travel to next stage (Copley, 2008). This drawback can be overcome by introducing a washing solution in the pre-separator when testing DPIs and pre-coating the surface of the ACI collection plates with a media that provides a tacky surface to minimise particle bounce and re-entrainment (Table 3.2). There is no standard approach in terms of either material used or method of applying the coating. Standardisation is unnecessary since each research group has independently selected and validated their procedure with their own formulations (Allen, 1990).

The particle loss in the stages part which is known as the ‘‘inter-stage loss’’ is not a major drawback of ACI use as it remains low compared to the recovery dose from the impactor. Some studies reveal that only 5% is the inter-stage loss in most of the cases (CDER, 1998, Byron et al., 2004, Kamiya et al., 2009).

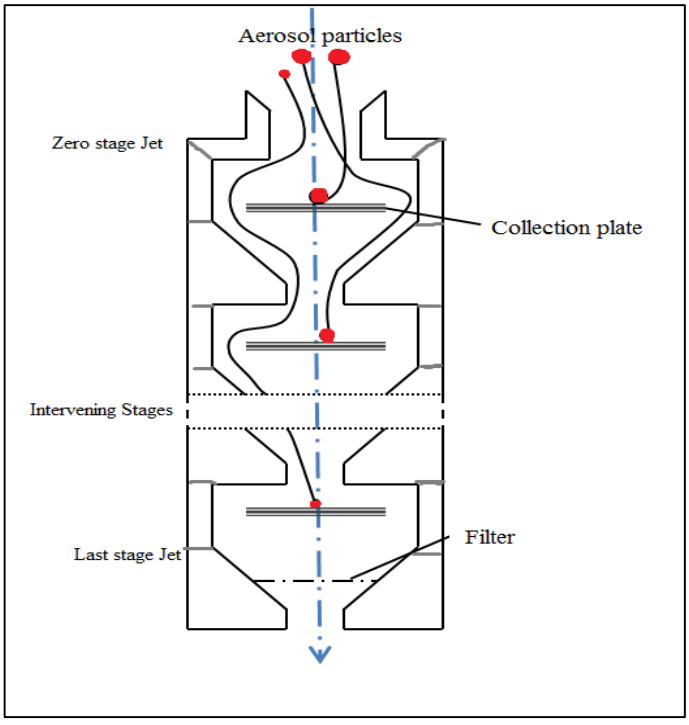

Figure 3.5: Principle of Andersen cascade impactor operation (USP, 2014)

Table 3-2: Coating materials and procedures used by European pharmaceutical aerosol group EPAG member organisation (Mitchell, 2003)

EPAG Member	Impactor	Inhaler	Surface	Coating material	Procedure
1	ACI	pMDIs	Stainless steel	Ethanolic solution of Brij35(polyoxyethylenelaurylether) is mixed with glycerol	Dip plate into coating solution, leave in a fume hood to allow solvent to evaporate before assembly of impactor
2	ACI	DPI	Stainless steel	11% polypropylene glycol in Hexane	Submerge plate, remove plate allowing the bulk to drain off, then air dry
3	ACI, NGI	DPI	Stainless steel	A solution of Brij, glycerol and ethanol (ethanol evaporates after applying, before analysis)	Apply the standard amount of coating material (thin) evenly (in practice done with a sponge).
4	ACI	pMDIs, DPI	Stainless steel or aluminium alloy	Glycerol or Brij 35. Applied only if required	Apply a standard quantity of solution to give a thin film of coating substance on each plate.
5	ACI	pMDIs, DPI	Stainless steel	1 ml of Brij 35 in 20 ml ethanol dissolved in 5 g glycerol	Distribute 50 µl per plate For DPI, distribute 100 µl in pre-separator
6	ACI, NGI	DPI	Stainless steel	n-hexane + 1% of SPAN 85 (sorbitan trioleate)	Immerse each plate in a crystalizer containing the coating material. Allow 0.5 hr for solvent evaporation in a fume hood before assembly of impactor
7	ACI, NGI	pMDIs, DPI	Stainless steel	1% w/v silicone oil in hexane	Dip plates in solution and allow hexane to evaporate prior to assembly of impactor
8	ACI, NGI	DPI	Inox	Glycerine	Coat with a thin layer of glycerine

3.1.4.3 Breath Simulator (BRS)

The breath simulator instrument was firstly introduced for testing the nebulisers with a maximum Vin of 1 L. In 2012, the breath simulator BRS 3000 was designed and launched to test the inhaler devices with a wider specification. Whereas an inhalation flow of up to 240L/min can be achieved, an acceleration rate of 25 L/s^2 and the capacity of the inhaled volume for testing was increased up to 5 L (Copley Scientific, 2015). The last version BRS 3000 is able to obtain a flow rate, volume and acceleration rate to test the inhaler devices such as DPIs and MDIs with more realistic approach to mimic the patient inhalation and exhalation pattern. Furthermore, it enables the testing of spacers, nebulisers and valve holding chambers. The BRS replays the *in-vivo* profiles that have been recorded from the patient with COPD and asthma, thereby providing an insight into the dose that the patient would inhale in routine use of the inhaler device. The BRS can also be used to generate either dose uniformity data using DUSA or aerodynamic particle size distribution data using ACI.

The compendial method of testing the inhalers using the vacuum pump clearly does not reflect the capabilities of a typical patient. The applied inhalation profile is a square wave taking the flow rate from 0 L/min to a pre-determined constant value (X L/min) for short duration of time and then back to 0 L/min. This profile is by no mean achievable by healthy subject never mind an asthmatic patient (Copley, 2015).

3.2 High performance liquid chromatography

3.2.1 HPLC Overview

High performance liquid chromatography is one of the chromatography types, which is highly used in the pharmaceutical field to separate a mixture of different components. It is also used in the others fields such as analytical chemistry and biochemical for the purification and the identification of a compound in a mixture (Snyder et al., 2012, Bhardwaj et al., 2015).

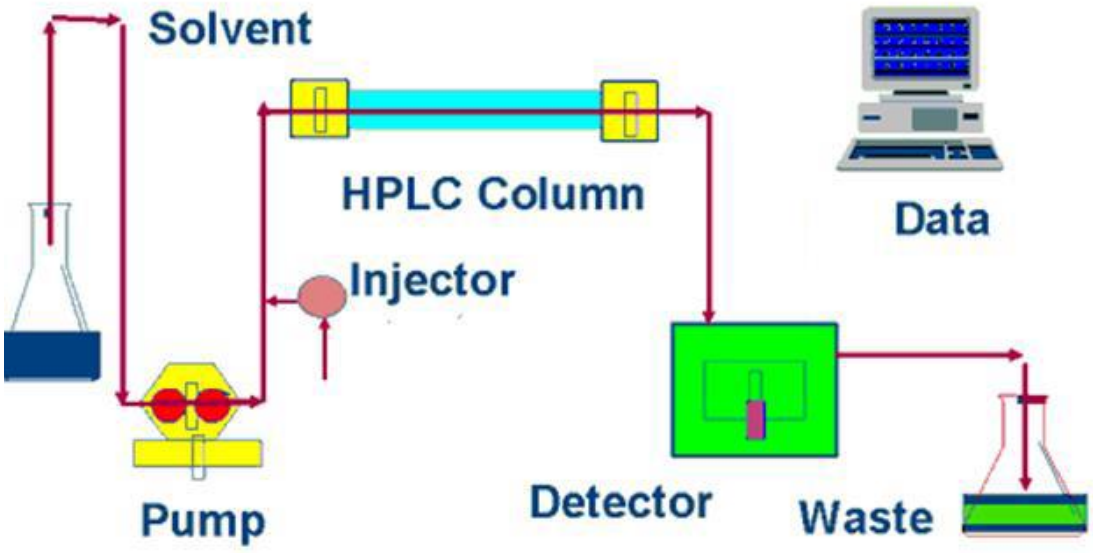

Figure 3.6: Schematic diagram of High performance liquid chromatography instrumentation presenting the main components (Bhardwaj et al., 2015)

It is a qualitative and quantitative technique, the mode of the system operation differ according to the solubility and the polarity of the compound as well as the polarity of the aqueous phase and the stationary phase. The principle of the compound separation depends on the affinity or the partitioning of the compound between the mobile phase and stationary phase. In different words, like attract the like which determine the speed the compound elution time. Therefore different compounds can be separated (McPolin, 2009a). The HPLC

types differ according to the polarity of the phase system used for the separation. The most common types are the normal phase and reversed phase (Table 3.3).

Table 3-3: The normal and reversed phase mode of HPLC (Snyder et al., 2012).

Normal phase HPLC	Reversed phase HPLC
• Non-polar mobile phase	• Polar mobile phase
• Hydrophilic silica particles in the solid phase	• Hydrophobic hydrocarbon chain in the solid phase
• Hydrophobic molecules elute first	• Hydrophilic molecules elute first
• Less common	• Most common
• Elution most to least hydrophobic	• Elution least to most hydrophobic

In the RP-HPLC, the non-polar molecules form an attraction to the hydrocarbon chain of the silica in the column due to Van der Waals force. These compounds are less soluble in the mobile phase. Therefore, the elution time will be longer compared to more polar compounds (Watson, 2015). The polar compounds are more soluble in the mobile phase and spend less time in the column, and tend to elute with the solvent of the mobile phase (McPolin, 2009b). The mobile phase for RP-HPLC consists of the aqueous solution, and organic modifier, the most commonly used aqueous solutions are water and buffer salts. The organic modifiers used in the mobile phase composition are acetonitrile, methanol and tetrahydrofuran. However the most common used are acetonitrile and methanol, and both solvents have many advantages such as water miscibility, availability, safety and compatibility with HPLC (Aguilar, 2004).

In RP-HPLC peak tailing resulting from the interaction of the unbonded silanol group (Si-OH) which is exposed on the surface of the silica in the column with the sample. This interaction is more strong with basic compounds that contain an amine group (positive

charge). The interaction can be explained as the positive- negative interaction between the sample and the silica (Snyder et al., 1997, Dolan, 2003). The problem of peak tailing can be fixed by adopting different strategies depending on the extent of peak tailing. Firstly, it can be fixed by using a buffer solution in a certain range (depending on the compound of interest PKa) to minimise the interaction between the compound of interest and the silica. Secondly, the use of low pH is an important solution to reduce the peak tailing. For example, at pH 3 in the separation of formoterol fumarate using phosphate buffer, silanol group were protonated, and peak tailing was minimised. Furthermore, the use of a pH lower or higher than the pKa of the compound has also been effective. Thirdly, the use of a surfactant such as sodium dodecyl sulphate SDS or a competing base such as Triethylamine (TEA) to reduce the interaction of silanol group with a compound of interest (Dolan, 2003, McPolin, 2009b).

In this thesis, two HPLC methods were used to quantify recovered samples of formoterol and indacaterol after aerosolisation into DUSA and ACI. The formoterol peak initially was not symmetrical, and peak tailing, as well as broadening, was observed. The peak tailing problem with formoterol fumarate was solved by combining all the above mentioned solutions together. The mobile phase of the HPLC method used for the quantification of formoterol was phosphate buffer with TEA, and the latter was adjusted to pH 3. The indacaterol peak was better than formtoerol peak therefore the addition of TEA was unnecessary. The column used in the separation of formoterol fumarate was phenomenex, Luna C18 (Sigma Aldrich Ltd, UK), which provides a very good peak shape with optimum pressure. It was the column of choice for the separation of the basic compounds

3.2.2 HPLC Apparatus

The HPLC system used for samples quantification was Prominence liquid chromatography LC-20 AT (Shimadzu, UK). The specifications of the system component are as follow

- ✓ Pump: LC-20AT prominence pump. Oven: column oven (CT0-10AS VP)
- ✓ Autosampler: Prominence Auto Sampler (SIL-20A HT) fitted with a 200 μL loop (Shimadzu Ltd, UK)
- ✓ Degasser: Prominence degasser (DGU-20A5) (Shimadzu Ltd, UK)
- ✓ Detector: SPD-20A prominence UV-vis detector (Shimadzu Ltd, UK)

3.2.3 Materials

3.2.3.1 Laboratory apparatus and Solvents for HPLC

- Nylon filter membranes pore size 0.45 μm (Sigma Aldrich, Ltd, UK)
- Filtration Apparatus for Mobile phase (Supelco® .Sigma Aldrich, Ltd, UK)
- Ultra-purified water apparatus (Thermo Scientific Ltd, UK)
- HPLC vials with screw caps made from borosilicate glass/PE pre-fitted with rubber/PTFE liner amber 2 mL (Fisher Scientific Ltd, UK)
- Acetonitrile and HPLC HPLC grade (Fisher Scientific Ltd, UK)
- Ultra purified water (Barnstead™ Nanopure™, Thermo Scientific Ltd, UK)
- Glass Pasteur Pipettes (Fisher Scientific Ltd, UK)
- Glassware: Volumetric Flasks and Beakers (Fisher Scientific Ltd, UK)

3.2.3.2 Buffer and salt additives

- Potassium dihydrogen orthophosphate (Fisher Scientific Ltd, UK)
- Disodium hydrogen orthophosphate (Fisher Scientific Ltd, UK)
- Orthophosphoric acid (Fisher Scientific Ltd, UK)
- Triethylamine (TEA) (Fisher Scientific Ltd, UK)

3.2.3.3 Pharmaceutical standard compounds

- Formoterol fumarate dihydrate and Dexamethasone (Sigma Aldrich Ltd, UK)
- Indacaterol maleate (MedChem, USA)

3.2.4 HPLC method for the determination of Formoterol

The formoterol HPLC method developed by (Assi et al., 2006) was adjusted and optimised to be used in this thesis for the quantification of formoterol. The pump flow rate was reduced from 1.5 mL/min to 0.7 mL/min and peak tailing was reduced by the addition of TEA. The optimised chromatographic conditions used are illustrated in Table 3.4

Table 3-4: The chromatographic condition of the RP-HPLC method used for Formoterol.

Stationary phase (Column)	Phenomenex, Luna 250 mm x 4,6 mm, 5 µm (Phenomenex, UK)
Mobile phase	Acetonitrile: Buffer (60:40% v/v) The Buffer used was 5mM Disodium Hydrogen Orthophosphate with 1% TEA, adjusted to PH 3.00 using Orthophosphoric acid. Then, the buffer was filtered using 0.45µm membrane filter.
Internal standard	400 ng/mL Dexamethasone
Flow rate	0.7 ml/min
Injection volume	10 µl
Wavelength	214 nm
Temperature	30ºC
Run time	7 minutes
Measurement	Formoterol and Dexamethasone peak area

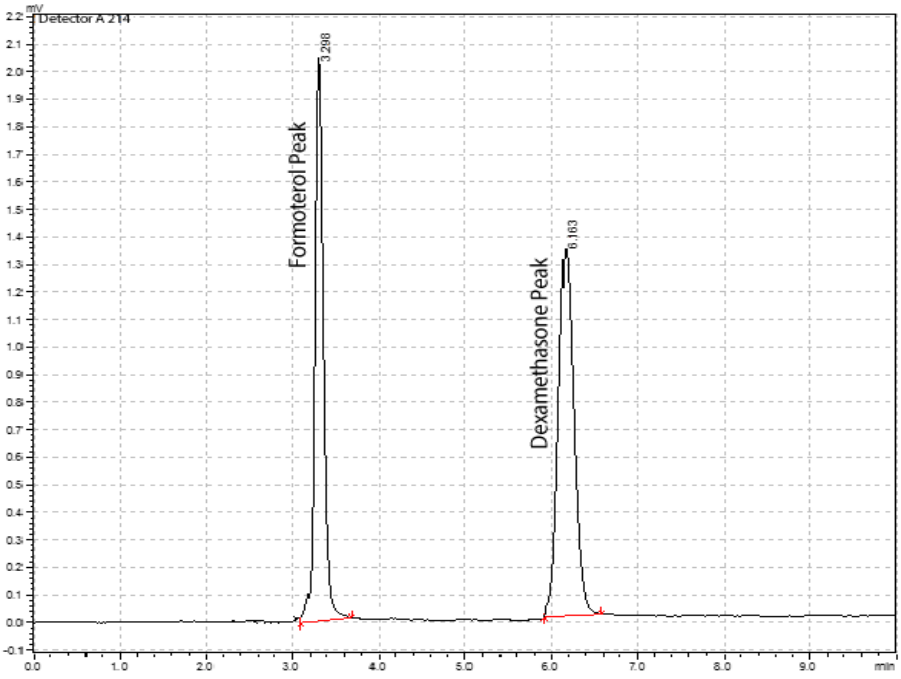

Figure 3.7: A typical chromatogram of formoterol (200 ng/mL) and dexamethasone (400ng/mL)

3.2.4.1 Method Validation

3.2.4.1.1 Calibration (Linearity)

The calibration curve of Formoterol was prepared using six points over the range (50-800 ng/mL). The samples were from the stock solution using mobile phase solution proportion 60:40 % (v/v) Acetonitrile: Water. The standard formoterol samples were injected three times on 3 different days. The average peak area was plotted against the nominal concentration (x) of the calibration standards. A straight line was fitted to the data using linear regression. A representative plot, described by the equation y = 107.8x - 1478.3 (R^2 =0.99), was obtained as shown in Figure 3.8.The detector response was found to be linear over the concentration range used with a correlation coefficient of ≥ 0.99. Dexamethasone 400 ng/mL was used as internal standard. The method was linear over the range of the concentrations used. The amount of the formoterol in the second emitted dose is expected to be low therefore; the calibration curve was plotted using a low concentration of 50 ng/mL formoterol standard.

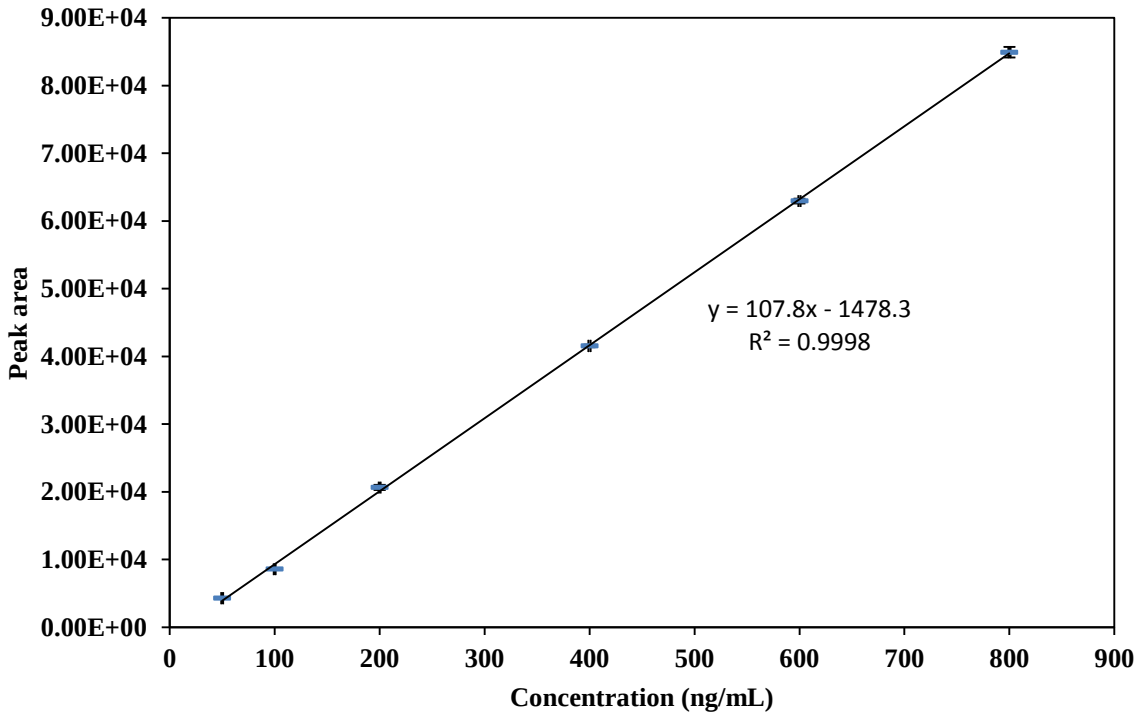

Figure 3.8: Calibration curve of formoterol at 214nm (n=3).

3.2.4.1.2 Precision

According to ICH and FDA guidelines, precision is the closeness of agreement; in other words, the degree of the scatter between a series of individual measurements is obtained from multiple sampling of the same homogeneous sample under the prescribed condition (ICH, 1994, Guideline, 2005).

The precision of the method was determined by analysing three concentrations of Formoterol fumarate (low 100 ng/mL, medium 400 ng/mL and high 800 ng/ml) on 3 different days in triplicate to determine both the intra-day and inter-day variation. The Intra-day and inter-day variations expressed as the coefficient of variation (% CV) which was calculated by the ratio of standard deviation to the mean. The results of intra-day and intra-day variations of formoterol fumarate are shown in Table 3.5.

Table 3-5: Intra-day and inter-day precision of formoterol [n=3].

Nominal Concentration of Formoterol (ng/ml)	Intra-day %CV	Inter-day %CV
100	0.61	0.94
400	0.56	0.78
800	0.24	0.89

3.2.4.1.3 Accuracy

The accuracy of an analytical method describes the closeness of the test result obtained by the method to the true value (concentration of the analyte). Accuracy is determined by a replicate analysis of a sample containing a known amount of the analyte and should be measured using a minimum of three determinations. A minimum of three concentrations in the range of the expected concentration is recommended (ICH, 1994). The accuracy was calculated as the percentage ratio of the measured concentration (obtained from the linear regression line over

the concentration range investigated) to the nominal concentration. The results of formoterol accuracy are shown in Table 3.6.

Table 3-6: Intra-day and inter-day accuracy results of Formoterol [n=3].

Nominal concentration (ng/ml)	Mean (SD) of Measured concentration	Mean (SD) % to nominal concentration
	Intra-day Variation	Intra-day Variation
100	101.7 (1.87)	101.7 (1.87)
400	402.9 (2.27)	100.7 (0.56)
800	795.3 (1.1)	99.4 (0.14)
	Inter-day Variation	Inter-day Variation
100	103.6 (1.7)	103.6 (1.7)
400	393.5 (10.2)	98.4 (2.55)
800	796.8 (16.4)	99.6 (2.05)

3.2.5 HPLC method for the determination of Indacaterol

The chromatographic condition of the indacaterol HPLC method is reported in Table 3.7. The method was developed using HPLC method development strategies reported by Koerner et al., 2013.

Table 3-7: Chromatographic condition of RP-HPLC method used for Indacaterol

Stationary phase (Column)	Phenomenex, Luna 250 mm x 4,6 mm, 5µm (Phenomenex, UK)
Mobile phase	Methanol: Buffer (60:40%v/v) The Buffer used was 25 mM potassium dihydrogen Orthophosphate adjusted to pH 2.50 using Orthophosphoric acid. Then, the buffer was filtered using 0.45 µm membrane filter.
Internal standard	1µg/mL Dexamethasone
Flow rate	1 ml/min
Injection volume	20 µl
Wavelength	259 nm
Temperature	40^{o}C
Run time	12 minutes
Measurement	Indacaterol and Dexamethasone peak area

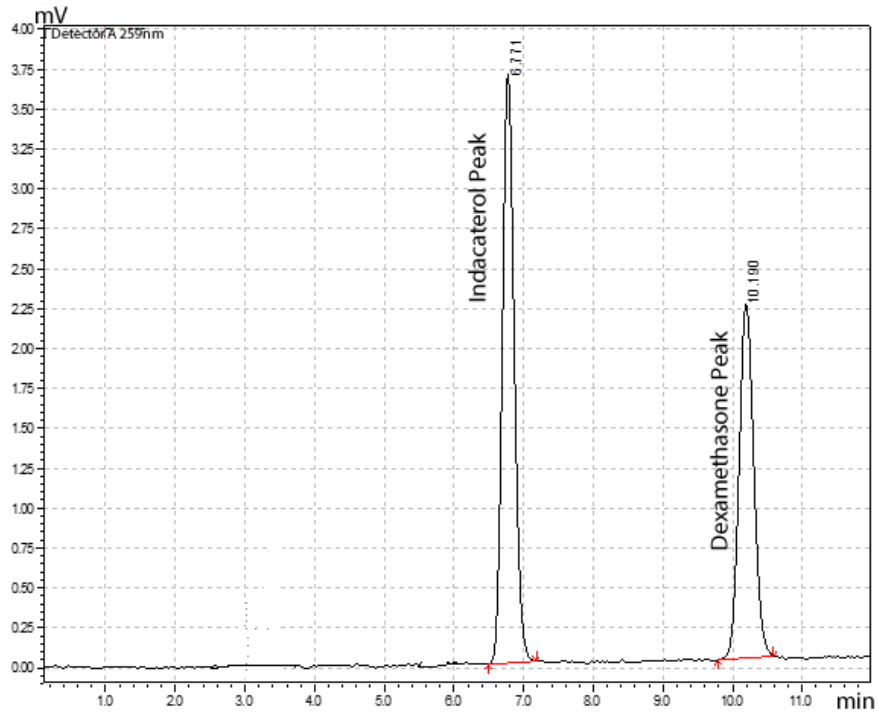

Figure 3.9: A typical chromatogram of indacaterol (2.5 µg/ mL) and dexamethasone (1µg/ mL)

3.2.5.1 Method development

The method development was firstly started with a general HPLC method using water: acetonitrile, then Acetonitrile was changed with Methanol because indacaterol was found be more soluble in methanol and gave a good absorbance intensity when compared with Acetonitrile (El-Sherbiny et al., 2015). Two different columns were used during the development of this method C18, RP-Luna 250×4.6 i.d (5µm particle size) and RP- Kinetex 250mm × 4.6mm i.d (5µm particle size) (phenomenex, UK). RP-Kinetex column gives high-resolution peaks with shorter elution time compared to RP-Luna. Three different aqueous mobile phases were used, firstly water was adjusted to pH 2.5 using formic acid the peaks were eluted early within the column's dead volume, then hexane sulfonic acid was used instead of water (formic acid), but the resolution between indacaterol peak and internal standard was not good. Afterwards, potassium hydrogen phosphate buffer adjusted to pH 2.5 was found the best aqueous mobile phase for the determination of indacaterol. The pH was

lowered to eliminate the interaction between the ionised silanol group and the solutes. The ratio of the aqueous phase to the organic modifier was investigated at 70% 60% and 50% methanol, the proportion of 60:40% (methanol: potassium dihydrogen orthophosphate buffer) was found to give the best peak in terms of resolution and sensitivity. The internal standard used was Dexamethasone. Initially, we tried different internal standards namely formoterol, bamethane and salbutamol but all these compounds were eluted within the dead volume of the column or overlap with an indacaterol peak (Snyder et al., 2012, Koerner, 2013).

3.2.5.2 Method Validation

Following the similar validations protocols, indacaterol HPLC method was validated and the results are as following:

3.2.5.2.1 Calibration (Linearity)

The method showed a good linearity throughout the range of 0.1 µg/ mL to 10µg/ mL with a correlation coefficient of R2= 0.999.

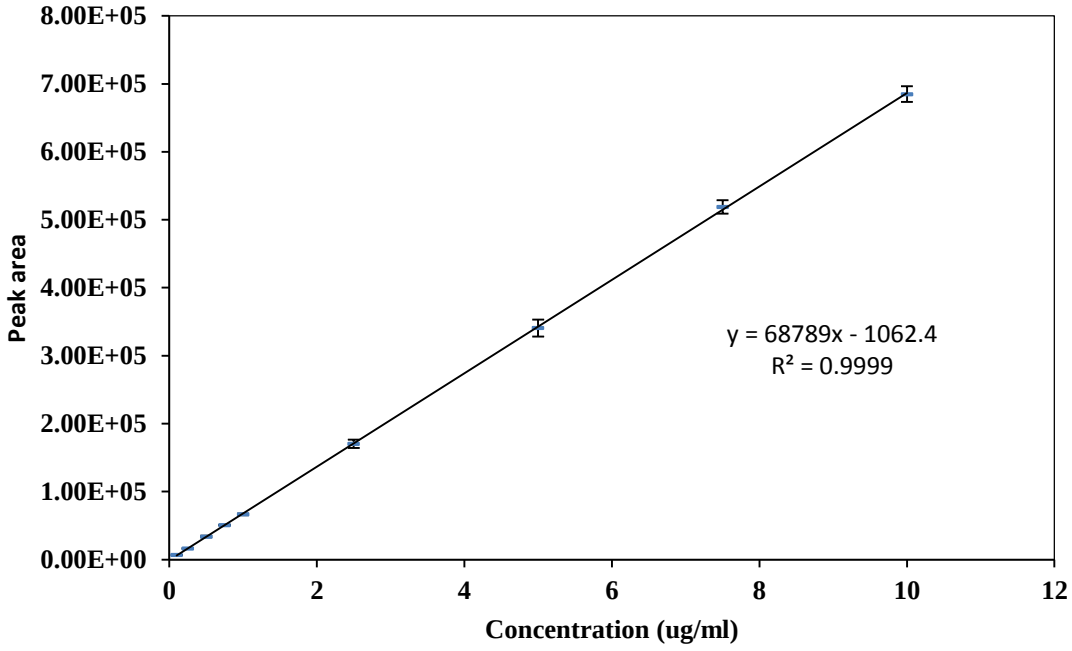

Figure 3.10: Calibration curve of indacaterol at 259nm (n=3).

3.2.5.2.2 Precision

Three different concentrations levels (low, medium and high) of indacaterol (2.0 µg/mL, 6.0 µg/mL and 9.0 µg/mL) were used to assess the Intra-day and inter-day precision of the method. Each concentration was injected 3 times on three different days. The results of method precision are shown in Table 3.8.

Table 3-8: Intra and inter-day precision of indacaterol [n=3].

Concentration (µg/mL)	Intra-Day %CV	Inter-Day %CV
2	0.37	0.58
6	0.20	0.37
9	0.66	0.83

3.2.5.2.3 Accuracy

The method accuracy was determined by injecting three concentrations of indacaterol 3 times (Table 3.9). The concentrations of (2, 6.0 and 9.0µg/mL) indacaterol were used. The calculated relative error was used to determine the accuracy of the method for each concertation.

Table 3-9: Intra and inter-day accuracy of indacaterol [n=3].

Nominal concentration (µg/ml)	Mean (SD) of measured concentration	Mean ± SD % to nominal concentration
	Intra-day Variation	Intra-day Variation
2.0	1.99 (0.01)	99.5 ± 0.5
6.0	5.98 (0.06)	99.6 ± 0.8
9.0	9.07 (0.03)	100.7 ± 0.3
	Inter-day Variation	Inter-day Variation
2.0	1.97 (0.03)	98.50 ± 1.5
6.0	5.95 (0.04)	99.29 ± 0.7
9.0	8.98 (0.12)	99.78 ±1.3

3.2.5.3 Detection and Quantification Limits

The limit of detection (LOD) can be defined as the lowest concentration of an analyte in a sample that can be detected but not quantified according to the ICH guidelines. In contrast, the limit of quantification (LOQ) is known as the lowest amount of an analyte in a sample that can be determined quantitatively with suitable precision and accuracy under the standard operating conditions of the method. According to the ICH, there are two options to determine the LOD and LOQ of an assay. Firstly, by calculating LOD and LOQ as a concentration at a specified signal to noise ratio, in which 3:1 for the signal to noise ratio can determine LOD, and it is 10:1 for LOQ. Secondly using the mathmatecial equation to calculate the LOD and LOQ based on the standard deviation (Standard error) and the slope of the calibration curve. The equations for calculation LOD and LOQ are shown as following:

LOD $$\frac{\text{Standard deviation}}{\text{Slope}} \times 3.3$$ **Eq. 3.2**

LOQ $$\frac{\text{Standard deviation}}{\text{Slope}} \times 10$$ **Eq. 3.3**

Indacaterol method **LOQ** and **LOD** were 0.17µg/mL and 0.07µg/mL

Formoterol method **LOQ** and **LOD** were 49.8 ng/ml and 17.9 ng/ml respectively.

3.2.5.4 Robustness

Both HPLC methods were considered robust and no change in the methods performance were observed when slight but deliberate changes were introduced to the flow rate, column oven temperature, buffer pH and % of the organic modifier. (See appendix for robustness results).

Chapter 4.*In-vitro* dose emission of formoterol Aerolizer® and indacaterol Breezhaler® at different MIFs and Vins using two separate inhalations

4.1 Chapter overview

DPIs are breath actuated devices relying on the inhalation effort generated by the patient during the inhalation manoeuvre to de-aggregate and disperse the powder formulation and release the dose for lung deposition (Chrystyn, 2003). The design of the device inhalation channel, metering cup and the air inlet determine the specific intrinsic resistance of the inhaler device (De Boer et al., 1997). The DPIs are classified into three main groups Low, Medium, and High depending on their intrinsic resistance. This classification is usually determined by the inhalation flow required to generate a pressure drop of 4 kPa across the inhaler device (Dal Negro, 2015). The patient's inhalation flow through the inhalers depends on the resistance of the device; a fast flow rate is produced through lower resistance device when compared to slow flow with high resistance devices (Al-Showair et al., 2007b, Azouz and Chrystyn, 2012, Chrystyn et al., 2016). Beside the MIF, Vin is another inspiratory parameter contributing to dose emptying from single dose capsule based DPIs. For this reason, the manufacturer patient information leaflet (PIL) instructs the patients to inhale twice for each capsule dose to empty the dose efficiently (Haughney et al., 2010, Laube et al., 2011). The patients with limited lung capacity have limited inspiratory capabilities along with low Vin and they are unable to generate sufficient inhalation manoeuvre, thus resulting in low dose being delivered to the lungs (Azouz et al., 2015a, Azouz et al., 2015b). Several studies have used the inhaled volume of 2 L and 4L to investigate the dose emission aerodynamic characteristics from DPIs. These studies demonstrate that DPIs dose emission performance show no significant improvement when using Vin $\geq$ 2 L (Abdelrahim, 2010, Alaboud et al., 2010, Yakubu et al., 2013).For *in-vitro* DPIs dose emission testing the pharmacopoeia recommended the use of a MIF corresponding to 4kPa pressure drop and a Vin of 4 L (EP, 2013, USP, 2014). The Food and Drug Agency (FDA) recommends the use of 2 L Vin when testing the DPIs (FDA, 1998). The Vin value recommended by FDA seems to be more

reasonable because majority of the patients cannot generate the pharmacopeial recommended 4 L inhaled volume (Azouz et al., 2015b). Each DPIs has a minimum flow requirement to efficiently de-aggregate the dose and release the medication when patient unable to achieve that certain flow dose de-aggregation will be incomplete resulting in less drug being delivered to the lungs (Laube et al., 2011, Haidl et al., 2016). Laube et al., (2011) pointed out in their task force report that ‘‘no manufacturer has stated the minimum flow for their DPIs although it is clear that this information is needed’’.

The inhaled aerosol dose quality emitted from DPIs depends on patient’s inspiratory flow, inhaler specific resistance and the formulation (Clark and Hollingworth, 1993, De Boer et al., 1997, Chrystyn, 2009, Hira et al., 2012). In the present chapter, the dose emission of two low resistance capsule based inhaler devices Foradil Aerolizer® (12 µg) and Onbrez Breezhaler® (150 µg) will be determined. Furthermore, Evaluating the impact of increasing the MIF and Vin on the dose emission after two separate inhalations. Therefore, the present chapter was designed to determine the minimum MIF and Vin of clinical relevance to operate both formoterol Aerolizer® and indacaterol Breezhaler®. A range of MIF and Vin was used [MIF of 28.3 L/min, 60 L/min, 90 L/min and 120 L/min] [Vin of 0.5 L, 0.75 L, 1 L, 1.5 L and 2 L]. The dose delivery uniformity (DDU) for both devices will be determined by using 16 randomly chosen capsule for each set of MIF and Vin (n=16).

4.2 Materials and methods

The materials and instrumentations for the determination of dose emission using DUSA were listed in Chapter 3 (section 3.1.2 and 3.1.4.1). The general laboratory material and apparatus used in the study were listed in Chapter 3 (section 3.1.1). For the quantification of formoterol and indacaterol, two HPLC methods were developed validated, explained in details Chapter 3 (section 3.2.4 and 3.2.5).

4.2.1 Experimental set-up

The method set-up for the determination of DPIs dose emission is shown in Figure 4.1. The compendial method described in the USP (2014) was used to determine the dose emitted from Foradil Aerolizer® and Onbrez Breezhaler® at different MIFs and Vins. Dose emission at high inhalation flow rate (up to 100 L/min) can be determined by using the high capacity vacuum pump (Copley Scientific, 2015). The mouthpiece adapter was used to avoid any loss of drug between the collection tube of DUSA and the device's mouthpiece. Inhalation flow up to 100L/min is achievable by using a high capacity vacuum pump, However, to produce higher stable inhalation flow ($P3/P2 \leq 0.5$) two vacuum pumps were connected in series together (Copley scientific Ltd, 2015. See section 3.1.4.1). In between flow controller and vacuum pump, a timer controlled two ways solenoid valve was connected.

The latter enables a set of volume air to be withdrawn from the device mouthpiece at the desired inhalation flow. Flow controller was used to ensure that critical (sonic) flow is achieved for the flow control valve, in other words, the downstream pressure of the valve should be equal or less than half of upstream pressure as $P3/P2 \leq 0.5$ (Copley, 2015).

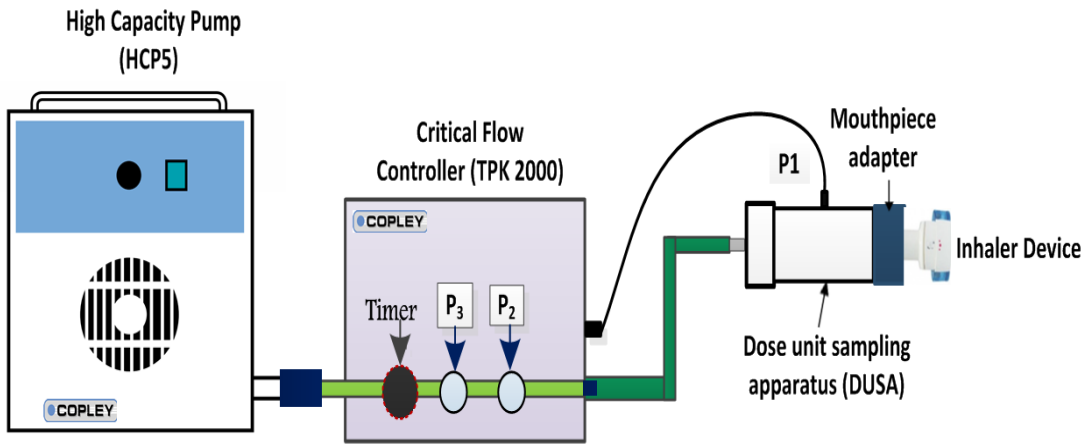

Figure 4.1: Schematic diagram of the instrument set-up to determine dose emission at different MIFs and Vins

The duration of inhalation was set for different Vins and MIFs by using the mathematical relationship that was described in the pharmacopoeia (USP, 2014), whereas the duration of inhalation was calculated using Eq. 5.1 relating to MIF, Vin and time.

$$\mathbf{T(sec) = \frac{Vin\ (L)}{MIF\ (\frac{L}{min})}\ x\ 60} \qquad \textbf{Eq. 4.1}$$

Where;

T = duration of inhalation for a given set of MIF and Vin

MIF = maximum inhalation flow

Vin = the volume to be drawn through the device

Table 4-1: Calculation of inhalation time (T sec) for each set of MIF and Vin

MIF (L/min)	Vin (L)				
	0.5	**0.75**	**1**	**1.5**	**2**
28.3	1.1s	1.6s	2.1s	3.2s	4.2s
60	0.5s	0.7s	1.0s	1.5s	2.0s
90	0.3s	0.5s	0.7s	1.0s	1.3s
120	0.2s	0.4s	0.5s	0.7s	1.0s

4.2.2 Dose emission procedure:

The emitted dose was determined using DUSA as shown in Figure 4.1. Instead of using the standard pharmacopeial condition Vin of 4 L and MIF corresponding to 4 kPa pressure drop, different values MIFs (28.3 L/min, 60 L/min , 90 L/min and 120 L/min) and Vins (0.5 L, 0.75 L, 1 L, 1.5 L and 2 L) were used. In this chapter, two DUSA (Copley Scientific Ltd, UK) were used for the determination of the emitted dose after the first and second inhalations *i.e.* DUSA1 to collect ED1 and DUSA2 to collect ED2.

Firstly, the DUSA was fitted with a glass fibre filter (47mm, Whatman Ltd, UK) to collect the aerosolised drug powder into the sampling apparatus. For each DUSA a rubber mouthpiece adapter (especially designed for each inhaler device, Copley Scientific Ltd, UK) was used to connect the inhaler device to the DUSA tube. A high capacity vacuum pump was used to produce the desired MIF during the experiment. The vacuum pump was switched on before each determination to ensure the flow stability would be maintained during the experiment. The inhalation flow through the device mouthpiece was set to 28.3, 60, 90 and 120 L/min with a calculated duration of inhalation (Table 4.1) corresponding to each set of MIFs and Vin to allow the inhaled volumes to be withdrawn through the Aerolizer® device.

Once the MIF and Vin were adjusted to the required conditions, a single capsule dose was loaded into the device capsule chamber as instructed in the patient instruction leaflet (PIL). The capsule was then pierced, and whilst keeping the fingers on the side buttons of the device it was horizontally connected to the DUSA1 tube. Thereafter, the TPK timer controlling the solenoid valve was started to aerosolise the dose (ED1) into the DUSA1. Then, using the same capsule (already being pierced) the ED2 was aerosolised into DUSA 2. For each set of MIF and Vin, 16 capsules randomly chosen (Table 4.2) were aerosolised to determine the dose delivery uniformity of both formoterol Aerolizer® and indacaterol Breezhaler®. The

procedure was carried out for all the 16 capsules using one inhaler device, and the dose retained in the device was recovered after finishing dose emission procedure for all the 16 capsules.

Table 4-2: The randomisation of the capsules chosen throughout lifetime of device

MIF (L/min)	Vin (mL)	Randomised capsules form Aerolizer® and Breezhaler® label claim of 60 Doses
28.3 60 90 120	500 750 1000 1500 2000	1 - 4 - 8 - 12 16 - 20 - 24 - 28 32 - 36 - 40 - 44 48 - 52 - 56 - 60

The dose emitted washing procedure was then taken place to collect the dose emitted into DUSA 1 and 2, mouthpiece 1 and 2, residual dose in both capsule and device. For formoterol, dose emission washing procedure of 60 % v/v Acetonitrile/ water was used, while for indacaterol washing solution of 60 % v/v methanol/ water was used. The selection of washing solution proportion is depending on the percentage of organic modifier in the HPLC method. In practical, using a higher percentage of diluent in sample preparation may lead to peak shape problems and affect the quantification accuracy of the HPLC method.

DUSA was washed by firstly sealing the metal end of the tube with Parafilm (Fisher Scientific Ltd, UK). Then, the cap of the DUSA was opened to add appropriate volume of washing solution, after that, the cap was closed tightly and shaken gently for 2 minutes to recover all the drug powder inside the DUSA tube. The solution with the drug powder was transferred into a beaker, and the DUSA filter was removed and immersed in the solution. The beaker was sealed with Parafilm (Fisher Scientific Ltd, UK) and sonicated for 15 minutes to ensure drug detachment from the filter.

The mouthpiece (MP) was washed by sealing the base of the MP with Parafilm, then appropriate volume of washing solution was added to collect drug particles deposited in the MP. The capsule was taken from the device, and then placed into clean beaker with appropriate volume of washing solution. The stirring plate was used to allow the washing solution to enter the capsule; therefore, drug lodged in the capsule would be recovered.

The device was washed only with water, because acetonitrile and methanol are harsh solvent and they may affect the linning layer of the device. After washing the device with water, 60% methanol or acetonitirle was added to the solution before soniction. The recovered samples were transferred into 20 mL glass vials, then all vials were sonicated for 15 miuntes to ensure solubility of drug particles in the solution. The sonicated samples were then transferred into amber 2 mL HPLC vials (Fisher Scientific Ltd, UK). A validate HPLC methods were used to quntify the amount of formoterol and indacaterol in the recovered samples (Chapter 3, section 3.2.4 and 3.2.5).

4.2.3 Data analysis

The ED1 and ED2 representing first and second inhalation respectively and were obtained from the amount of the drug emitted into the DUSA1 and DUSA2. The RA was the residual amount retained in the capsules (16 caps) and the inhaler device. The TED was obtained as the sum of the dose emitted after first and second inhalations. A one-way and Two-ways ANOVA were used to determine the difference in the ED1, ED2, TED, and RA. Tuckey test was used to determine the difference between the different parameters used. The hypothesis of (P-value = 0.05) was used to determine the significant difference.

4.3 Results

4.3.1 *In-vitro* formoterol dose emission results at different MIFs and Vins.

The results were classified according to the MIF into 4 levels (28.3 L/min, 60 L/min, 90 L/min and 120 L/min). **ED1** was the dose emitted into DUSA 1 + MP 1; **ED2** was the sum of DUSA 2 + MP2. **TED** was calculated as the sum of ED1 + ED2. **TRA** was obtained by adding the amount retained in the capsule to that retained in the device (residual amount in capsule + residual amount in device). **TRD** total dose recovery (TED + TRA).

The results show that the increase of the MIF result in a significant increase ($p < 0.05$) in ED1 for all the Vins used. The impact of MIF was higher when low Vins (0.5 L and 0.75 L) were used in comparison to high Vins (1L, 1.5 L and 2 L) (Figure 4.6).

At low MIFs 28.3 L/min and 60 L/min the dose emission after first inhalation (ED1) increased significantly ($p < 0.05$) with the increase of the Vins, In contrast, the dose emission after second inhalation (ED2) decreased significantly ($p < 0.05$) Tables 4.3 and 4.4. At high MIFs 90 L/min and 120 L/min the ED1 increased with increasing the Vin, most of the dose was emitted from the capsule during first inhalation and amount left for second inhalation was below the detection limit of the HPLC method Table 4.5 and 4.6.

TRA decreased significantly ($p < 0.05$) with the increase of MIF from 28.3 to 120 L/min (Tables 4.3 to 4.6). TED significantly increased ($p < 0.05$) with the increase of the MIF (Figure 4.6). The Vin increase has a significant effect ($p < 0.05$) on the TED only at low MIF 28.3 and 60 L/min. In contrast, at high MIF 90 and 120 L/min increasing the Vin result in insignificant difference in the TED. The dose delivery was consistent for all 16 capsules used with an average of 11.5µg TRD. [See appendix for DDU results]

Table 4-3: Mean (SD) of the amount of formoterol in (µg) dose emission results at MIF of 28.3L/min using different Vins. [n=16]. [TRA (ED1+ED2) TRD (Cap +Dev) highlighted are the main parameters]

Dose emission (µg)	Inhalation Volume (Vin)				
	500 mL	**750 mL**	**1000 mL**	**1500 mL**	**2000 mL**
ED1	5.51 (0.56)	5.86 (0.32)	6.05 (0.54)	6.21 (0.21)	6.44 (0.32)
ED2	2.16 (0.54)	2.06 (0.72)	2.02 (0.19)	1.92 (0.50)	1.71 (0.46)
TED	7.67 (0.23)	7.92 (0.30)	8.07 (0.55)	8.13 (0.51)	8.16 (0.61)
MP1	0.07 (0.01)	0.11 (0.03)	0.19 (0.05)	0.24 (0.08)	0.27 (0.09)
Cap	3.56 (0.43)	3.21 (0.44)	3.18 (0.53)	2.90 (0.65)	2.57 (0.35)
TRA	**4.13 (0.24)**	**3.89 (0.16)**	**3.75 (0.1)**	**3.58 (0.10)**	**3.47 (0.10)**
TRD	**11.80 (1.00)**	**11.81 (1.10)**	**11.82 (0.80)**	**11.71(1.20)**	**11.63 (0.70)**

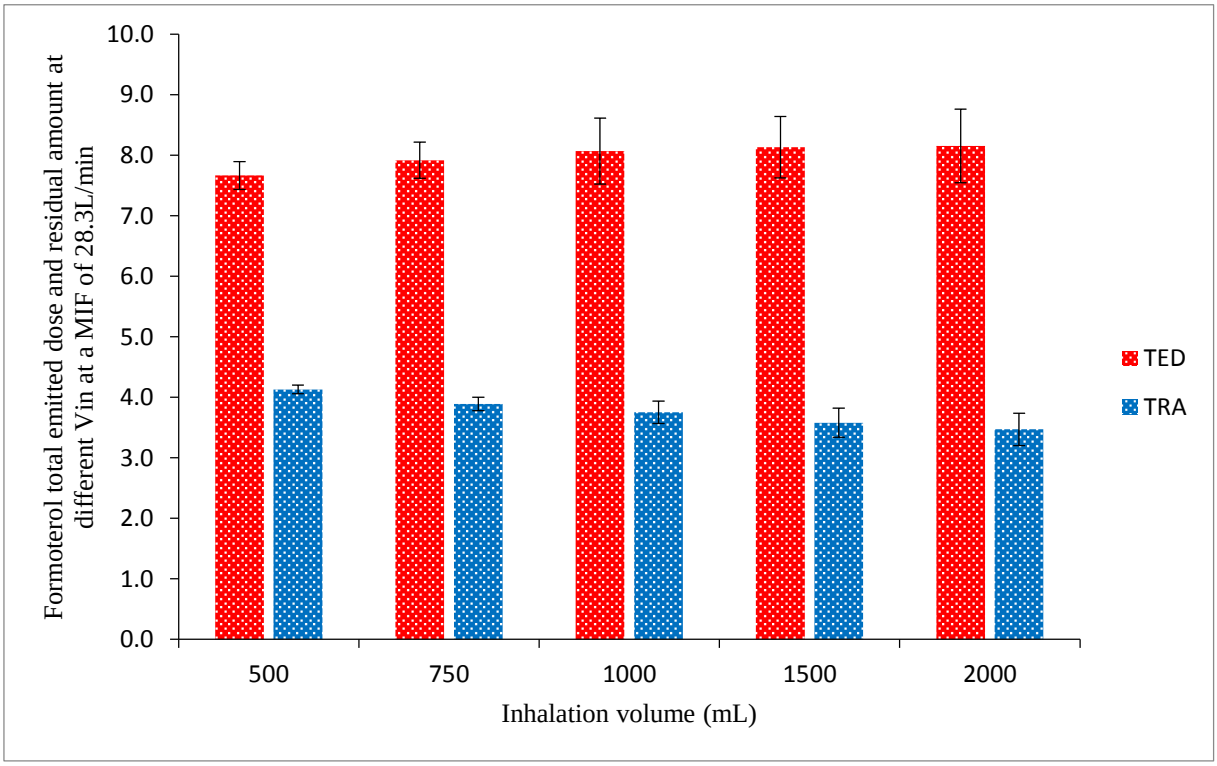

Figure 4.2: Mean (SD) of the total emitted dose (TED) and total residual amount (TRA) of formoterol emitted from Aerolizer® at different Vins using a MIF of 28.3L/min.

Table 4-4: Mean (SD) of the amount of formoterol in (µg) dose emission results at MIF of 60L/min using different Vins. [n=16]. [TRA (ED1+ED2) TRD (Cap +Dev) highlighted are the main parameters]

Dose emission (µg)	Inhalation Volume (Vin)				
	500 mL	**750 mL**	**1000 mL**	**1500 mL**	**2000 mL**
ED1	6.33 (0.32)	6.51(0.47)	6.91 (0.72)	6.91 (0.72)	7.22 (0.47)
ED2	1.40 (0.21)	1.34 (0.16)	1.29 (0.20)	1.29 (0.20)	1.10 (0.21)
TED	7.73 (0.35)	7.84 (0.46)	8.20 (0.79)	8.20 (0.79)	8.32 (0.59)
MP1	0.12 (0.03)	0.18 (0.04)	0.23 (0.09)	0.23 (0.09)	0.26 (0.03)
Cap	2.81(1.08)	2.61 (0.86)	2.31 (0.86)	2.32 (0.86)	2.46 (0.56)
TRA	**3.59 (0.86)**	**3.49 (0.56)**	**3.37 (1.00)**	**3.31 (1.03)**	**3.28 (0.99)**
TRD	**11.32 (0.99)**	**11.33 (1.03)**	**11.57 (1.31)**	**11.51 (1.31)**	**11.60 (1.00)**

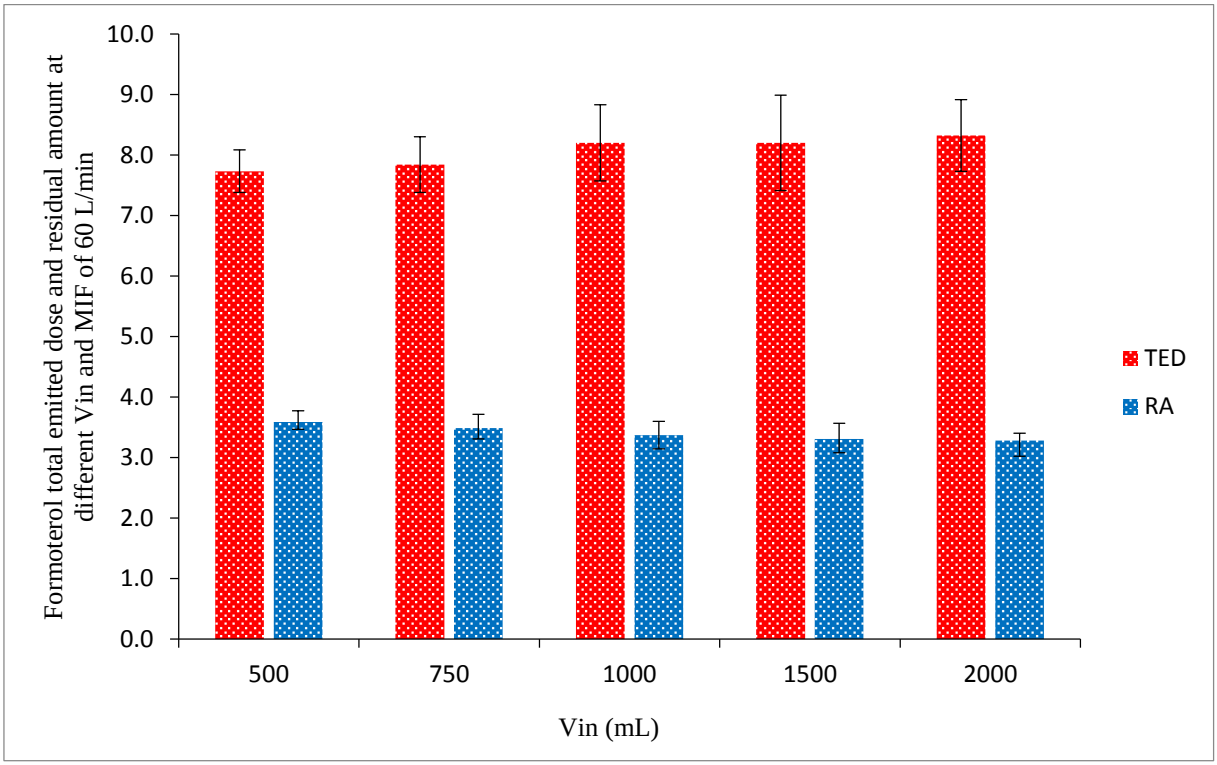

Figure 4.3: Mean (SD) of the total emitted dose (TED) and total residual amount (TRA) of formoterol emitted from Aerolizer® at different Vins using a MIF of 60 L/min.

Table 4-5: Mean (SD) of the amount of formoterol in (µg) dose emission results at MIF of 90 L/min using different Vins. [n=16]. [TRA (ED1+ED2) TRD (Cap +Dev) highlighted are the main parameters]

Dose emission (µg)	Inhalation Volume (Vin)				
	500 mL	750 mL	1000 mL	1500 mL	2000 mL
ED1	7.89(0.36)	8.42(0.80)	8.60(0.19)	8.63(0.40)	8.68(0.67)
ED2	0.47(0.22)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
TED	8.35(0.30)	8.42(0.80)	8.60(0.19)	8.63(0.40)	8.68(0.67)
MP1	0.18(0.04)	0.22(0.06)	0.27(0.02)	0.25(0.07)	0.28(0.08)
Cap	2.12(0.56)	2.12(0.36)	2.01(0.35)	1.96(0.21)	1.93(0.28)
TRA	**3.21(0.36)**	**3.09(0.40)**	**2.96(0.30)**	**2.86(0.07)**	**2.78(0.37)**
TRD	**11.56(0.65)**	**11.51(0.56)**	**11.68(0.45)**	**11.49(0.33)**	**11.46(0.60)**

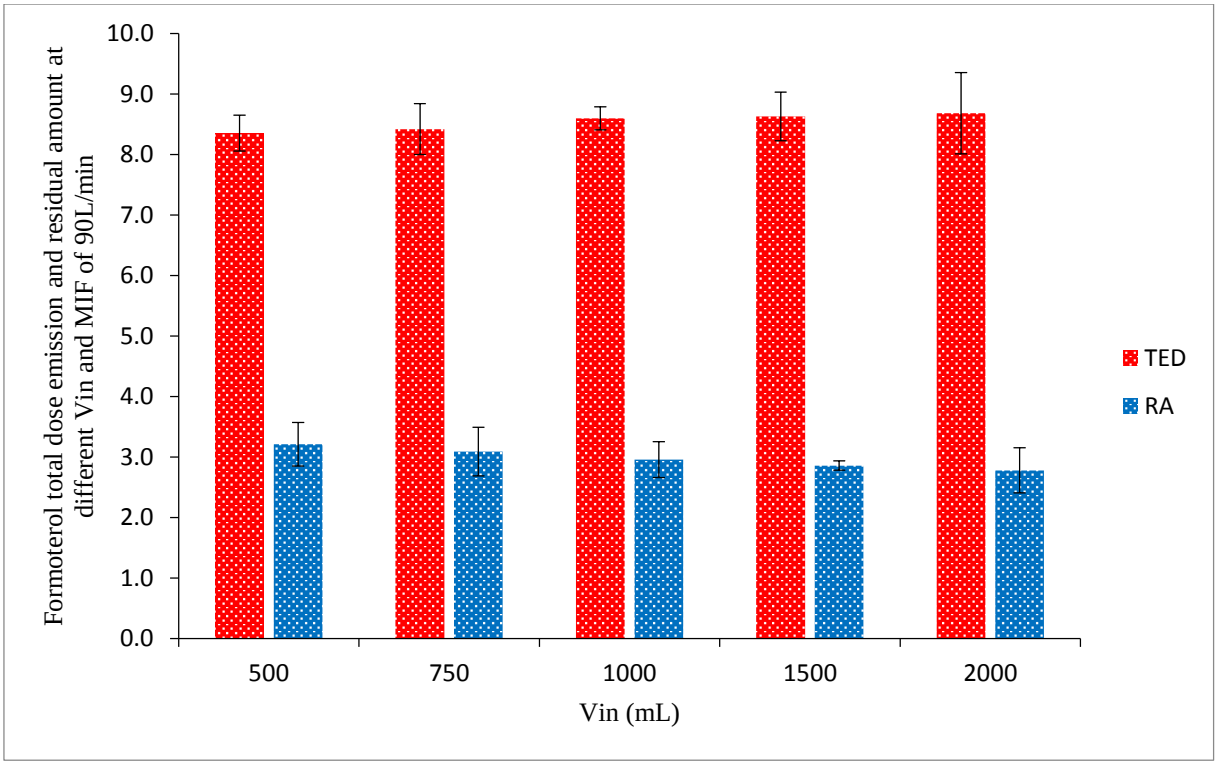

Figure 4.4: Mean (SD) of the total emitted dose (TED) and total residual amount (TRA) of formoterol emitted from Aerolizer® at different Vins using a MIF of 90 L/min.

Table 4-6: Mean (SD) of the amount of formoterol in (µg) dose emission results at MIF of 120L/min using different Vins. [n=16]. [TRA (ED1+ED2) TRD (Cap +Dev) highlighted are the main parameters]

Dose emission (µg)	Inhalation Volume (Vin)				
	500 mL	**750 mL**	**1000 mL**	**1500 mL**	**2000 mL**
ED1	8.49(0.37)	8.60(0.38)	8.72(0.40)	8.81(0.60)	8.85(0.77)
TED	8.49(0.37)	8.60(0.38)	8.72(0.40)	8.81(0.60)	8.85(0.77)
MP1	0.29(0.04)	0.33(0.06)	0.34(0.06)	0.36(0.07)	0.37(0.07)
Cap	2.31(0.35)	2.28(0.35)	2.22(0.45)	2.05(0.34)	2.02(0.41)
TRA	**2.75(0.13)**	**2.64(0.32)**	**2.51(0.05)**	**2.35(0.16)**	**2.30(0.10)**
TRD	**11.24(0.59)**	**11.24(0.50)**	**11.22(0.62)**	**11.16(0.74)**	**11.15(0.67)**

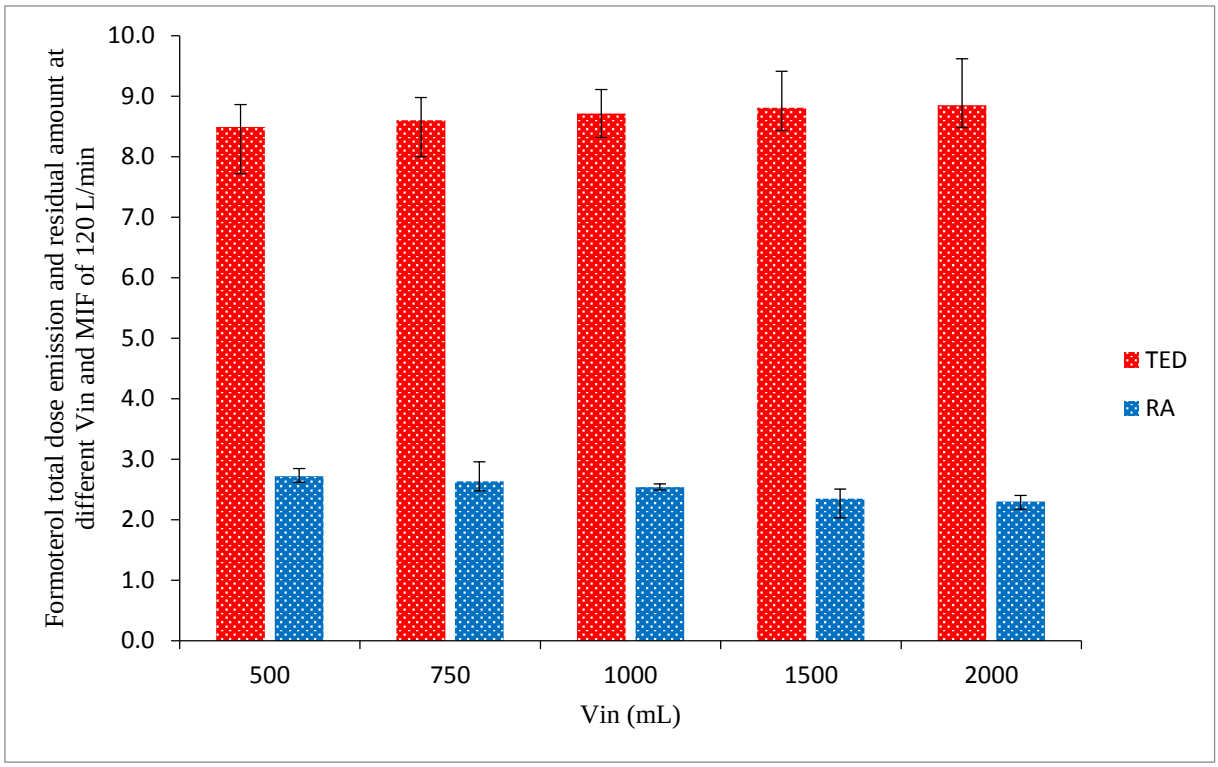

Figure 4.5: Mean (SD) of the total emitted dose (TED) and total residual amount (TRA) of formoterol emitted from Aerolizer® at different Vins using a MIF of 120 L/min

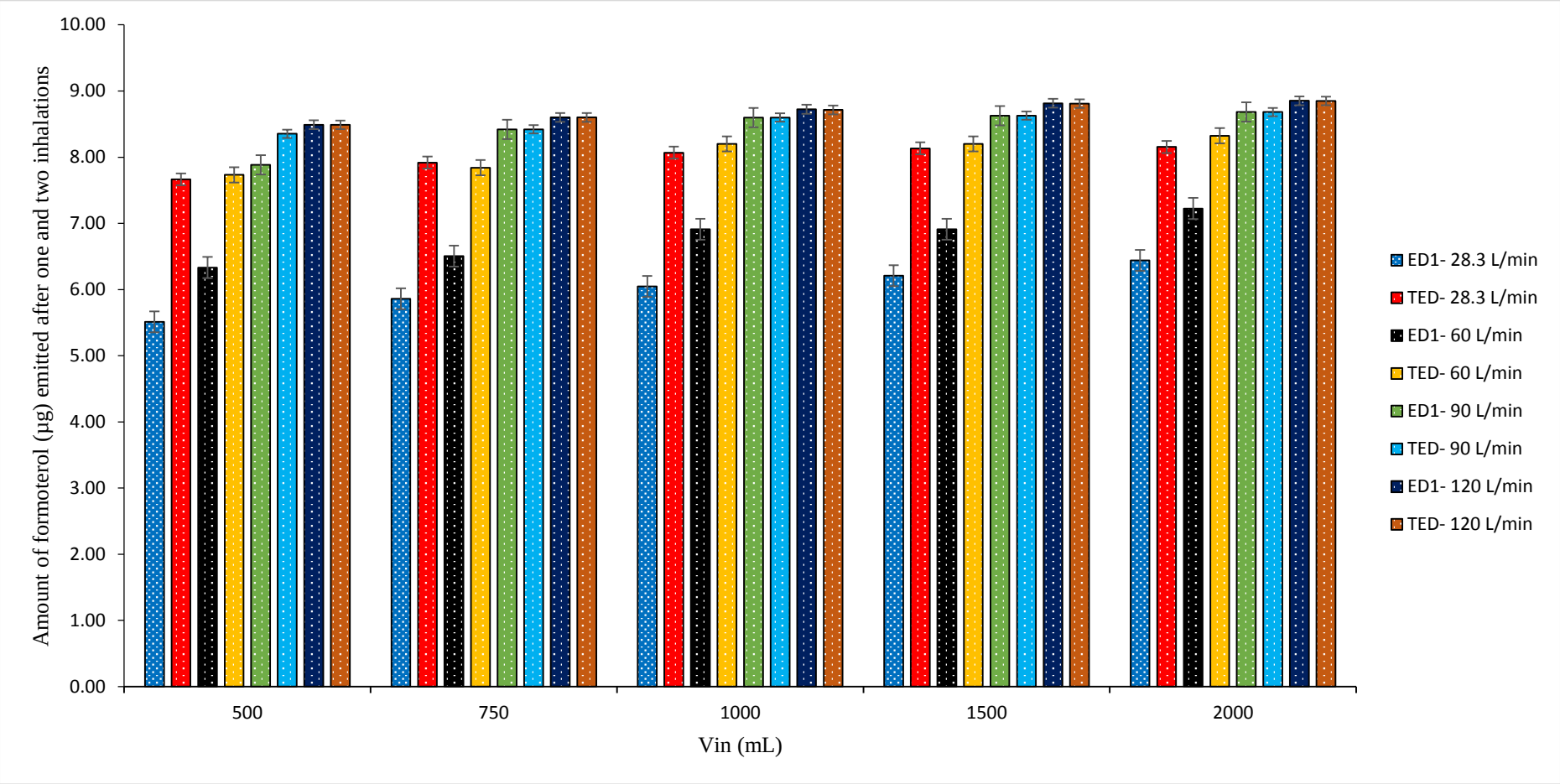

Figure 4.6: Mean (SD) of formoterol dose emitted after one and two inhalations at different MIF and Vin values

4.3.2 *In vitro* indacaterol dose emission at different MIFs and Vins

The dose emission performance of indacaterol Breezhaler® using wide range of MIFs and Vin was determined after two separate inhalations. Furthermore, the dose delivery uniformity of indacaterol was also determined by randomly chosen 16 doses out of 60 capsules pack for each set of MIF and Vin.

The results demonstrate that ED1, TED increased significantly ($p < 0.05$) with increasing the MIF from 28.3 L/min to 120 L/min for all Vins used. The impact of the MIF was more pronounced when low Vin values (0.5, 0.75 and 1 L) were used (Figures 4.7, 4.8, 4.9 and 4.10). In contrast, dose retained in capsule, TRA and ED2 decreased significantly ($p < 0.05$) with increase the MIF (Tables 4.7 to 4.10) (Figures 4.8, 4.10, 4.12 and 4.14).

Similarly, ED1 increased and ED2 decreased significantly ($p < 0.05$) with increasing the Vin. The impact of the Vin was more observed at low MIFs 28.3 and 60 L/min (Tables 4.7 and 4.8) , at high MIFs most of the dose delivered after first inhalation and no much dose left for the second inhalation (Tables 4.9 and 4.10). TRD of indacaterol Breezhaler® was consistent with an average dose delivery of 149 µg was recovered for all Vin and MIF used. [See appendix DDU results]

Table 4-7: Mean (SD) of the amount of Indacaterol in (µg) dose emission results at MIF of 28.3 L/min using different Vins. [n=16].

Dose emission (µg)	Inhalation Volume (mL)				
	500 mL	**750 mL**	**1000 mL**	**1500 mL**	**2000 mL**
ED1	99.85 (5.06)	108.21(7.00)	113.89 (6.58)	119.47 (6.47)	127.73 (5.73)
ED2	24.02 (5.08)	19.76 (5.02)	16.01(3.31)	11.90 (3.74)	5.90 (2.67)
MP1	1.92 (0.70)	2.04 (1.04)	2.19(0.78)	2.51(0.60)	2.76 (0.45)
MP2	0.88 (0.62)	0.84 (0.57)	0.66 (0.57)	0.35 (0.10)	0.21 (0.08)
TED	123.87 (4.87)	127.96 (7.91)	129.90 (8.25)	131.37 (7.41)	133.63 (6.00)
% TED*	82.58 (3.25)	85.31(5.27)	86.60(5.50)	87.58(4.94)	89.09(4.00)
CAP	9.04 (2.88)	6.22 (1.69)	5.53 (1.83)	4.89 (1.01)	3.69 (1.24)
TRA	30.68 (2.31)	25.00 (2.67)	18.89 (1.35)	17.43 (1.65)	15.31(0.83)
TRD	150.42 (2.35)	152.97 (5.00)	148.78 (4.36)	148.80 (5.43)	148.94 (3.17)

*= Total emitted dose as % of the nominal dose 150µg indacaterol from Onbrez Breezhaler®

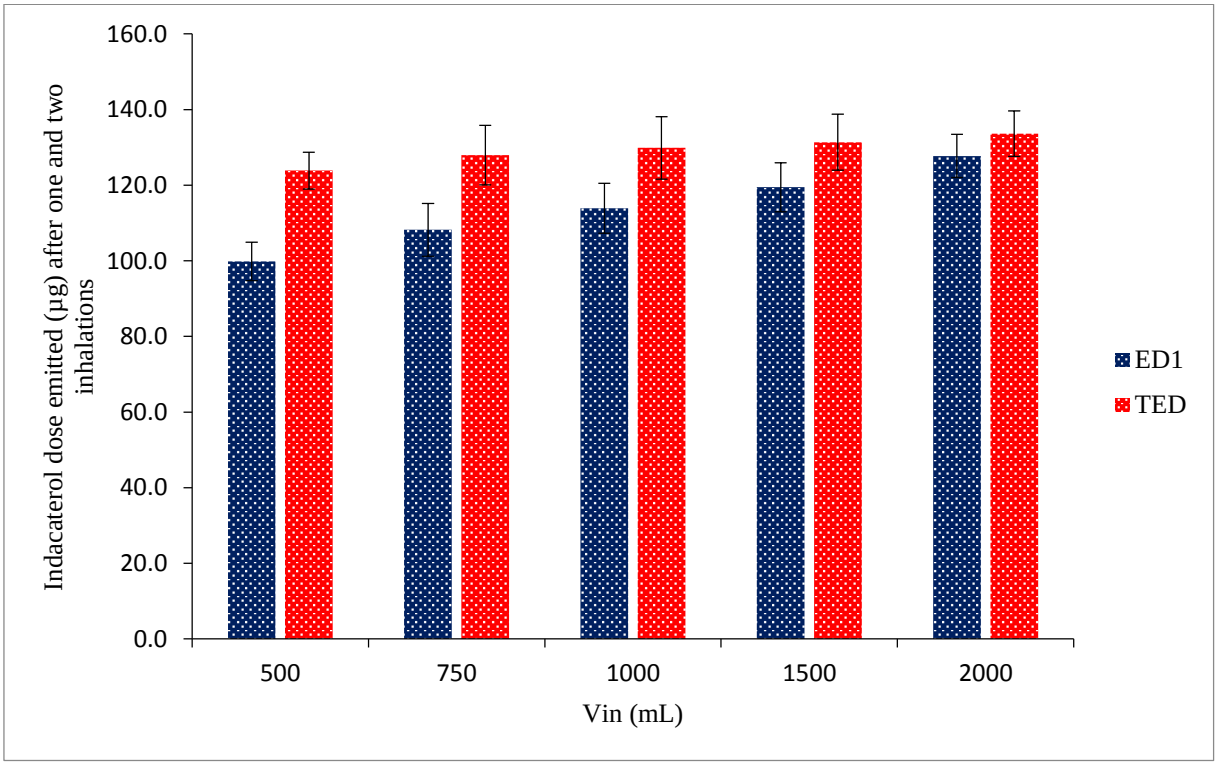

Figure 4.7: Mean (SD) of the emitted dose after one inhalation (ED1), and two (TED) inhalations at different Vins using a MIF of 28.3 L/min.

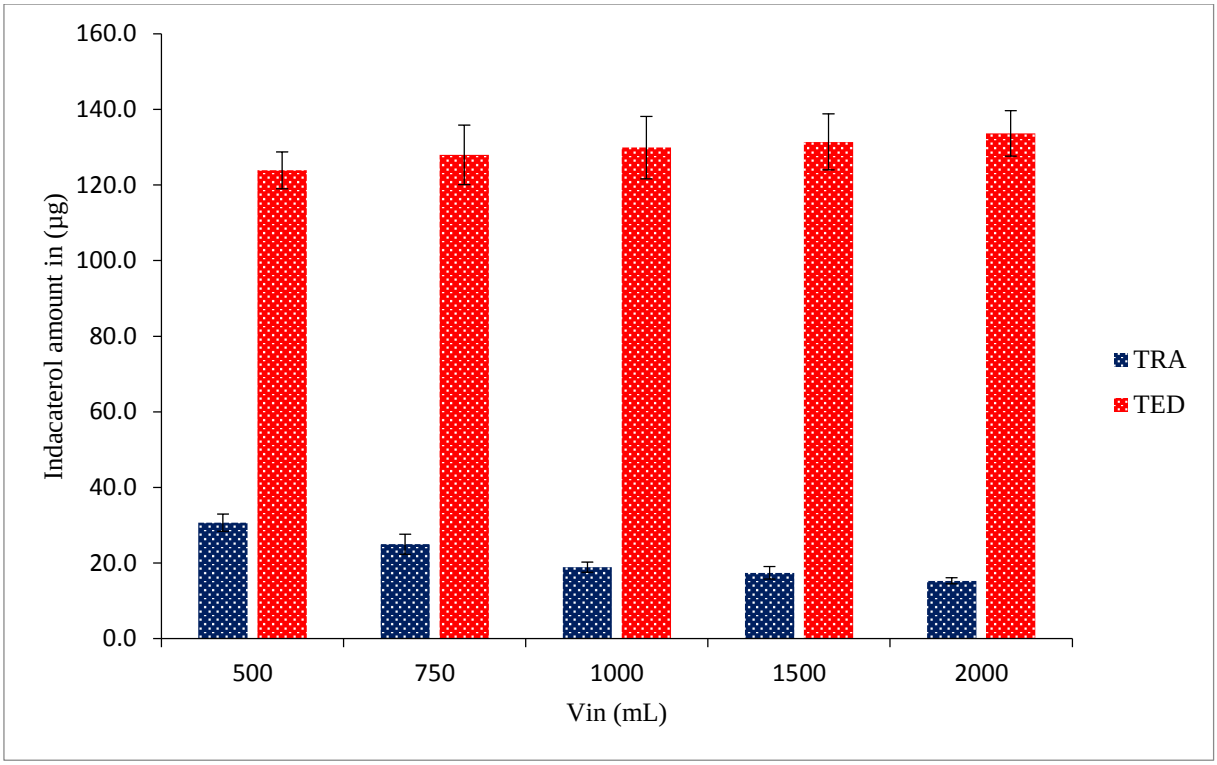

Figure 4.8: Mean (SD) of the total emitted dose (TED) and total residual amount (TRA) at different Vins using a MIF of 28.3 L/min.

Table 4-8: Mean (SD) of the amount of Indacaterol in (µg) dose emission results at MIF of 60 L/min using different Vins. [n=16].

Dose emission (µg)	Inhalation volume (mL)				
	500 mL	**750 mL**	**1000 mL**	**1500 mL**	**2000 mL**
ED1	112.32(7.45)	120.73(8.77)	126.41(6.99)	130.52(8.58)	133.96(4.23)
ED2	14.09(6.09)	10.56(4.95)	8.90(3.50)	5.55(2.28)	3.87(1.58)
MP1	2.76(1.17)	2.96(0.90)	3.74(1.17)	4.55(0.77)	4.57(0.88)
MP2	0.74(0.35)	0.70(0.45)	0.34(0.40)	0.22(0.06)	0.21(0.12)
TED	126.42(8.99)	131.29(8.78)	135.31(6.95)	136.07(7.80)	137.83(4.04)
% TED*	84.28(6.00)	87.53(5.85)	90.21(4.63)	90.72(5.20)	91.89(2.69)
CAP	5.86(2.39)	4.64(1.09)	3.78(1.59)	3.49(0.89)	2.90(0.46)
TRA	19.06(0.95)	16.42(1.02)	15.84(0.86)	13.84(1.28)	12.79(0.66)
TRD	145.48(6.80)	147.71(5.68)	151.15(6.88)	149.91(7.71)	150.62(4.12)

*= Total emitted dose as % of the nominal dose 150µg indacaterol from Onbrez Breezhaler®

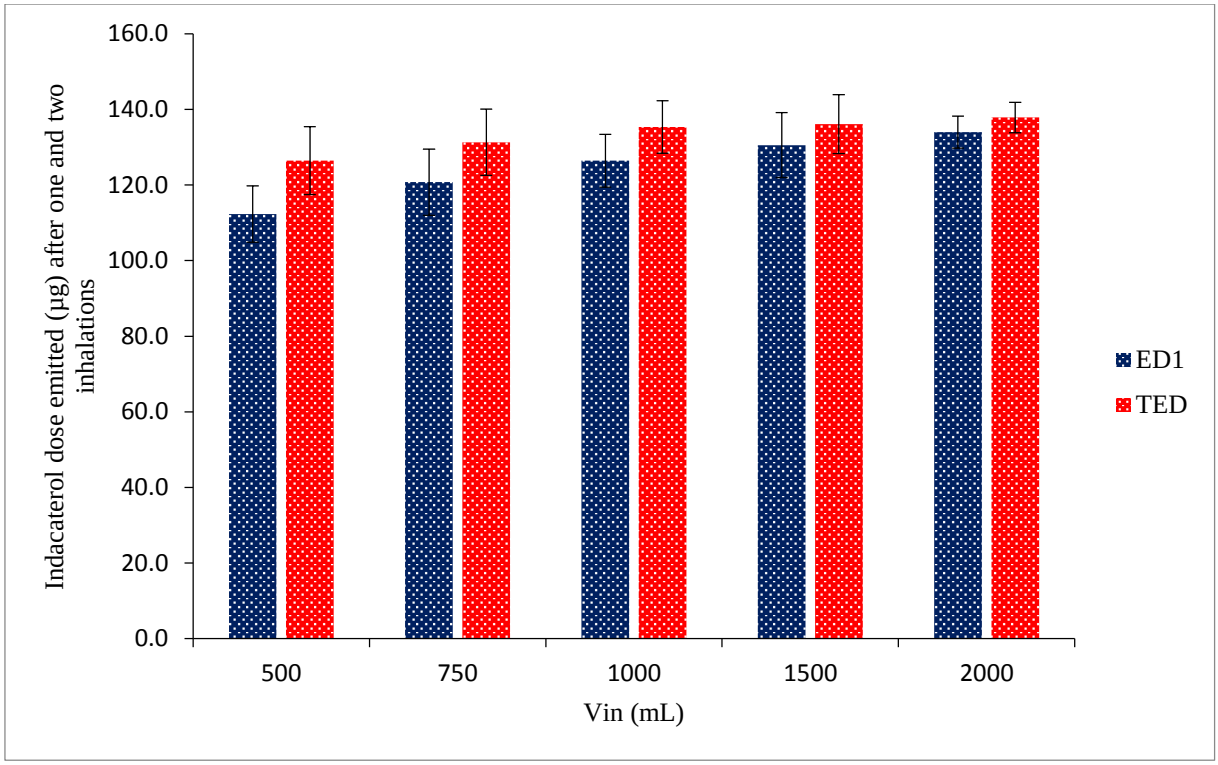

Figure 4.9: Mean (SD) of the emitted dose after one inhalation (ED1), and two inhalations (TED) at different Vins using a MIF of 60 L/min.

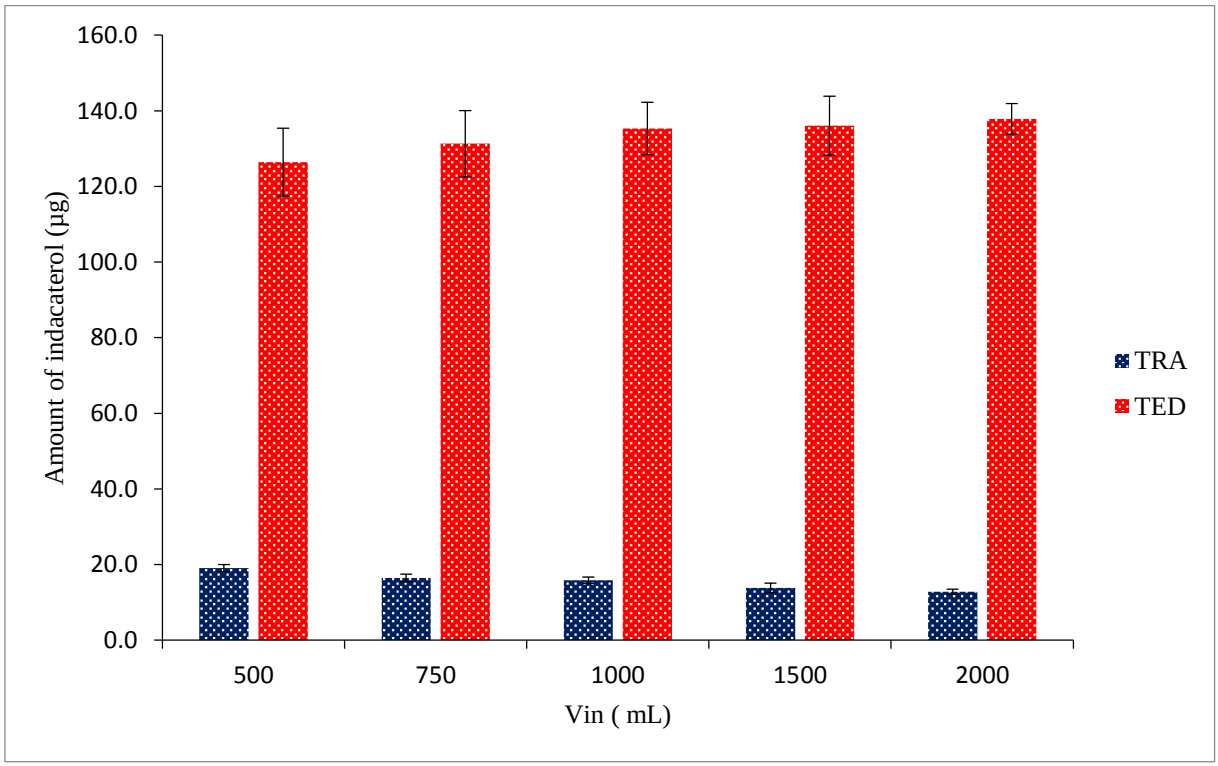

Figure 4.10: Mean (SD) of the total emitted dose (TED) and total residual amount (TRA) at different Vins using a MIF of 60 L/min.

Table 4-9: Mean (SD) of the amount of Indacaterol in (µg) dose emission results at MIF of 90 L/min using different Vins. [n=16].

Dose emission (µg)	Inhalation volume (mL)				
	500 mL	**750 mL**	**1000 mL**	**1500 mL**	**2000 mL**
ED1	126.24(7.71)	130.70(8.59)	135.41(7.59)	137.12(8.73)	138.94(6.73)
ED2	10.87(4.57)	6.95(2.94)	3.19(1.25)	2.36(1.01)	2.10(1.59)
MP1	3.56(0.53)	4.23(1.35)	4.36(0.41)	4.68(1.01)	4.78(0.91)
MP2	0.33(0.22)	0.23(0.12)	0.15(0.11)	0.13(0.09)	0.09(0.07)
TED	137.11(7.46)	137.65(8.02)	138.61(8.01)	139.49(8.11)	141.04(6.26)
% TED*	91.41(4.97)	91.77(5.35)	92.40(5.34)	92.99(5.41)	94.02(4.17)
CAP	4.14(2.65)	2.83(1.41)	2.68(1.47)	2.74(1.71)	2.57(1.46)
TRA	13.83(0.84)	12.44(0.54)	11.21(0.32)	10.32(0.41)	10.17(0.65)
TRD	150.94(4.13)	150.09(5.96)	149.82(3.81)	149.80(5.63)	151.20(6.86)

*= Total emitted dose as % of the nominal dose 150µg indacaterol from Onbrez Breezhaler®

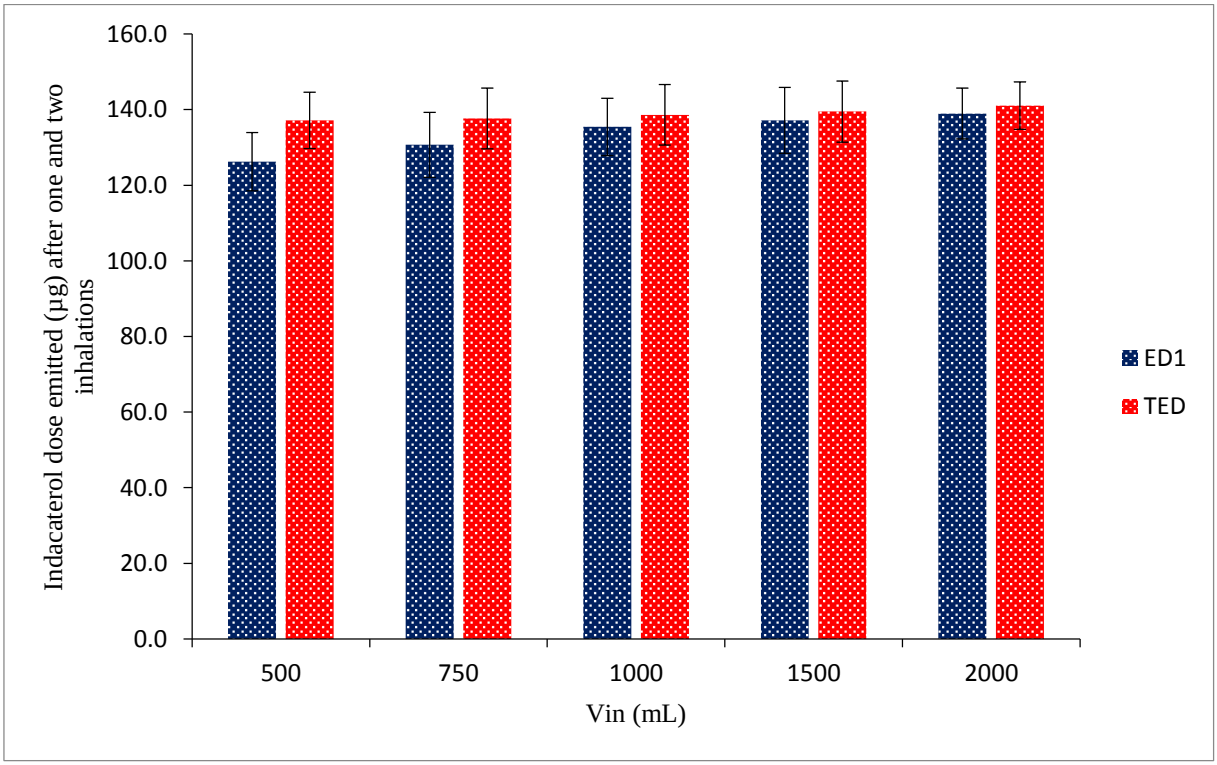

Figure 4.11: Mean (SD) of the emitted dose after one inhalation (ED1), and two inhalations (TED) at different Vins using a MIF of 90 L/min.

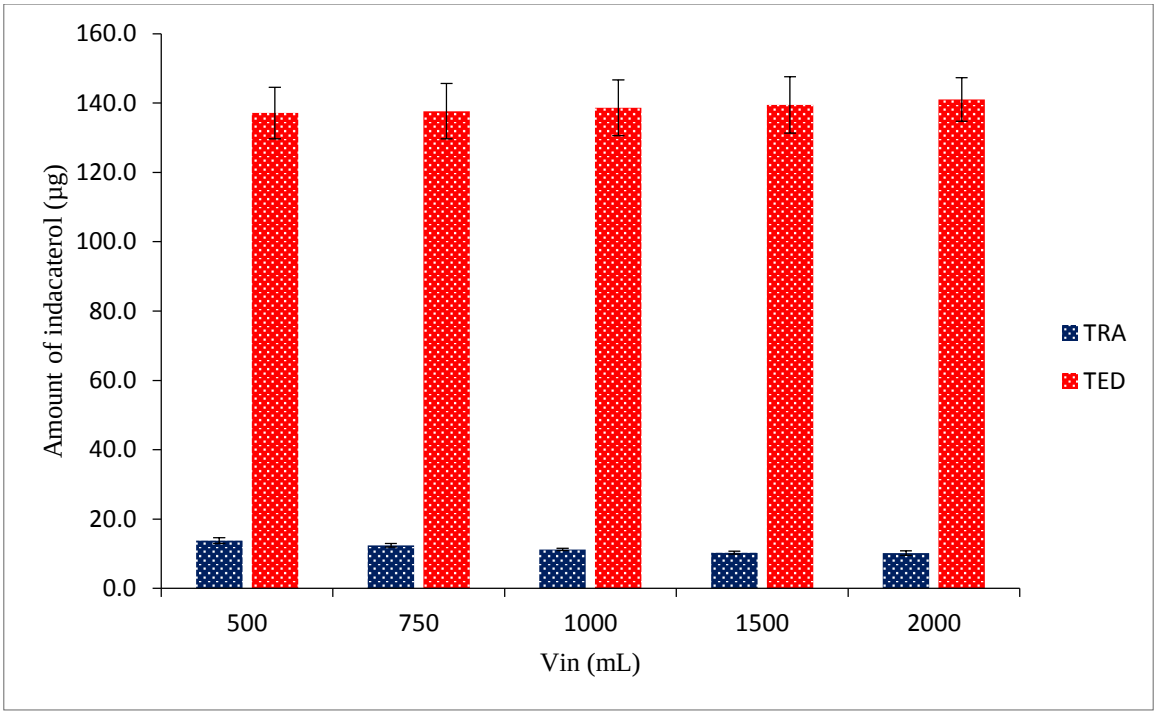

Figure 4.12: Mean (SD) of the total emitted dose (TED) and total residual amount (TRA) at different Vins using a MIF of 90 L/min.

Table 4-10: Mean (SD) of the amount of Indacaterol in (µg) dose emission results at MIF of 120 L/min using different Vins. [n=16].

Dose emission (µg)	Inhalation volume (mL)				
	500 mL	**750 mL**	**1000 mL**	**1500 mL**	**2000 mL**
ED1	129.44(5.39)	136.16(6.38)	138.73(7.46)	140.75(6.22)	142.85(8.68)
ED2	8.73(4.00)	4.48(1.62)	3.01(0.77)	2.28(0.87)	1.67(0.88)
MP1	3.84(1.53)	4.55(0.86)	4.78(0.92)	4.80(1.01)	5.28(0.50)
MP2	0.54(0.43)	0.24(0.16)	0.25(0.20)	0.14(0.07)	0.07(0.04)
TED	138.18(4.62)	140.64(6.08)	141.74(7.66)	143.03(6.18)	144.52(8.85)
% TED*	92.12(3.08)	93.76(4.06)	94.50(5.11)	95.35(4.12)	96.35(5.90)
CAP	2.38(1.44)	2.20(1.10)	2.26(1.25)	2.08(1.12)	1.75(0.80)
TRA	11.56(0.49)	8.71(0.21)	7.79(0.35)	6.57(0.19)	6.10(0.26)
TRD	149.74(4.22)	149.35(5.51)	149.54(7.67)	149.60(6.53)	150.62(3.04)

*= Total emitted dose as % of the nominal dose 150µg indacaterol from Onbrez Breezhaler®

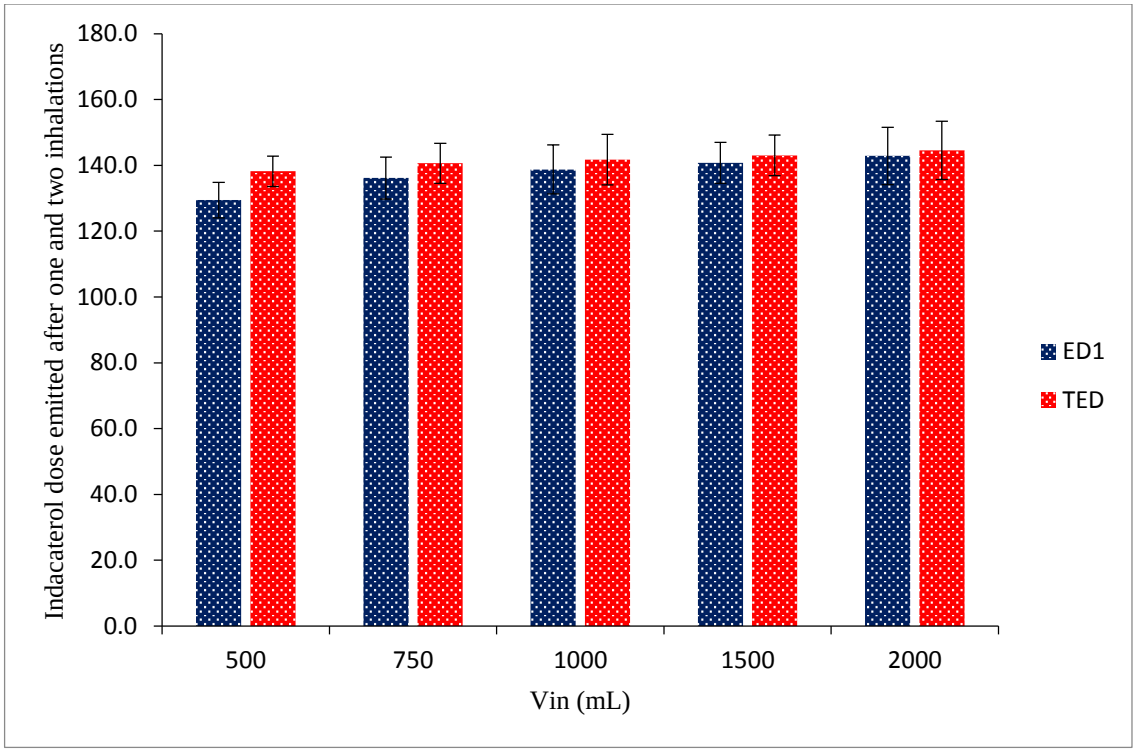

Figure 4.13: Mean (SD) of the emitted dose after one (ED1), and two inhalations (TED) at different Vins using a MIF of 120 L/min.

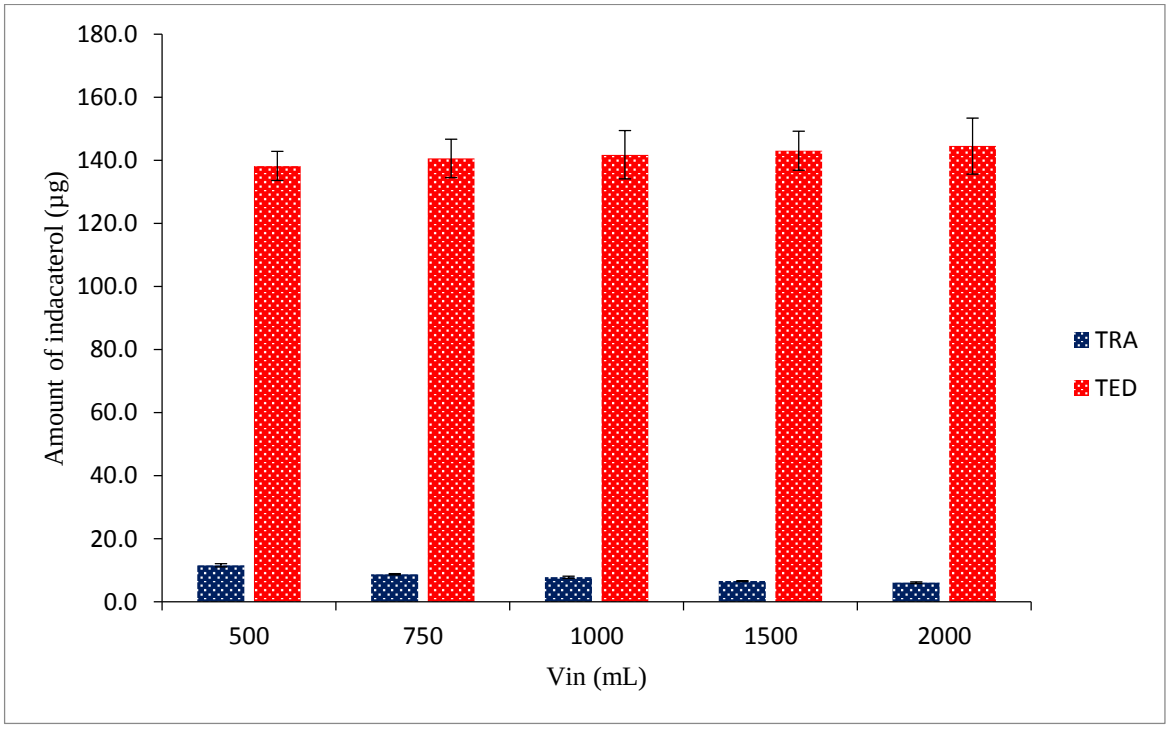

Figure 4.14: Mean (SD) of the total emitted dose (TED) and total residual amount (TRA) at different Vins using a MIF of 120 L/min.

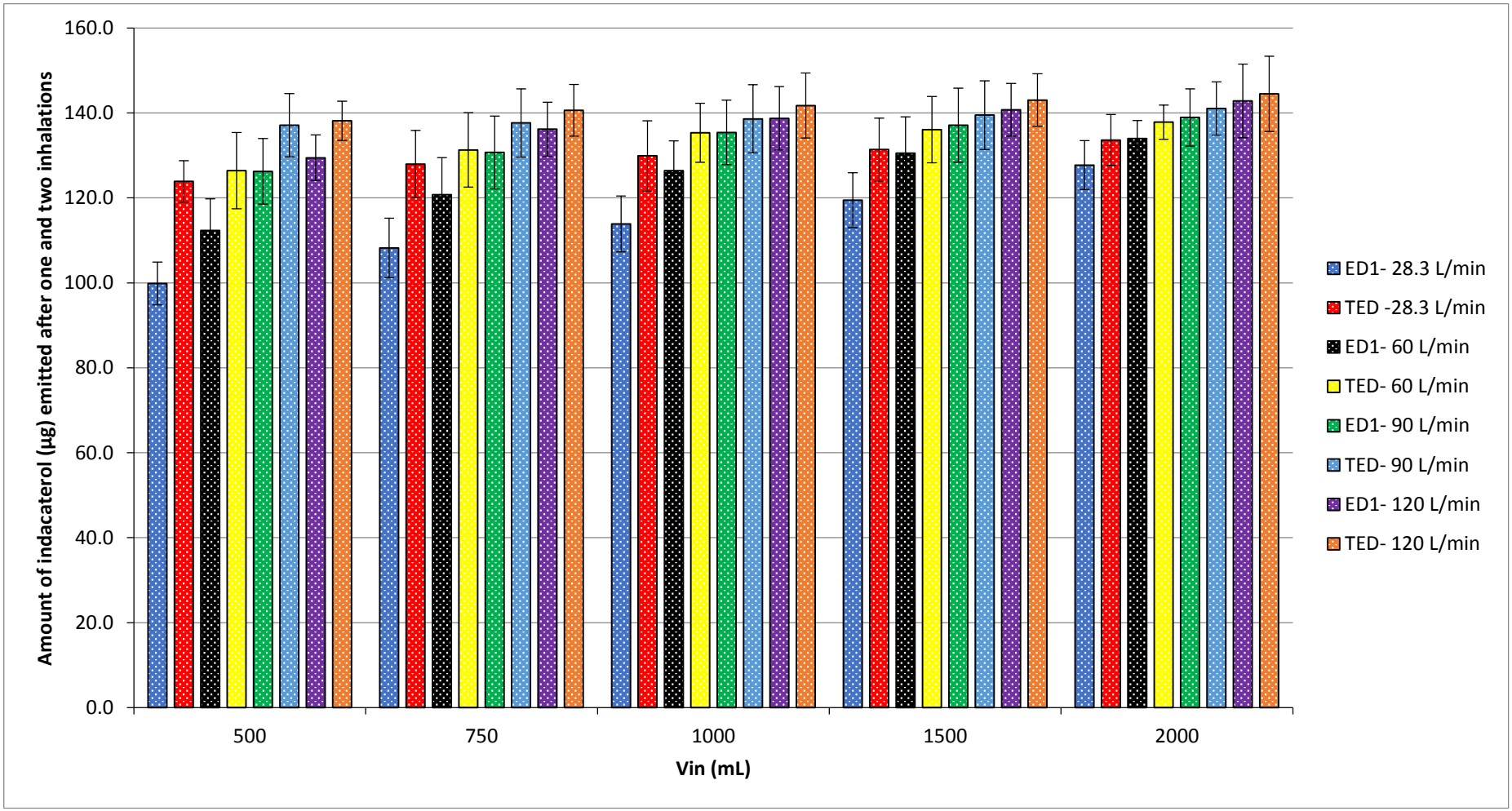

Figure 4.15: Mean (SD) of the dose emitted after one (ED1) and two (TED) inhalation at different MIF and Vin values

4.4 Discussion

The effect of increasing the MIF and Vin on the dose emission performance of both formoterol Aeroliser® and indacaterol Breezhaler® was determined using two separate inhalations. The dose delivery uniformity was determined for each set of MIF and Vin using 16 randomly chosen capsules.

Aeroliser® and Breezhaler® are low resistance devices and a MIF exceeding 100 L/min is required to achieve a pressure drop inside device equivalent to 4kPa when using both devices (Dal Negro, 2015). Inhaling fast from the start of the inhalation manoeuvre would lead to a sufficient turbulent inside the inhaler causing the aggregates to break up into particle small enough to be carried into the lower airways and also to separate drug particles from the surface of the carrier (Labiris and Dolovich, 2003, Al-Showair et al., 2007b, Laube et al., 2011). The fast inhalation achieved from low resistance device such as Aerolizer® may not de-aggregate the formulation inside the inhaler which is along with the high momentum of the particles in the fast moving airstream causes a substantial amount of drug particles being deposited in the oropharyngeal region resulting in less therapeutic effect (Haughney et al., 2010). The pharmacopoeia methods for *in-vitro* dose emission testing of DPIs are that a vacuum pump is used to generate a constant MIF corresponding to 4kPa pressure drop with an inhalation volume of 4 L or 2 L (EP, 2013, USP, 2014). The patients with asthma and COPD are not able to achieve these parameters when inhaling though DPIs in real life (Azouz et al., 2015b).

Most DPIs are breath actuated and are highly dependent on the patients' MIF. The threshold inhalation flow required to de-aggregate and disperse the drug powder is not determined for each DPI, and more effort is needed to shade the light on the minimum flow required to operate each DPI more efficiently to benefit the patients (Scichilone, 2015, Haidl et al., 2016).

4.4.1 Formoterol Aeroliser®

The results show that the dose emission after the first inhalation (ED1) significantly ($p < 0.05$) increased with increasing the MIF from 28.3 to 120 L/min while dose emission after the second inhalation reduced significantly ($p < 0.05$) with increasing the MIF (Figure 4.6) (Tables 4.3 to 4.6). Suggesting that increasing the MIF was corresponding to an increase in the turbulent energy inside the device and therefore more powder emitted when MIF increased. The results also demonstrate the reverse relationship between TED (ED1+ ED2) and RA (Cap +Dev) where the increase in the MIF significantly ($p < 0.05$) lead to an increase in the TED and therefore a reduction in the RA (Figures 4.2 to 4.5). The flow dependent dose emission of formoterol from Aerolizer® demonstrated in the present chapter is in line with the findings of previous studies (Nielsen et al., 1997, Weuthen et al., 2002, Alaboud et al., 2010).

The marked decrease in the RA at a flow rate above 60 L/min suggesting this would be the minimum flow rate to operate Aerolizer® efficiently. This study showed that formoterol emitted dose from an Aerolizer increased with the MIF especially at a MIF $\geq$ 60 L/min suggesting the effectiveness of the Aerolizer at high flow. This finding is in line with Haidle et al., (2016) who demonstrated that minimum MIF of 65 L/min is required for optimum dose de-aggregation from Aerolizer®. It is understood that the relationship between the inhalation flow and the turbulent force inside the inhaler is not linear and increasing the inhalation flow above 60 L/min suggest an increase in the level of the turbulent force de-aggregating the powder formulation. It is widely accepted that breath activated DPIs are often associated with flow rate dependent changes in the emitted dose (Johal et al., 2013).

Patient with asthma and COPD inhaling through the Aerolizer® were reported to achieve a Mean (SD) MIF and Vin of 86.5 (26.3) and 1.8 (0.8) respectively(Azouz et al., 2015b). The Vin is another inspiratory parameter that affects the dose emission of DPIs especially those

with the capsule based design. The capsule based inhalers require a prolonged inhalation manoeuvre to empty the capsule (Kamin et al., 2002, Chrystyn and Price, 2009, Laube et al., 2011).The design of the inhaler device suggests whether dose emission occurs at the start of the inhalation or require a prolonged inhalation. Failure to achieve the required inhaled volume may result in incomplete dose emission and dispersion resulting in a lower dose of drugs to reach the lungs and compromising therapeutic efficacy (Abadelah et al., 2017). Therefore, determination of the minimum inhaled volume required to operate each DPI is needed.

The results show that ED1 increased and ED2 decreased with increasing the Vin from 0.5 L to 2 L especially at low MIF 28.3 and 60 L/min (Figures 4.6) while at high MIF 90 and 120 L/min most of the dose was emitted from the device after the first inhalation (Tables 4.5 and 4.6). Suggesting that, when low MIF was used more dose was left for the second inhalation, Therefore, increasing the Vin result in an increase in the ED1. In contrast, at high MIF most of the dose was emitted from the Aerolizer® after the first inhalation.

Chan and Chew (2001) have measured eformoterol dose emission uniformity from Foradil Aerolizer® using ten separate capsules for set of MIF values (30-90L/min) using standard pharmacopeial method, the results showed that comparing to Turbuhaler®, Foradil Aerolizer® was less dependent on the MIF as TED (% of nominal dose) were 80% and 90% at 30 L/min and 90L/min respectively . The Aerolizer® showed less variation in the normalized emitted dose uniformity than the Turbuhaler®. The results of present chapter are in line, and 16 capsules dose emission from Aerolizer® was consistent with Mean (SD) of 97% (2.13) of the nominal dose of 12µg formoterol Aerolizer®.

The total residual amount (TRA) decreased significantly ($p < 0.05$) with an increase of both MIF and Vin. At low MIF and Vin, the TRA was approximately 39% of the nominal dose.

While at high MIF and Vin it was approximately 16 % of the nominal dose (Figures 4.2 to 4.5). Foradil Aerolizer® showed a flow dependent dose emission especially at a flow rate > 60 L/min, and Vin becomes of less importance. Suggesting that, 60 L/min may be considered as the minimum MIF required for successful dose de-aggregation.

4.4.2 Indacaterol Breezhaler®

Indacaterol Breezhaler® has flow dependent dose emission after first inhalation (ED1) at all Vins used. These results are in line with previously published data on indacaterol dose emission (Pavkov et al., 2010, Chapman et al., 2011, Colthorpe et al., 2013). Emitted dose after the second inhalation (ED2) decreased with the inhalation flow rate significantly ($p < 0.05$) however the most significant decrease in the emitted dose occurred at MIF of 28.3 and 60 L/min (Table 4.7 and 4.8). At these low MIFs, more indacaterol was left in the capsule after the first inhalation and second inhalation needed to withdraw most of the dose remained in the capsule and device. The results also show that (TED) which is the sum of two inhalations increased with the increase of inhalation flow rate (Figure 4.15).

The Vin was shown to have an impact on the dose emptying, and total emitted dose especially for capsule based devices (Kamin et al., 2002, Laube et al., 2011). The dose emission after the first inhalation (ED1) significantly increased ($p < 0.05$) with increasing the inhaled volume (Vin) at low MIF 28.3 and 60 L/min (Figures 4.7 and 4.9). At high MIF values > 60 L/min increasing the Vin did not bring much difference in ED1 as shown in Figures 4.11 and 4.13. The ED2 showed a reverse trend and significantly decrease ($p < 0.05$) with increasing the Vin, suggesting that dose de-aggregation was incomplete at low MIF and therefore the dose remained in the capsule and device for second inhalation (ED2). At high MIF most of the dose was emitted after one inhalation and the second inhalation showed less impact on the overall TED (ED1 + ED2) (Figure 4.15). The patients with COPD are liable to have reduced inhalation capacity hence to get the most benefit of the inhaled dose it would be

better to inhale twice for each capsule dose when using Breezhaler® mainly for those patients with small Vins and cannot generate high MIF as demonstrated in Tables 4.7 to 4.10. For Breezhaler® the dose is emitted latter, and inhalation quality depends on the inhaled volume (Figure 4.15). However, at high MIF values > 60 L/min and Vin of 1 L there was not much difference between emitted dose after one inhalation (ED1) and two inhalations (TED). Therefore, it is important when using the Onbrez Breezhaler® to inhale as fast, deep and for as long as possible.

Onbrez Breezhaler is a low resistance device, and the turbulent energy inside the device relies more on the flow rate. Furthermore, the increase in the pressure drop inside the device is less pronounced at MIF of ≤ 60 L/min. Thus, the flow dependent dose emission of indacaterol Breezhaler® could be due to the incomplete de-aggregation of the indacaterol from the device at low MIF as was previously shown by De Boer et al., (1996) or the insufficient emptying of the capsule and device at low MIF as shown from the TRA results (Figures 4.8 and 4.10). It is clear from the results (Tables 4.7 to 4.10) that the residual amount left in the device is higher compared to the capsule. Therefore, it is important to clean the device after use to avoid overdosing during next inhalation.

TRD results approached the nominal dose of 150µg indacaterol at all MIF and Vin suggesting that the procedure of washing DUSA, mouthpiece, device and capsule, and that the validated HPLC method for indacaterol quantification was accurate and reproducible.

4.5 Conclusion

In the present *in-vitro* dose emission of formoterol Aeroliser® and indacaterol Breezhaler® delivery systems has shown stable and consistent drug delivery characteristics. The results for both devices have demonstrated the need for second inhalation especially at low MIF to ensure dose emptying and maximise dose emission. For both devices, dose emission was shown to be dependent on the MIF generated by the patients with high MIF producing the greater dose emission. The Vin has shown an impact on the dose emission especially at low MIF, furthermore, a minimum MIF of 60 L/min and Vin of 1 L was required for efficient dose aerosolisation. Consequently, patients should be encouraged to inhale twice and as hard and deep as they can for each single dose especially for patients with low MIF or limited lung capacity

Chapter 5. Effect of inhalation flow rate and inhaled volume on formoterol drug deposition in-vitro from an Easyhaler® dry powder inhaler

5.1 Chapter overview

DPIs have been extensively used for delivering medication to the lungs, especially for bronchodilators and corticosteroids, in the management of asthma and chronic obstructive pulmonary disease (COPD) (Price and Chrystyn, 2015). Most of the pharmaceutical DPIs on the market are passive devices, relying on the patient's inhalation manoeuvre as an energy source to de-aggregate and disperse the static bulk powder formulation into an aerosol for lung deposition (Chrystyn, 2003). Beside the MIF and ACIM, another important aspect of the inhalation manoeuvre to consider is the Vin. This parameter ensures that the entire dose leaves the dose metering cup during an inhalation. Pharmacopoeia Methods (EP, 2013, USP, 2014) for dose emission recommend that a Vin of 4 L should be drawn through each DPI with a MIF that corresponds to a pressure change of 4 kPa inside the DPI. *In-vitro* dose emission studies have traditionally used 2 L (FDA, 1998) or 4 L Vin (Abdelrahim, 2010, EP, 2013, USP, 2014).

However, most of the patient with asthma and COPD are not able to achieve neither 4kPa pressure drop inside the inhaler device nor 4L inhaled volume (Azouz et al., 2015b). The design of the inhaler device suggests whether dose emission occurs at the start of the inhalation manoeuvre or requires a prolonged inspiration. Thus, a patient inhaling through a reservoir or blister based device would require a smaller Vin to withdraw the dose compared with the single-dose capsule based devices (Chrystyn, 2009, Laube et al., 2011). Devices with a short inhalation channel between the metered dose and the mouthpiece exit will require a lower Vin. Also, the presence of a cyclone in the inhalation channel of the DPI, to help de-aggregate the formulation, would have an effect on the required inhaled volume (Azouz and Chrystyn, 2012, Haidl et al., 2016). Failure to achieve the required inhaled volume may result in incomplete dose emission and dispersion which may result in a lower dose of the drug delivered to the lungs and compromising therapeutic efficacy (Broeders et al., 2003). In

addition to the traditional focus on the influence of the MIF, attention should be directed towards identifying the minimum required an inhaled volume to ensure that the entire metered dose is emitted from the DPI during routine use. For each DPI there will be a minimum Vin for the emission of a dose with sufficient potential for lung deposition. Therefore, this work was aimed to investigate the effect of Vin, when using a low and high MIF, on the dose emission and drug deposition of formoterol fumarate from an Easyhaler® DPI.

The present chapter was designed to investigate the effect of wide range of MIFs and Vins on the dose emission as well as the aerodynamic characteristics of the emitted dose of formoterol fumarate from 12 μg Easyhaler® DPI device. The inspiratory condition of both MIF and Vin were 28.3 L/min, 60 L/min and 90 L/min and 240 mL, 750 mL, 1500 mL and 2000 mL respectively.

5.2 Material and Methods

5.2.1 Dose emission material and instrumentation

The materials and instrumentations for DUSA experiment were provided in Chapter 3 (sections 3.1.2 and 3.1.4.1). The general laboratory apparatus and material used were listed in Chapter 3 (see section 3.1.1).

5.2.2 Dose emission methodology

The methodology and experimental set-up used for the determination of DPIs dose emission using dose unit sample apparatus (DUSA) *in vitro* was explained in the previous Chapter 4 (section 4.2.1 and 4.2.2).

5.2.3 Easyhaler® resistance to airflow

The specific resistance to airflow (R), of 10 Easyhaler devices were determined by calculating the slopes of the linear relationships between the square root of pressure drop (ΔP) against the volumetric flow rate (Q) between 28.3 and 90 L/min) according to Clark and Hollingsworth (1993): $\sqrt{\Delta P} = R \times Q$

Each of the 10 inhalers was assessed at different MIFs, and the corresponding pressure drops were recorded. The mean ± SD pressure drop of Easyhaler® at different MIF 28.3 L/min, 60 L/min and 90 L/min were 2.01 ± 0.04, 5.99 ± 0.04 and 13.96 ± 0.07 respectively. These result showed that there is a low variability in airflow resistance between separate inhalers

5.2.4 Aerodynamic Particle Size Distribution (APSD) characteristics of the emitted dose and collection of the residual amount of formoterol fumarate.

Figure 5.1 shows the ACI schematic diagram used to determine the APSDs of formoterol fumarate from an Easyhaler at MIF values of 28.3, 60 and 90 L/min with Vin values of 240,

750, 1500 and 2000 mL using the Andersen Cascade Impactor (ACI ;Copley Scientific Ltd, UK). To enable determinations at 28.3 L/min stages 0 to 7 and the pre-separator designed for use at 28.3 L/min were used. For 60 L/min stage 7 was replaced by stage -1, for 90 L/min, stages 0, 6 and 7 were replaced by stages -2A, -1A and -0 and the pre-separator designed for use at 90 L/min was used. The collection plates were sprayed with silicone lubricant (Pro-Power Premier Farnell plc, UK) and allowed to dry at room temperature before use.

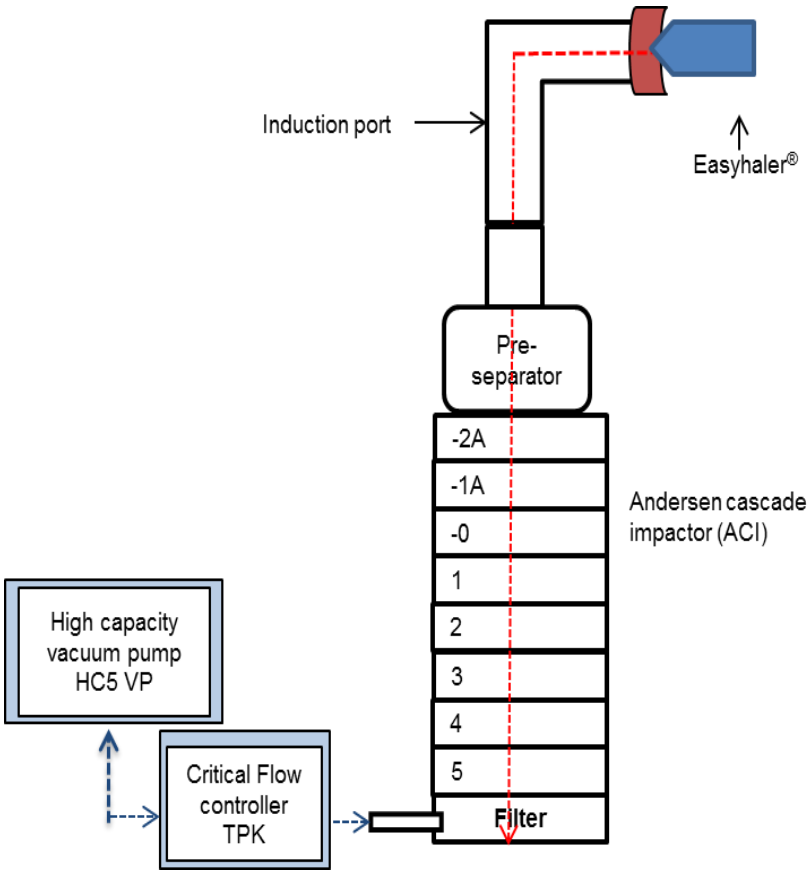

Figure 5.1: Andersen cascade impactor (ACI) set-up for the determination of formoterol aerodynamic characteristics emitted from an Easyhaler®.

The ACI was connected to a vacuum pump (HCP5, Copley Scientific Ltd, UK) via the critical flow controller (model TPK ; Copley Scientific Ltd, UK). The ACI stages were assembled with 10 mL of 60 % acetonitrile: 40 % ultra-purified water (% v/v) in the pre-separator and a

glass fibre GF50 (Whatman; UK) filter was placed in the final stage. For each determination, one dose from 10 separate inhalers was used. Each dose was prepared according to the manufacturer's recommended patient instructions. Three separate determinations were made for each set of MIFs and Vin. Once one dose was discharged into the ACI, and then the residual amount remaining inside the device was captured using a MIF of 90L/min and a 4L Vin using a dose unit sampling apparatus (DUSA; Copley Scientific Ltd, UK).

The MIF and Vin values were chosen because they reflect the maximum and minimum values achieved by patients (Azouz et al., 2015b). The amount of formoterol after each determination was determined using a validated HPLC method (Explained in Chapter 3 section 3.2.4).

5.2.5 Data Analysis

The Copley Inhaler Testing Data Analysis Software (CITDAS version 2.0, Copley Scientific Ltd, UK)) was used to calculate the aerodynamic dose emission parameters.

The total emitted dose (TED_{ACI}) was obtained from the cumulative amounts of formoterol fumarate deposited in the induction port (IP), using the USP throat, the pre-separator (PS) and all the stages of the ACI. The fine particle dose (FPD) was the mass associated with particles < 5 µm, and the extra-fine particle dose (EFPD) was the mass of powder associated with particles less than 2 µm . The fine particle dose was expressed as a % of the label claim, and the fine particle fraction (FPF) was the FPD divided by the TED_{ACI}. The total recovered dose (TRD_{ACI}) was the sum of the total emitted dose (TED_{ACI}) and the residual amount (RA_{ACI}). A one way and two ways ANOVA, as well as Tuckey test, were used to determine significant difference the hypothesis of ($p < 0.05$) was used.

5.3 Results

The aerodynamic dose emission characteristics of formoterol Easyhaler® was determined using both DUSA and ACI at wide range of MIF and Vin. A summary of the deposition data for ACI are shown in Tables 5.1, 5.2 and 5.3 respectively, in addition to DUSA results in Table 5.5. The use of DUSA was to evaluate the DDU of the device.

Table 5-1: Deposition profile data (µg/actuation) from Easyhaler at different inhalation volumes using a MIF of 28.3 L/min (n=3).

Vin	**240 mL**	**750 mL**	**1500 mL**	**2000 mL**
Deposition location	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Mouthpiece (MP)	0.71 (0.28)	0.54 (0.06)	0.55 (0.01)	0.56 (0.02)
Induction port (IP)	0.94 (0.16)	1.15 (0.08)	1.02 (0.13)	1.03 (0.03)
Pre-separator (PS)	5.14 (0.64)	4.49 (0.17)	4.48 (0.19)	5.38 (0.20)
LPM (µg)	**6.08 (0.76)**	**5.64 (0.18)**	**5.50 (0.29)**	**6.41 (0.17)**
S 0	0.28 (0.09)	0.27 (0.01)	0.26 (0.02)	0.26
S 1	0.27 (0.06)	0.45	0.43 (0.04)	0.45 (0.01)
S 2	0.19 (0.03)	0.47 (0.03)	0.49 (0.02)	0.51(0.02)
S 3	0.20 (0.04)	0.90 (0.05)	1.01(0.05)	1.02 (0.05)
S 4	0.26 (0.11)	1.00 (0.13)	1.06 (0.06)	1.08 (0.03)
S 5	0.14 (0.06)	0.45 (0.06)	0.43 (0.06)	0.45 (0.01)
S 6	0.01 (0.01)	0.05 (0.01)	0.04	0.04
S 7	0	0	0.01 (0.01)	0
Filter	0.04	0.05	0.06	0.07 (0.01)
FPD (µg)	0.86 (0.25)	2.93 (0.26)	3.11 (0.17)	3.16 (0.09)
EFPD (µg)	0.19 (0.07)	0.55 (0.07)	0.54 (0.07)	0.56 (0.02)
MMAD [µm]	4.93 (0.15)	3.70 (0.17)	3.67 (0.06)	3.67 (0.06)
GSD	2.20	1.80	1.70	1.70

FPD; Fine particle dose, EFPD; Extra fine particle dose, LPM; Large particle mass, MMAD; mass median aerodynamic diameter, GSD; Geometric standard deviation.

Table 5-2: Deposition profile data (µg/actuation) from Easyhaler® at different inhalation volumes using a MIF of 60 L/min (n=3).

Vin	240 mL	750 mL	1500 mL	2000 mL
Deposition location	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Mouthpiece (MP)	0.62(0.32)	0.57(0.12)	0.50(0.12)	0.55(0.06)
Induction port (IP)	0.99(0.12)	1.21(0.06)	1.12(0.10)	1.08(0.13)
Pre-separator (PS)	5.08(0.58)	4.54(0.11)	4.62(0.14)	5.22(0.24)
LPM (µg)	**6.07 (0.76)**	**5.75 (0.18)**	**5.74 (0.29)**	**6.30 (0.17)**
S -1	0.31(0.07)	0.25(0.03)	0.27(0.01)	0.24(0.01)
S 0	0.26 (0.05)	0.38(0.06)	0.32 (0.02)	0.32 (0.01)
S 1	0.20 (0.04)	0.36 (0.04)	0.29 (0.04)	0.46 (0.01)
S 2	0.21(0.01)	0.84 (0.15)	0.94 (0.03)	0.95 (0.10)
S 3	0.27 (0.09)	1.03 (0.09)	1.02 (0.11)	0.99(0.08)
S 4	0.16 (0.01)	0.58 (0.02)	0.78(0.05)	0.63(0.02)
S 5	0.02 (0.01)	0.06	0.08 (0.01)	0.09
S 6	0.00	0.04	0.04	0.08 (0.01)
Filter	0.03	0.06 (0.01)	0.05	0.06 (0.02)
FPD (µg)	0.89 (0.17)	2.97 (0.14)	3.20 (0.18)	3.26 (0.19)
EFPD (µg)	0.21 (0.09)	0.74 (0.12)	0.95 (0.10)	0.86 (0.09)
MMAD [µm]	4.80 (0.06)	3.20 (0.15)	3.10 (0.17)	3.10 (0.06)
GSD	2.40	2.10	2.10	1.9

FPD; Fine particle dose, EFPD; Extra fine particle dose, LPM; Large particle mass, MMAD; mass median aerodynamic diameter, GSD; Geometric standard deviation.

Table 5-3: Deposition profile data (µg/actuation) from Easyhaler at different inhalation volumes using a MIF of 90 L/min (n=3).

Vin	240 mL	750 mL	1500 mL	2000 mL
Deposition location	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Mouthpiece (MP)	0.57 (0.04)	0.59 (0.10)	0.65 (0.04)	0.56 (0.01)
Induction port (IP)	1.37 (0.07)	1.18 (0.18)	1.02 (0.14)	0.96 (0.01)
Pre-separator (PS)	4.66 (0.20)	4.63 (0.29)	4.79 (0.22)	4.92 (0.28)
LPM (µg)	**6.03 (0.67)**	**5.81 (0.46)**	**5.81 (0.35)**	**5.88 (0.29)**
S -2A	0.46 (0.01)	0.26 (0.01)	0.21 (0.06)	0.22 (0.01)
S -1A	0.29 (0.03)	0.14	0.17 (0.07)	0.12
S -0	0.25 (0.01)	0.27 (0.04)	0.25 (0.01)	0.24 (0.02)
S 1	0.27	0.51 (0.02)	0.58 (0.03)	0.56 (0.03)
S 2	0.37 (0.01)	0.68 (0.02)	0.78 (0.05)	0.79 (0.02)
S 3	0.57 (0.03)	1.09 (0.01)	1.26 (0.04)	1.29 (0.04)
S 4	0.25 (0.02)	0.57 (0.02)	0.64 (0.04)	0.67 (0.02)
S 5	0.04	0.12	0.12	0.13 (0.01)
Filter	0.16	0.29 (0.01)	0.34 (0.03)	0.34 (0.02)
FPD (µg)	2.2 (0.09)	3.68 (0.02)	4.14 (0.25)	4.14 (0.09)
EFPD (µg)	1.02 (0.05)	2.07 (0.04)	2.36 (0.11)	2.43 (0.09)
MMAD [µm]	3.30 (0.2)	2.50 (0.1)	2.41 (0.1)	2.40 (0.02)
GSD	2.10	2.20	2.10	2.00

FPD; Fine particle dose, EFPD; Extra fine particle dose, LPM; Large particle mass, MMAD; mass median aerodynamic diameter, GSD; Geometric standard deviation

Table 5-4: Effect of inhalation volume on the total emitted dose (TED_{ACI}), residual amount (RA_{ACI}) and total recovered dose (TRD_{ACI}) of formoterol left in the Easyhaler at low (28.3 L/min), medium (60 L/min) and high (90 L/min) inhalation MIF values. Results expressed as mean (SD) [n=3]

MIF	28.3 L/min			60 L/min			90 L/min		
Vin [mL]	TED [µg]	RA [µg]	TRD [µg]	TED [µg]	RA [µg]	TRD [µg]	TED [µg]	RA [µg]	TRD [µg]
240	7.47 (0.27)	1.95 (0.29)	9.42 (0.50)	7.53(0.24)	2.03 (0.11)	9.56(0.42)	8.69 (0.23)	0.62 (0.12)	9.31 (0.33)
750	9.28 (0.06)	1.71 (0.17)	10.99 (0.18)	9.35 (0.19)	1.69(0.09)	11.04(0.12)	9.74 (0.19)	0.49 (0.09)	10.22 (0.27)
1500	9.29 (0.51)	1.68 (0.04)	10.97 (0.51)	9.53(0.27)	1.58(0.18)	11.11(0.10)	10.16 (0.37)	0.48 (0.08)	10.64 (0.43)
2000	10.29 (0.24)	1.64 (0.15)	12.00 (0.10)	10.12(0.63)	1.52(0.08)	11.64(0.09)	10.24 (0.18)	0.49 (0.06)	10.71 (0.13)

Table 5-5: Effect of inhalation volume on the total emitted dose (TED_{DUSA}), residual amount (RA_{DUSA}) and total recovered dose (TRD_{DUSA}) of formoterol left in the Easyhaler at low (28.3 L/min), medium (60 L/min) and high (90 L/min) MIF. Results expressed as mean (SD) [n=3]

MIF	28.3 L/min			60 L/min			90 L/min		
Vin [mL]	TED [µg]	RA [µg]	TRD [µg]	TED [µg]	RA [µg]	TRD [µg]	TED [µg]	RA [µg]	TRD [µg]
240	7.59 (0.17)	3.65 (0.69)	11.23 (0.86)	8.50 (0.27)	2.68 (0.11)	11.18 (0.16)	9.24 (0.20)	1.84 (0.71)	11.07 (0.90)
750	8.11 (0.60)	2.93 (0.12)	11.04 (0.72)	9.13 (0.07)	2.18 (0.64)	11.31(0.56)	10.13 (0.24)	1.19 (0.05)	11.32 (0.19)
1500	8.59 (0.38)	2.46 (0.13)	11.04 (0.13)	9.79(0.02)	1.86 (0.08)	11.65 (0.10)	10.49 (0.24)	0.77 (0.16)	11.26 (0.40)
2000	9.26 (0.12)	1.77 (0.16)	11.03 (0.28)	10.18(0.48)	1.39 (0.07)	11.57 (0.40)	10.67 (0.06)	0.62 (0.17)	11.29 (0.23)

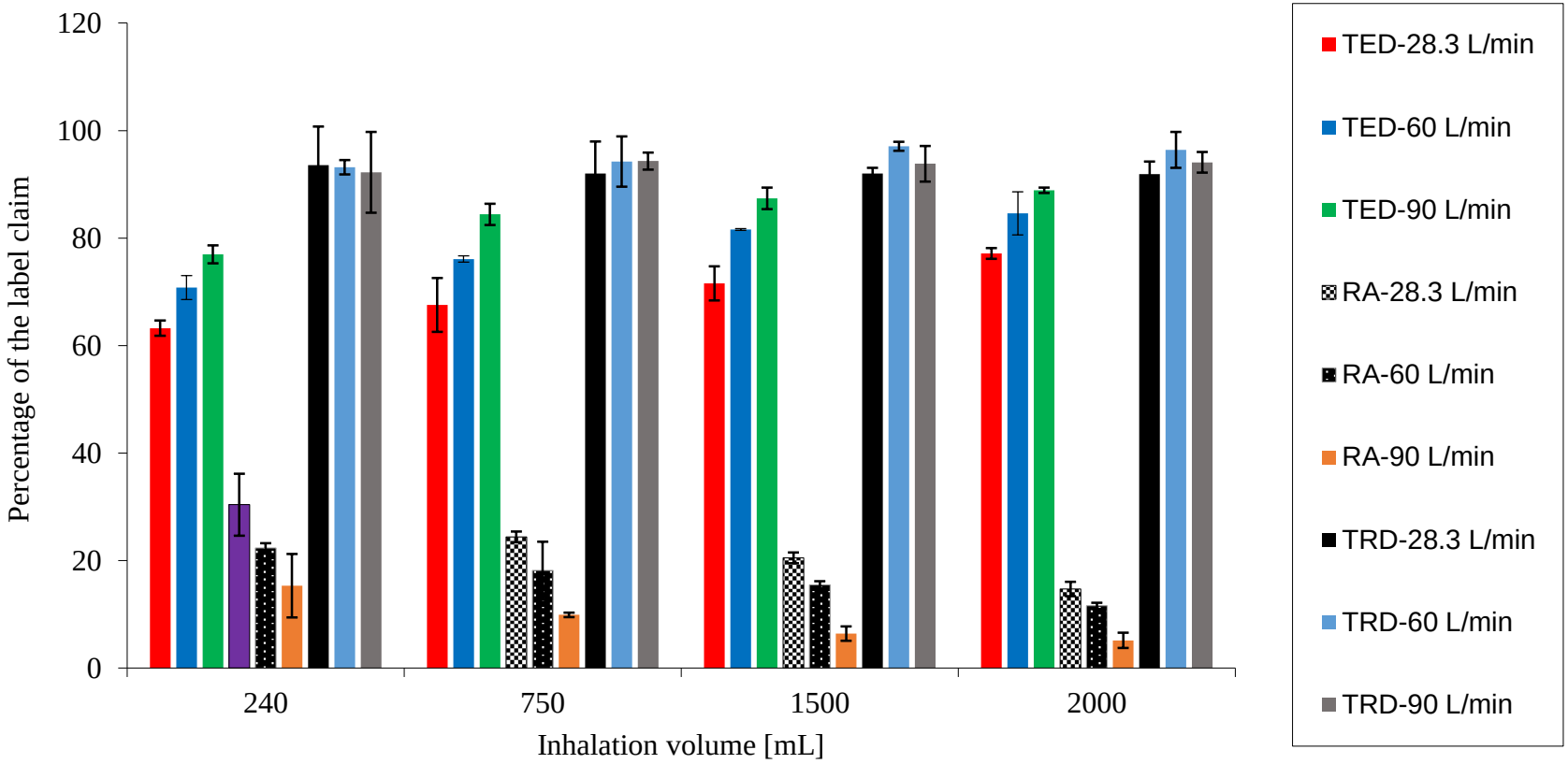

Figure 5.2: Mean (SD) of formoterol dose emission using dose unit sampling apparatus (DUSA). [n=10]. The results presented as % of the nominal dose.

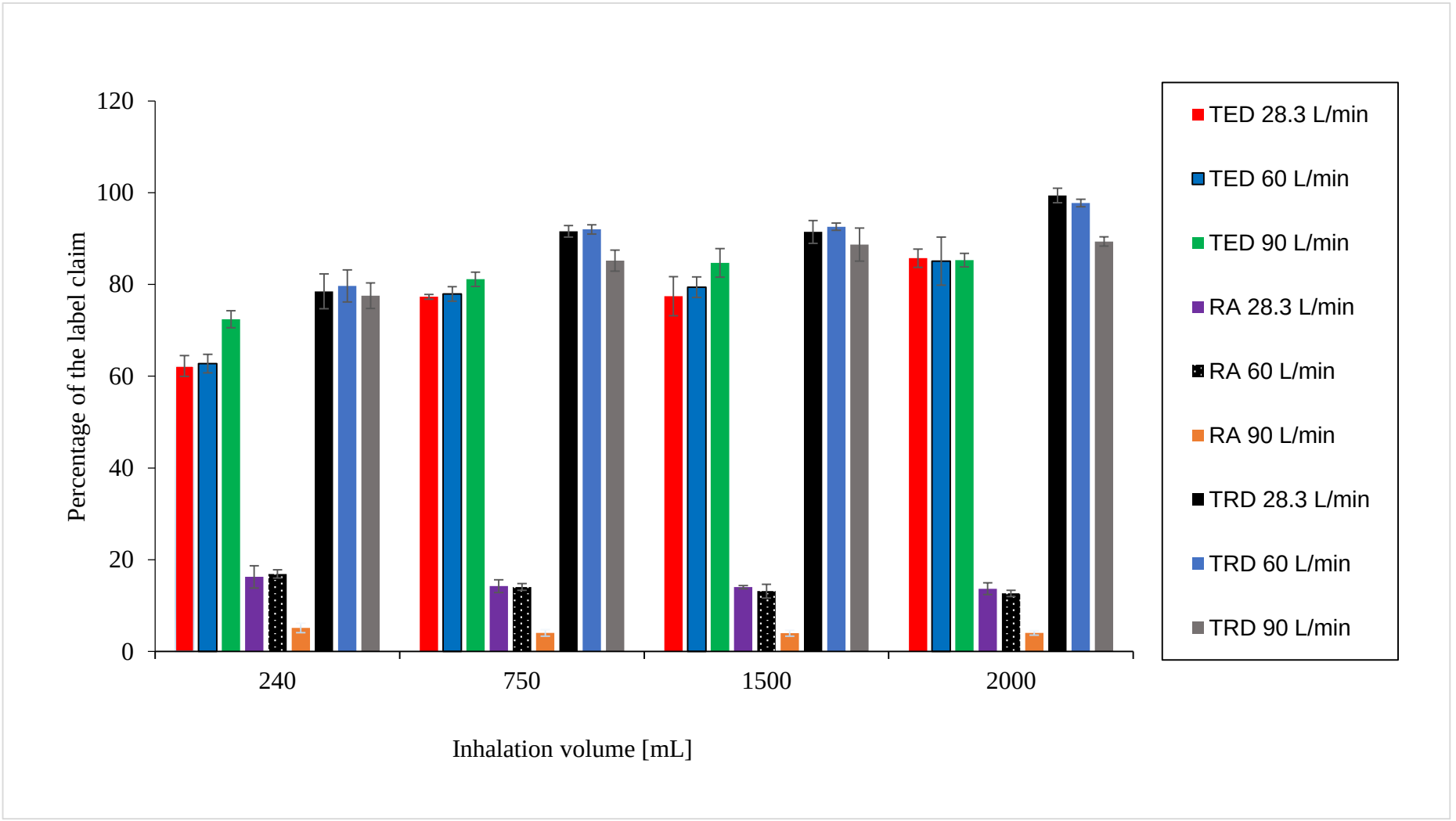

Figure 5.3: Mean (SD) of formoterol aerodynamic dose emission characteristics using Andersen cascade impactor (ACI). [n=3]. The results represented as % of the nominal dose 12 µg formoterol Easyhaler®.

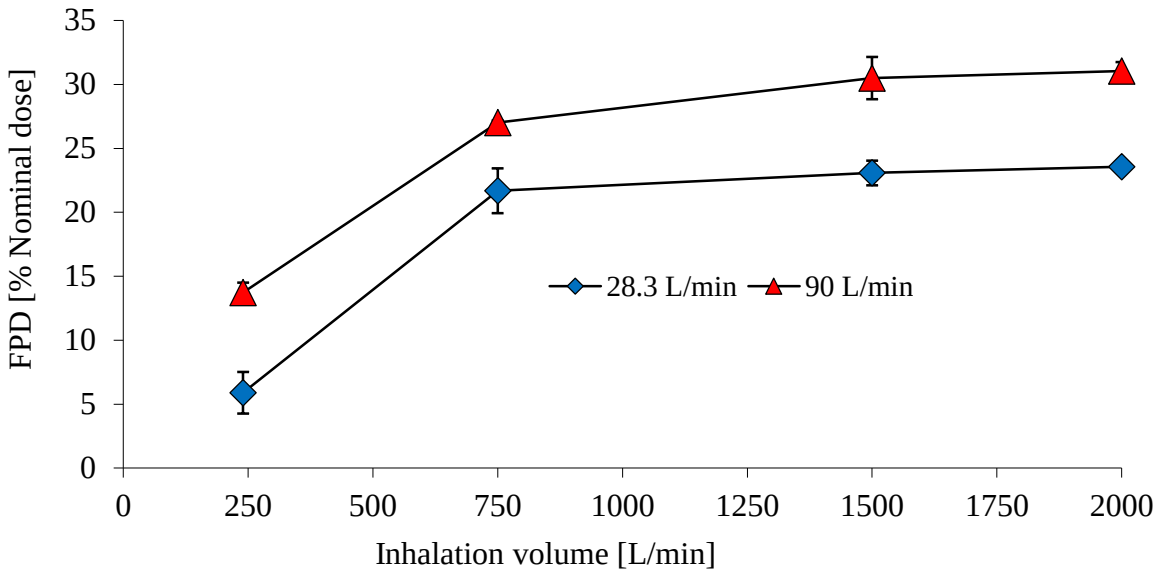

Figure 5.4: Mean (SD) of fine particle dose (FPD) of formoterol emitted from Easyhaler® at different MIF and Vin. [Results presented as % of the nominal dose 12µg formoterol]

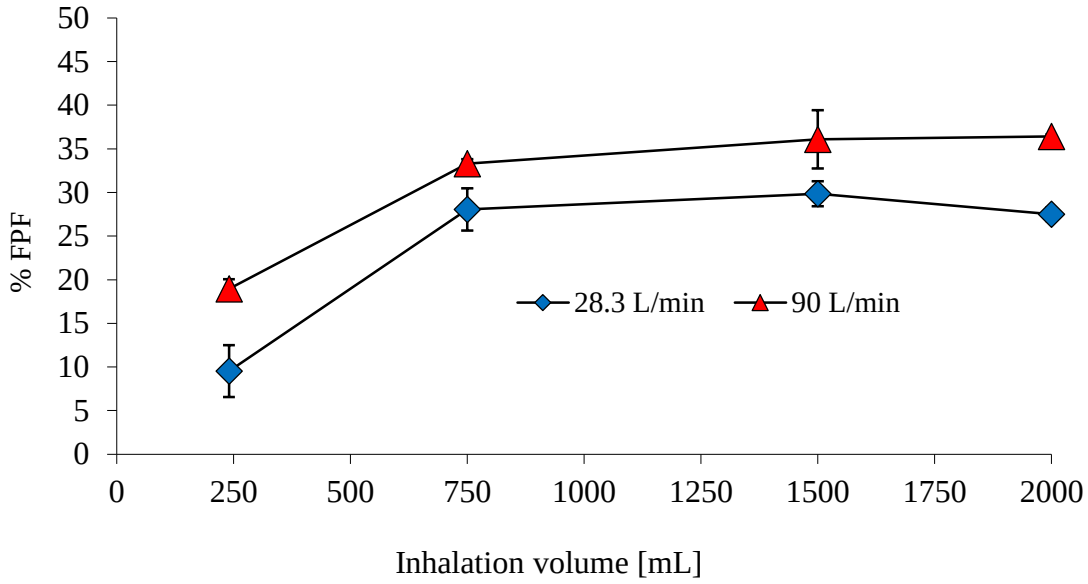

Figure 5.5: Mean (SD) of fine particle Fraction (%FPF) of formoterol emitted from Easyhaler® at different MIF and Vin.

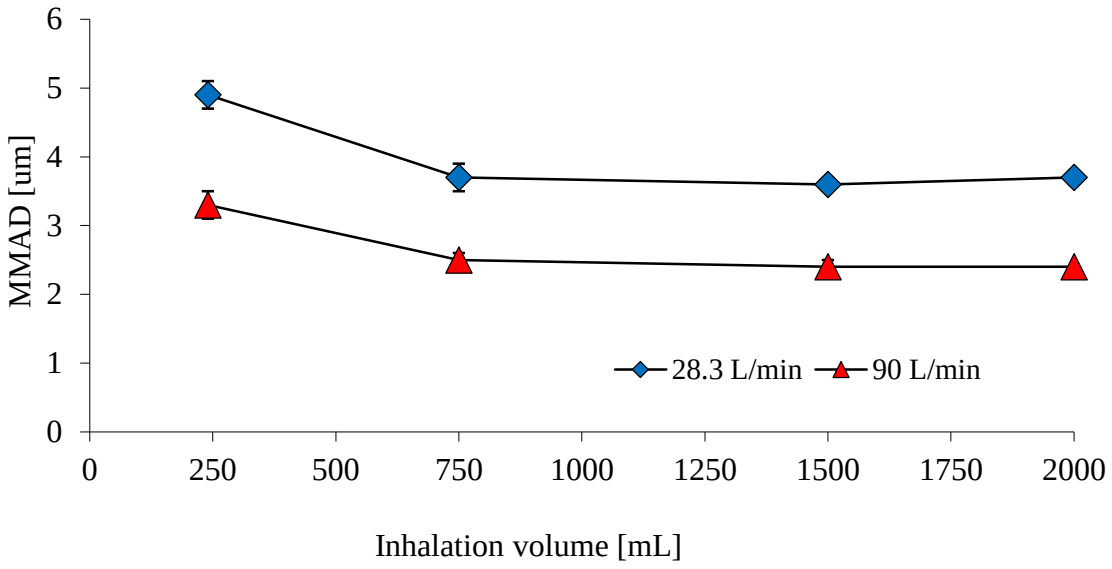

Figure 5.6: Mean (SD) of mass median aerodynamic diameter (MMAD) of formoterol emitted dose from an Easyhaler® at different MIF and Vin.

5.4 Discussion

New inhaler devices are being developed to improve compliance, targeting and uniformity of the delivery of the emitted dose of the drug for routine use by the patient. In the present chapter, reliability testing of Easyhaler DPIs was carried out using 10 devices chosen randomly. Each of the 10 inhalers was assessed at different MIFs, and the corresponding pressure drops were recorded . The calculated airflow specific resistance ranged from 0.1333-0.1355 $(cm.H_2O)^{0.5}$ *min. L^{-1} (0.0417-0.0424 $(kPa)^{0.5}$ *min. L^{-1}), with a coefficient of variation (%CV), within the 10 inhaler devices, of < 0.5%. These result showed that there is a low variability in airflow resistance between separate inhalers. DPI devices are classified into 4 groups based on their resistance: low, medium, medium-high and high resistance devices (Laube et al., 2011, Dal Negro, 2015) and each device have its own unique inhalation technique which is recommended in each patient information leaflet (Azouz and Chrystyn, 2012). To achieve a 4 kPa pressure drop across the Easyhaler, an inhalation flow of about 48 L/min would be required. The results suggest that the Easyhaler is a high resistance device

(Dal Negro, 2015). Patients with greater airflow obstruction will achieve a lower inhalation flow rate when using a high-resistance inhaler device. Azouz and Chrystyn (2012) showed that an inhaler device with a high resistance and low flow rate had the same turbulent energy, as a device that had a low resistance and high flow rate hence the amount of de-aggregation should not be affected.

It is widely accepted that breath-activated DPIs are often associated with flow rate-dependent changes in the emitted dose and also the aerodynamic characteristics such as the fine particle dose (FPD), fine particle fraction (%FPF) and mass median aerodynamic diameter (MMAD) (Hill and Slater, 1998, Taylor and Gustafsson, 2005, Telko and Hickey, 2005, Johal et al., 2013). The emitted dose from an Easyhaler® was measured at different inhalation flow rates 28.3, 60 and 90 L/min using both DUSA and ACI Figures 5.2 and 5.3. The difference in the total dose emission between 28.3 and 60 L/min was pronounced with the DUSA (Figure 5.2) than with the ACI (Figure 5.3), and the total recovered dose was more consistent with the DUSA. Increasing the MIF from 28.3 L/min to 90 L/min resulted in a significant increase ($p < 0.05$) in the emitted dose (Figures 5.2 and 5.3). FPD (Figure 5.4), % FPF (Figure 5.5) and a decrease in the MMAD (Figure 5.6). Although this de-aggregation was significantly ($p < 0.05$) more efficient at 90L/min the differences were relatively small. Whereas, the difference in the dose emission results (TED_{ACI} and FPD) between 28.3 L/min (Table 5.1) and 60 L/min (Table 5.2) was insignificant. The dose emission characteristics (TED_{ACI}, RA_{ACI}, TRD_{ACI}, FPD and MMAD) results using ACI at 90 L/min (Table 5.3) was slightly higher than both 28.3 L/min and 60 L/min. The shallow flow dependent dose emission of formoterol in our study is similar to that previously reported for the salbutamol Easyhaler (Palander et al., 2000). Our results at the low MIF highlight why the Easyhaler has been shown to be effective at inhalation flows of 28 L/min (Koskela et al., 2000).

There was an inverse relationship between the FPD, %FPF and MMAD. As MMAD decreased, the FPD and %FPF increased. The steepest increase in pressure drop above 60 L/min. suggests an increase in the level of the turbulent force, which is in accordance with the relationship between the pressure changes and the flow described by Clark and Hollingsworth (1993). This turbulent force would be able to generate sufficient shear forces overcoming the adhesive forces between drug/carrier and/or cohesion forces between drug-drug particles. Such a phenomenon would be causing further de-aggregation and dispersion of drug particles in the emitted dose as demonstrated by the shift towards much smaller particles with increasing the flow rate. MMAD values ranged between 1.9 and 4.9 µm (Figure 5.6). An MMAD < 5 µm is considered to be necessary for sufficient airway deposition (Mitchell et al., 1987). The present results suggest that inhaled formoterol from Easyhaler® should reach the lungs irrespective of the Vin and MIF used, but with a high dose expected to reach the lungs at a high flow rate (Figures 5.2, 5.3 and Figures 5.4 and 5.5).

Examination of the residual amounts (RA_{DUSA} and RA_{ACI}), presented in Table 5.4 and Table 5.5 from DUSA and ACI, as well as the FPD (Tables 5.1, 5.2 and 5.3), suggests that when using an inhaled volume of 240 mL not all of the formoterol dose was emitted. Residual amounts above 750 mL show that an adequate volume was passed through the device to empty the dose. Although Azouz et al., (2015b) have shown that some patients (especially children) can only achieve a Vin between 200 and 750 mL, the delivered dose and the fine particle dose would be sufficient to provide an adequate therapeutic response. The data confirms why the Easyhaler provides an adequate response when used by young children (Koskela et al., 2000). A 240 mL volume was used because we measured that the dead space in the horizontal section of the induction port (from the mouthpiece of the adapter to the 90 degree bend) was 40 mL and so the APSD results represented a true volume of 200 mL inhaled through the Easyhaler and passed through the ACI.

Drug retention inside the inhaler continues to be a factor plaguing the performance of novel inhalers (Tajber et al., 2009). Drug retention varies between inhaler devices in that some studies have reported between 30-50% of the nominal dose is retained within the device (Heng et al., 2013). It is important that the complete dose is released from the inhaler to maximise the therapeutic effect, minimising drug wastage and avoiding potential dosage errors during the next inhalation. Our results showed that the amount of drug retained in the Easyhaler was smaller than those reported in the above studies, *i.e.*, between 14-16% at 28.3 L/min for 2000 mL and 240 mL Vin, respectively, whereas, the amount of formoterol retained in the Easyhaler device at 90 L/min was ≤ 5% (Figures 5.2 and 5.3). The ACI with the induction port (IP) and the pre-separator (PS) has an internal volume of 1155 mL (Copley et al., 2005). An inhaled volume of 240 mL, therefore, represents only about 21% of the entire internal volume of the ACI. Hence, this small volume of air should not be sufficient to pull the entire aerosol bolus through the impactor causing a premature deposition of the aerosol in the inhalation channel of the device or in the upper parts of the impactor. Our results showed that the amount of formoterol deposited as a large particle mass was almost unaffected by the Vin (Tables 5.1, 5.2 and 5.3), but the amount of aerosol (especially the extra fine particle dose) reaching the lower stages of the impactor was less when a 240 mL was used. The lower TED_{ACI} (Figure 5.3), as well as the FPD (Figure 5.4) and % FPF (Figure 5.5) at this volume of 240 mL, are more likely to be due to insufficient air pumped through the ACI. The MMAD results (Figure 5.6) do suggest that deposition into the lower stages may not have been efficient when 240 mL was pulled through the ACI. However, as mentioned above, the high residual amount at both MIFs when using a 240 mL suggests that the results could also be due to incomplete emptying of the dose. The results show that a volume of 750 ml is sufficient when using the ACI. This volume is lower than the 1 L inhaled volume previously reported (Mohammed et al., 2012, Mohammed et al., 2014) and the internal capacity of the ACI. The

results show that a volume lowers than the internal capacity of the ACI can be used. The reason for this is unknown so required investigating. Hence, the ACI could be used to study the effect of low Vin and MIF values on the dose emission characteristics of DPIs. This method could be used if facilities to study *in-vitro* dose emission by replaying patients' inhalation profiles (Olsson et al., 2013, Chrystyn et al., 2015) are not available.

At higher MIFs, increasing the Vin above 750 mL had a less significant effect on the residual amount. The Vin was found to be different between DPI devices; some devices require more inhaled volume than others. For example, the Diskus® requires a Vin as low as 150 mL in contrast to the Turbuhaler® which require a minimum of 1 Litre Vin (Azouz et al., 2015b). Genuair® inhaler showed no difference in the total emitted dose and fine particle dose using 2 and 4 L inhaled volume (Chrystyn and Niederlaender, 2012). The Ellipta® DPI showed no variation in the total emitted dose and fine particle dose by increasing the inhaled volume from 0.7 L to 4.2 L (Hamilton et al., 2015).

The difference in the inhaled volume between DPIs was attributed to their internal design and the intrinsic resistance, for example a significant increase in the fine particle fraction was shown with the Aerolizer® when the air inlet size was reduced and the results were explained by the increase in the air velocity and turbulent inside the inhaler device (Zhou et al., 2013, Shur et al., 2015). The difference in the Vin between the Diskus® and Turbuhaler® was attributed to the length of the inhalation channel. The inhalation channel in the Turbuhaler® is relatively long and includes a cyclone, whereas the inhalation channels of the Diskus® is very short. The low resistance devices have also been reported to require larger inhaled volume than higher resistance devices (Azouz and Chrystyn, 2012). The Easyhaler® design, *i.e.*, short inhalation channel together with a high intrinsic resistance may have both accounted for only a small inhaled volume been required by this device, thus allowing the dose to leave the

inhaler. To minimise drug wastage and avoiding potential dosage errors during the next inhalation, it is therefore essential that patients inhale as hard as they can from the start of the inhalation manoeuvre to inhale the entire dose when using an Easyhaler.

5.5 Conclusion

An inhaled volume of 750 mL was sufficient to study the dose emission characteristics of formoterol from an Easyhaler. When using the Andersen Cascade Impactor, it is likely that an inhaled volume down to 750mL can be used to study the aerodynamic particle size characteristics. The *in-vitro* results suggest that although the Easyhaler demonstrates some flow dependent dose emission, as demonstrated by both the ACI and DUSA results, it was effective at low inhalation flows and inhaled volumes.

Chapter 6. The effect of dose strength on the dose emission of indacaterol Breezhaler® in-vitro using different inhalation flow rates and inhalation volumes

6.1 Chapter overview

Inhaled therapy is the mainstay treatment of choice to control the symptoms of asthma and chronic obstructive pulmonary disease (COPD) (Laube et al., 2011) using either pMDIs or DPIs. The quality and the amount of the emitted dose from a DPI depend on patient's inhalation manoeuvre, the type of device and its formulation (Clark and Hollingworth, 1993, Chrystyn, 2003). Each inhalation manoeuvre is characterised by a flow versus time profile, which includes the inhaled volume. Upon use, inhalation flow interacts with the resistance inside the DPI to generate a 'force' that deaggregates the formulation such that active drug particles, with the greatest likelihood of lung deposition, are entrained in the air stream as it leaves the device. The inhaled volume ensures that the dose is emptied from the metering cup/capsule (the latter applies if the DPI is a single dose capsule product). When inhaling from a single dose capsule DPI product it is essential that the inhaled volume is sufficient to empty the entire dose out of the capsule, It is for this reason that the patient information leaflet (PIL) for these DPIs should direct patients to make two separate inhalation manoeuvres from each prepared capsule dose (Haughney et al., 2010, Laube et al., 2011).

The Breezhaler® is a low resistance device and has a resistance of 0.0177 $(kPa)^{0.5}(min\ L^{-1})$ (Dal Negro, 2015). Patients with COPD have reduced inspiratory capacities together with low inhaled volumes (Azouz et al., 2015b), and so many use sub-optimal inhalations when using DPIs which means that the dose they inhale is reduced.

Inhalers play an important role in the management of patients with COPD, and it is being recognised that the choice of the inhalation device appears to be as important as that of drug molecule (Chapman et al., 2011, Lavorini and Usmani, 2013). Inhaled long-acting bronchodilators are used for the treatment of patients with moderate and more severe COPD (Cazzola and Page, 2014), and now agents are available for once-daily administration. Indacaterol is an inhaled, once-daily, ultra-long-acting β2-agonist (Cazzola et al., 2005). The

micronised indacaterol is mixed with lactose (to increase the bulk and enhance powder flow of the formulation). It is packaged in transparent hard gelatine capsules each containing 75 µg, 150 µg or 300 µg of indacaterol inhalation powder inhaled from a Breezhaler® inhaler. Published data shows that patients with stable COPD were able to generate, on average, a MIF of 72 L/min with a range of 47-99L/min to 94.8 L/min (range, 52–133 L/min) and mean inhaled volumes (Vin) of 1.7L, range of 1 to 2.2 L (Pavkov et al., 2010, Chapman et al., 2011, Colthorpe et al., 2013). *In-vitro* dose emission studies focus on identifying the optimal inhalation flow rate for dose de-aggregation; however, this optimal flow may not be achievable in the real life use by the patient.

The multivariate statistical approach study carried out by Buttini et al., (2016) demonstrate that the fine particle mass emitted from the single dose inhaler device had significantly decreased when a minimum inspiratory volume was used to operate the device (Buttini et al., 2016b). The information that is of most clinically relevant is to identify the type of dose emitted when low inhalation flows and inhaled volumes are used. This is very important for patients with COPD because these patients can only use low inhalation flows and low inhaled volumes (Azouz et al., 2015a, Azouz et al., 2015b).

Therefore, the primary aim of this work was to investigate a range of inhalation flow rates (28.3, 60, 90 and 120 L/min) and volumes (500, 750, 1000, 1500 and 2000 mL) on the dose emission from 150 µg and 300 µg Indacaterol Onbrez Breezhaler® (Novartis Pharmaceuticals Ltd, UK) using two separate inhalations. Secondly, to determine the aerodynamic characteristics of indacaterol emitted dose using an ACI at a MIF of 28.3, 60 and 90 L/min and Vin of 1, 1.5, 2 and 4 L.

6.2 Materials and Methods

6.2.1 Material and instrumentation for DUSA

The dose emission material and instrumentation used for *in-vitro* dose emission study were explained in Chapter 3 (see sections 3.1.2 and 3.1.4.1). A List of the material and other laboratory apparatus used in the study were listed in Chapter 3 (see section 3.1.1).

6.2.2 Dose emission methodology

The methodology and experimental set-up used for the determination of indacaterol Breezhaler® dose emission using dose unit sample apparatus (DUSA) *in vitro* was explained in the previous Chapter 4 (section 4.2.1 and 4.2.2). For dose strength study, dose emission experiment was carried out in triplicate (n=3) for each set of MIF and Vin.

6.2.3 Determination of the aerodynamic characteristics of the emitted dose and collection of the residual amount of indacaterol

Figure 6.1 shows schematic diagram of the method for the determination of aerodynamic dose emission characteristics of indacaterol 150 µg and 300 µg from an Onbrez Breezhaler® .

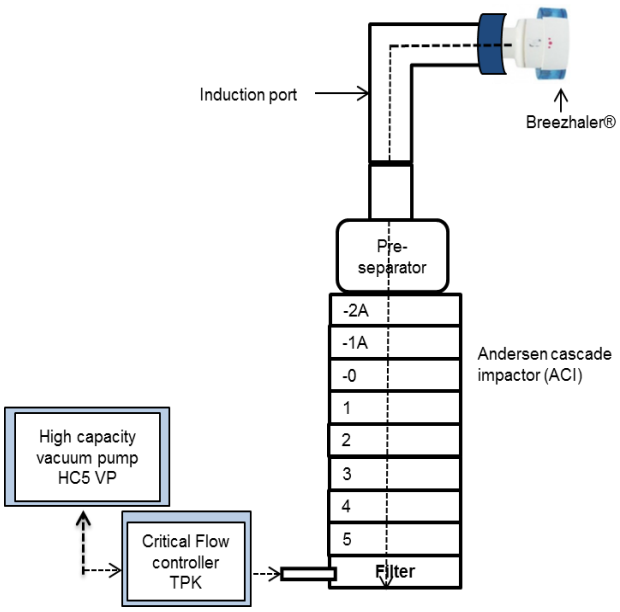

Figure 6.1: Schematic diagram of a pharmacopeial compendial method of Andersen cascade impactor for DPIs testing

6.2.4 Characterisation of the indacaterol Onbrez Breezhaler® 150 and 300 µg powder formulations by Scanning Electron Microscope (SEM).

SEM investigations of the shape, particle size and surface texture of indacaterol and the lactose in the Onbrez Breezhaler® 150 µg and 300 µg (Novartis Pharmaceuticals, CH) products was carried out using a Jeol 6060 LV SEM (Jeol Ltd., UK). A sample of the powder formulation was scattered onto the aluminium stage stub, by a gentle tapping motion to provide a thin layer that was suitable to view the particles. The movement of the sample was restricted by adhering the sample to a double-sided conductive carbon adhesive tape (Agar Scientific, UK). As the sample is non-conductive, it was required to coat the sample with a thin layer of gold (15 – 20 nm) (Quorum Technologies Ltd., UK) using a Quorum SC7620 Sputter Coater (Quorum Technologies Ltd., UK).

6.2.5 Characterisation of the indacaterol Onbrez Breezhaler® 150 and 300 µg powder formulations by differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA).

DSC experiments were conducted using a DSC 822^e instrument (Mettler-Toledo, UK), with a refrigerated cooling system (RCS). Nitrogen was used as the purge gas, flowing at a rate of 50 ml min^{-1} through the DSC cell and at 150 ml min^{-1} through the RCS units. Aluminium non-hermetic DSC pans were used through the study. The mass of each empty sample pan was matched with the mass of the empty reference pan to ±0.1 mg. The instrument was calibrated using an indium standard; approximately 2.5 ± 0.2 mg of sample (Indacaterol 150, 300 µg or indacaterol powder) was used for each run. After sealing the pans, they were placed in the DSC furnace, which had been pre-equilibrated at 25 °C. Before each measurement, the samples were allowed to equilibrate for 5 min at 25°C and were then heated to 250°C at a heating rate of 10°C min^{-1}. Each batch was analysed in duplicate. The DSC results were analysed using the STAR^e SW 9.01 version (Mettler-Toledo, Leicester, UK).

6.3 Results

6.3.1 Dose emission results for both dose strengths 150 and 300µg indacaterol Breezhaler®.

The dose emission of indacaterol from Breezhaler® was investigated using a different range of MIFs and Vins. The dose emission was measured after first (ED1) and second (ED2) inhalation and the total emitted dose was the sum of ED1+ ED2.

Table 6-1: Mean (SD) of Dose emission data of 150 µg indacaterol dose after two separate inhalations [n=3].

Vin (mL)	ED1 (µg)	ED2 (µg)	TED (µg)	TRA (µg)	TRD (µg)
a). Inhalation flow rate 28.3L/min					
500	96.26 (1.89)	22.68 (1.74)	118.94(2.72)	31.63 (2.51)	150.57 (0.37)
750	104.90 (2.01)	18.40 (1.65)	123.30(0.76)	28.41(1.52)	151.71 (1.86)
1000	114.98 (2.71)	9.72 (0.66)	124.70(3.27)	20.05(0.23)	144.75 (3.24)
1500	121.23 (3.08)	6.58 (1.92)	127.81(1.16)	17.78(0.38)	145.59 (1.12)
2000	123.13 (3.07)	4.44 (0.56)	127.57(2.68)	17.88(0.80)	145.45 (5.03)
b). Inhalation flow rate 60L/min					
500	120.14 (5.26)	10.50(1.27)	130.64(6.46)	19.43(0.65)	150.07 (7.04)
750	123.90 (2.41)	7.72(2.86)	131.62(1.57)	18.15(0.88)	149.77 (2.27)
1000	129.16 (3.36)	4.75 (0.66)	133.91(3.11)	15.97(0.61)	149.88 (3.11)
1500	131.73 (4.10)	3.72 (0.91)	135.45(4.65)	13.790.70)	149.24 (5.28)
2000	134.27 (4.32)	2.50 (0.70)	136.77(3.96)	13.37(0.75)	150.14 (4.25)
C). Inhalation flow rate 90L/min					
500	125.83 (4.58)	8.76 (2.02)	134.59(3.84)	14.76(1.54)	149.35 (2.97)
750	128.16 (3.62)	7.19 (1.61)	135.35(5.23)	13.37(0.14)	148.72 (5.10)
1000	133.87 (1.63)	3.07 (1.19)	136.94(2.38)	13.33(0.09)	150.27 (2.45)
1500	136.08 (4.45)	2.13 (0.63)	138.21(3.92)	12.50(0.24)	150.71 (5.52)
2000	137.15 (4.93)	1.92 (0.95)	139.07(5.76)	12.22(0.73)	151.29 (3.48)
d). Inhalation flow rate 120 L/min					
500	130.18 (4.37)	6.24 (1.59)	136.42(5.32)	12.42(0.92)	148.84 (6.18)
750	136.03 (4.77)	3.41 (0.94)	139.45(5.40)	11.33(1.80)	150.77 (7.12)
1000	138.07 (7.98)	3.41(1.64)	141.48(3.39)	11.71(1.63)	153.19 (8.00)
1500	140.42 (6.98)	2.76(0.47)	143.17(6.55)	9.94(0.54)	153.12 (6.62)
2000	142.43 (1.94)	1.28(0.73)	143.71(1.74)	8.65(0.89)	152.36 (2.53)

Table 6-2: Mean (SD) of Dose emission data of 300 µg indacaterol dose after two separate inhalations [n=3].

Vin (mL)	ED1 (µg)	ED2 (µg)	TED (µg)	TRA (µg)	TRD (µg)
a). Inhalation flow rate 28.3L/min					
500	199.15(12.86)	35.07(5.51)	234.21(9.46)	57.20(1.40)	291.41 (13.84)
750	214.89(12.81)	32.25(1.84)	247.14(14.20)	47.71(1.45)	294.85 (10.03)
1000	242.03(11.55)	11.49(5.13)	253.52(11.00)	36.16(0.84)	289.68 (11.42)
1500	244.82(12.01)	9.68(3.34)	254.51(10.47)	35.50(1.60)	290.01 (14.74)
2000	249.43(11.15)	5.74(1.77)	255.17(11.98)	31.47(1.13)	286.63 (13.10)
b). Inhalation flow rate 60L/min					
500	228.89 (9.28)	19.66 (8.79)	248.55(13.59)	42.94(1.40)	291.49 (12.91)
750	242.91 (5.23)	9.34 (2.13)	252.25(6.91)	38.74(1.33)	290.99 (8.22)
1000	253.20 (11.25)	5.17 (1.05)	258.37(10.25)	35.42(0.44)	293.79 (10.32)
1500	255.77 (7.15)	4.03 (2.07)	259.80(15.83)	32.70(0.78)	292.50 (11.07)
2000	259.17 (9.53)	3.40 (0.56)	262.57(9.61)	30.47(2.07)	293.04 (11.89)
c). Inhalation flow rate 90L/min					
500	253.98 (3.46)	11.78 (2.44)	265.76(1.91)	33.07(1.17)	298.83 (7.84)
750	264.76 (7.36)	8.75 (5.51)	273.51(8.65)	26.65(1.14)	300.16 (9.60)
1000	270.32 (6.58)	5.04 (1.79)	275.36(4.79)	24.63(1.69)	299.99 (6.48)
1500	274.72 (7.18)	2.81 (0.72)	277.53(6.73)	22.94(1.20)	300.47 (7.00)
2000	276.45 (8.63)	2.83 (1.56)	279.28(7.19)	23.77(1.15)	303.05 (6.24)
d). Inhalation flow rate 120 L/min					
500	261.25 (11.38)	8.84 (0.16)	270.10(19.22)	22.46(1.72)	292.55 (1.87)
750	268.31 (4.21)	5.61 (0.64)	273.92(4.77)	19.82(1.71)	293.75 (6.44)
1000	270.58 (3.57)	5.02 (0.37)	275.59(3.19)	19.02(0.80)	294.62 (2.99)
1500	278.02 (12.89)	3.18 (0.98)	281.20(13.51)	15.60(1.90)	296.80 (10.69)
2000	280.87 (12.04)	2.91 (0.99)	283.79(13.03)	12.80(1.17)	296.58 (13.20)

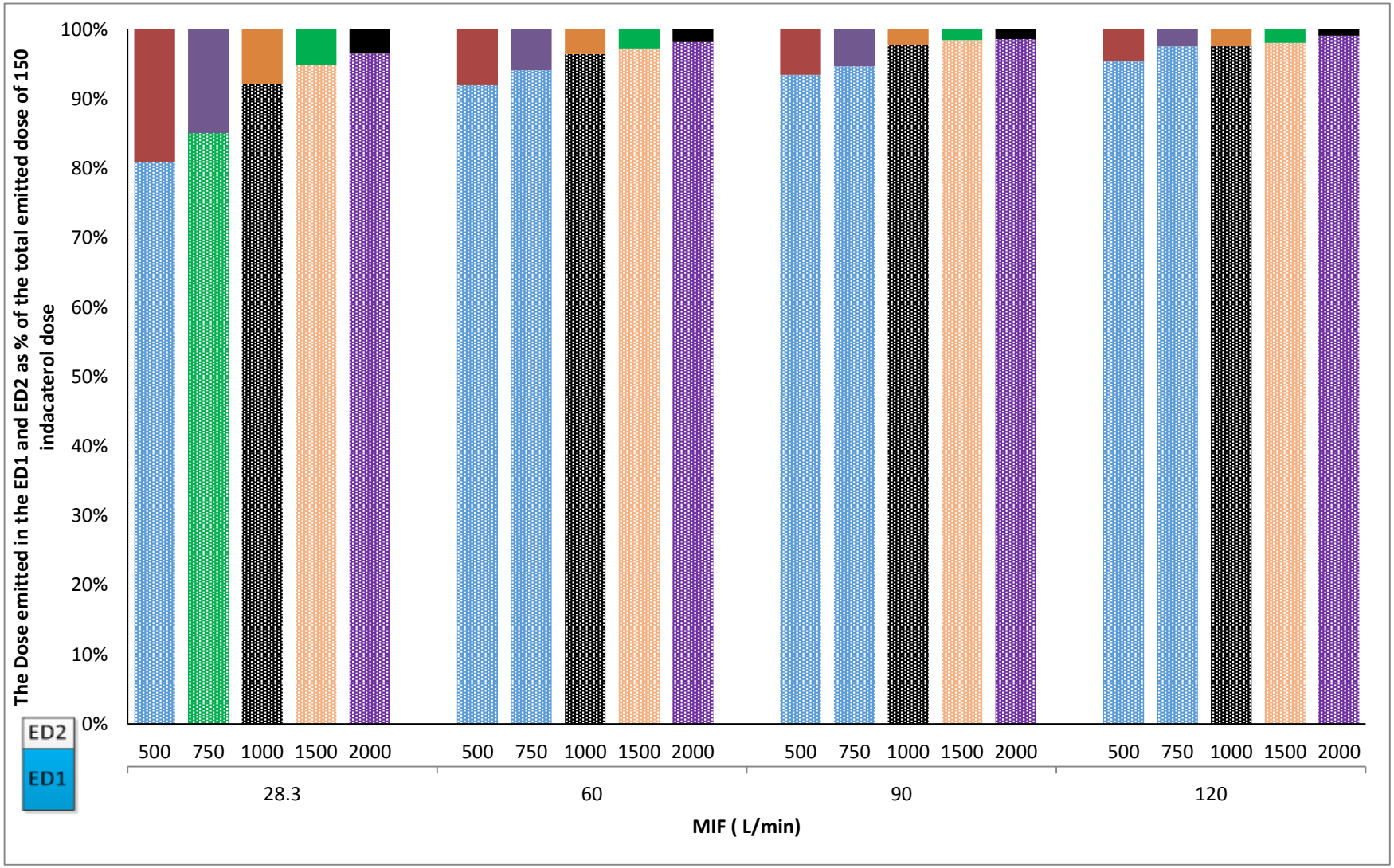

Figure 6.2: Mean of indacaterol 150µg (ED1) and (ED2) as % of the nominal dose [n=3].

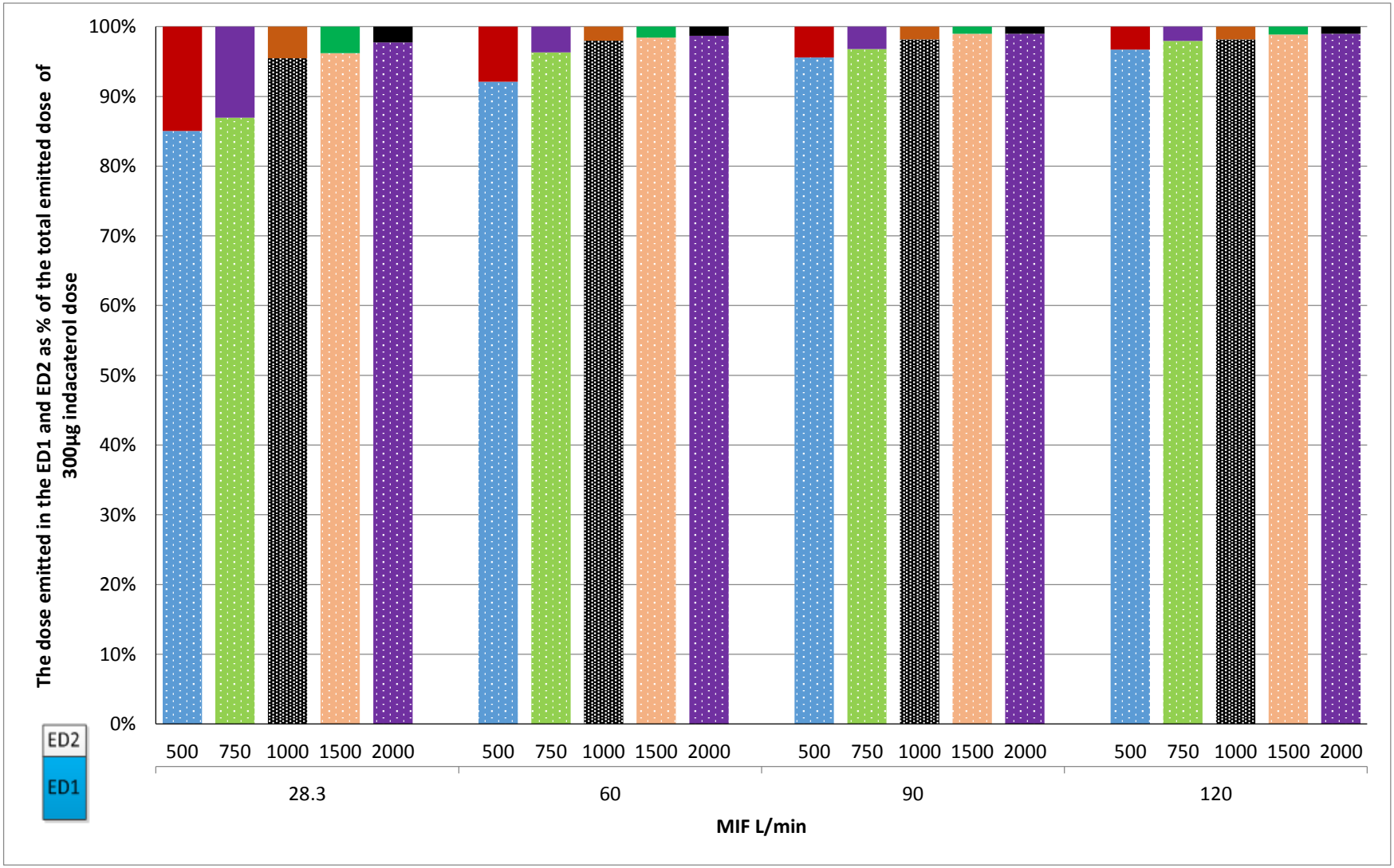

Figure 6.3: Mean of indacaterol 300µg emitted dose after first (ED1) and second (ED2) inhalation as % of the nominal dose [n=3].

6.3.2 Aerodynamic dose emission characteristics of indacaterol Breezhaler® using Andersen cascade impactor.

The aerodynamic characteristics of the emitted dose of indacaterol 150 and 300 µg dose strengths were investigated. The range of MIF and Vin used for the study was chosen to comply with the specification of the internal impactor volume (approximately 1L) and the clinical inhalation profiles data of patients with COPD (mild to very severe condition)

Table 6-3: Mean (SD) of the aerodynamic characteristics of the indacaterol 150 µg dose emission at different MIF and Vin [n=3].

	MIF = 28.3 L/min							
Vin (L)	FPD (µg)	TED (µg)	%FPF	EFPD (µg)	RA(µg)	%TRD	MMAD (µm)	GSD
1.0	36.4(0.4)	105.7(1.5)	34.5(0.2)	20.1(0.1)	29.3(1.3)	90.0(1.4)	3.5(0.1)	1.8(0.1)
1.5	40.5(0.2)	111.6(0.9)	36.3(0.4)	21.5(0.4)	22.3(0.8)	89.3(0.9)	3.4(0.0)	1.9(0.0)
2.0	42.9(0.1)	115.4(1.3)	37.2(0.3)	25.2(0.1)	21.7(1.3)	91.4(1.1)	3.3(0.1)	1.8(0.1)
4.0	46.8(0.5)	120.0(0.8)	39.1(0.1)	27.6(0.0)	18.9(0.4)	92.6(0.7)	3.2(0.1)	1.8(0.1)
	MIF = 60 L/min							
1.0	43.2(0.5)	119.4(1.1)	36.2(0.7)	28.6(0.2)	21.7(2.1)	94.1(1.0)	2.9(0.1)	1.9(0.1)
1.5	45.7(0.3)	120.4(0.7)	37.9(0.3)	30.7(0.3)	18.6(0.6)	92.7(0.6)	2.9(0.1)	2.0(0.1)
2.0	47.7(0.9)	121.2(0.3)	39.4(0.5)	31.6(0.3)	18.1(0.7)	92.9(0.3)	2.9(0.0)	2.1(0.0)
4.0	50.9(0.1)	123.9(0.4)	41.1(0.5)	34.1(0.1)	17.5(0.4)	94.3(1.2)	2.8(0.1)	2.0(0.1)
	MIF =90 L/min							
1.0	45.7(0.2)	121.0(0.2)	37.8(0.6)	34.2(0.6)	17.8(0.6)	92.5(0.8)	2.8(0.1)	2.0(0.1)
1.5	47.7(0.1)	123.8(0.8)	38.5(0.4)	35.1(0.3)	15.7(0.4)	93.0(1.2)	2.8(0.1)	2.1(0.1)
2.0	53.9(0.4)	124.6(0.7)	43.2(0.6)	38.4(0.2)	13.3(0.3)	91.9(0.7)	2.7(0.0)	2.0(0.0)
4.0	54.7(0.4)	126.2(0.3)	43.4(0.9)	39.1(0.9)	12.7(0.7)	92.6(1.5)	2.7(0.0)	2.0(0.0)

Table 6-4: Mean (SD) of the aerodynamic characteristics of the indacaterol 300 µg dose emission at different MIF and Vin [n=3].

	MIF= 28.3 L/min							
Vin (L)	FPD (µg)	TED (µg)	%FPF	EFPD (µg)	RA(µg)	%TRD	MMAD (µm)	GSD
1.0	72.9(1.2)	218.9(1.7)	33.3(1.2)	40.5(0.2)	49.7(0.9)	89.5(2.1)	3.3(0.0)	1.8(0.1)
1.5	83.0(0.2)	231.0(1.4)	36.0(0.3)	45.3(0.5)	39.2(1.1)	90.0(0.9)	3.3(0.1)	1.9(0.1)
2.0	86.3(0.4)	232.0(1.2)	37.2(0.2)	48.3(0.3)	38.1(0.7)	90.1(1.1)	3.2(0.0)	1.9(0.0)
4.0	89.8(1.3)	236.1(1.0)	38.0(0.3)	50.0(0.1)	35.5(1.3)	90.5(1.6)	3.0(0.1)	1.8(0.0)
	MIF = 60 L/min							
1.0	92.5(0.4)	240.9(2.0)	38.4(0.4)	62.6(0.5)	36.3(1.0)	92.4(2.0)	2.8(0.0)	2.0(0.1)
1.5	96.0(0.5)	245.4(1.3)	39.1(0.6)	64.4(0.3)	33.1(1.2)	92.8(1.1)	2.8(0.1)	2.1(0.1)
2.0	99.8(0.9)	247.8(1.5)	40.3(0.7)	66.1(0.3)	32.9(0.8)	93.6(1.3)	2.7(0.1)	2.0(0.1)
4.0	106.4(0.6)	249.5(0.7)	42.7(0.5)	68.8(0.8)	31.5(0.6)	93.6(1.9)	2.7(0.0)	2.1(0.0)
	MIF= 90 L/min							
1.0	95.5(1.2)	244.1(1.1)	39.1(0.8)	74.0(0.6)	34.6(0.9)	92.9(1.2)	2.7(0.1)	2.0(0.1)
1.5	105.3(0.8)	246.5(0.9)	42.7(0.7)	79.0(0.3)	30.5(0.4)	92.3(1.0)	2.7(0.0)	1.8(0.0)
2.0	108.5(0.4)	247.4(0.7)	43.9(0.6)	81.1(0.2)	31.1(0.8)	92.8(0.7)	2.6(0.1)	1.9(0.1)
4.0	112.6(0.7)	252.1(1.0)	44.7(0.9)	82.1(0.9)	29.1(0.5)	93.7(0.8)	2.6(0.1)	1.9(0.1)

FPD= Fine particle dose (≤5µm), TED= Total emitted dose, FPF= fine particle mass as % of the TED, EFPD= Extra fine particle dose (≤ 3µm), RA= Residual amount (retained in capsule and device), % TRD= Total recovery dose as % of the nominal dose 300 µg indacaterol, MMAD= Mass median aerodynamic diameter, GSD= Geometric standard deviation, MIF= maximum inhalation flow, Vin= Inhaled volume.

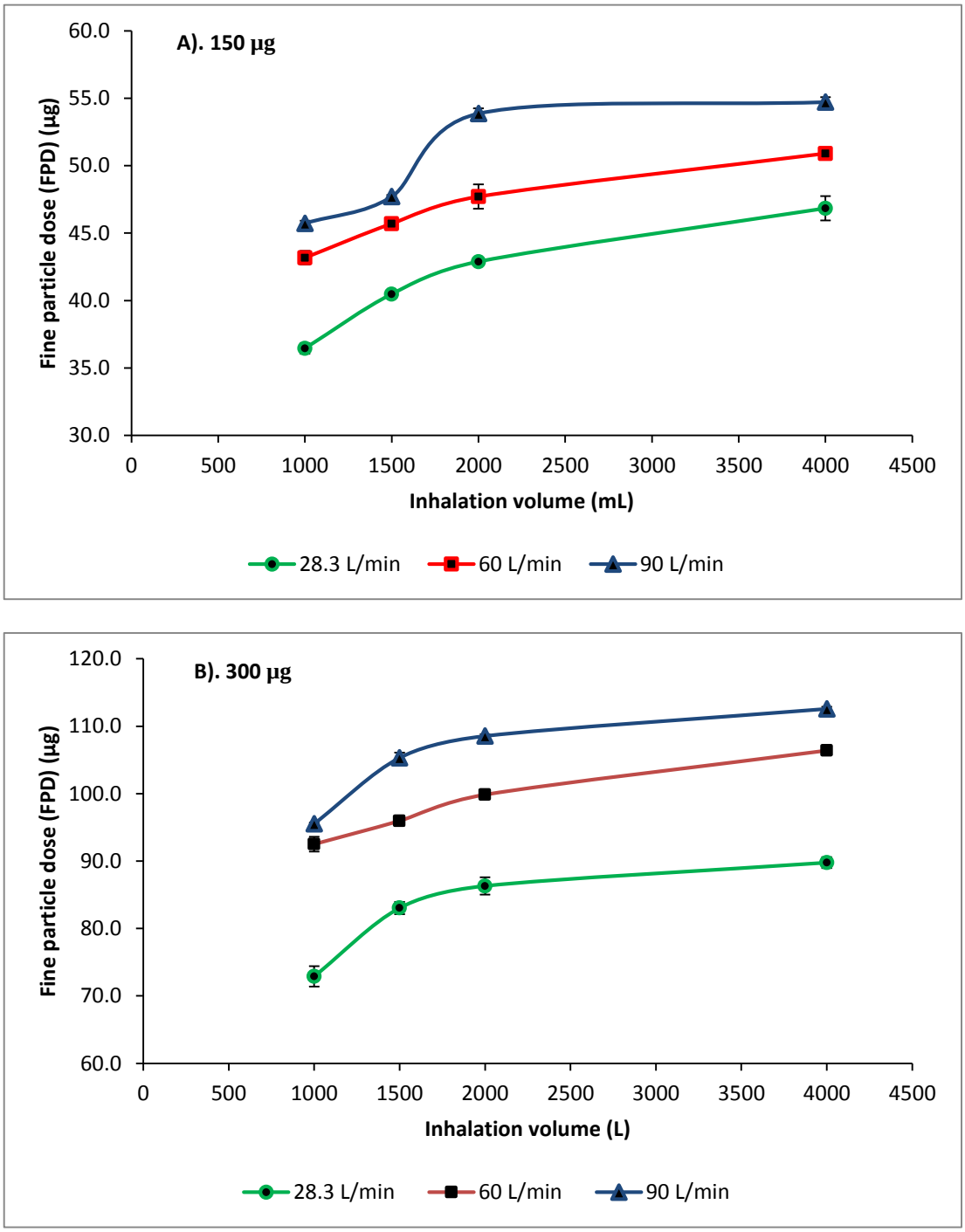

Figure 6.4: The fine particle dose (FPD) of indacaterol emitted from the Onbrez Breezhaler® at different MIF of (28.3, 60 and 90L/min) and Vin of (1, 1.5, 2 and 4L) for both A) 150µg dose strength B) 300 µg dose strength

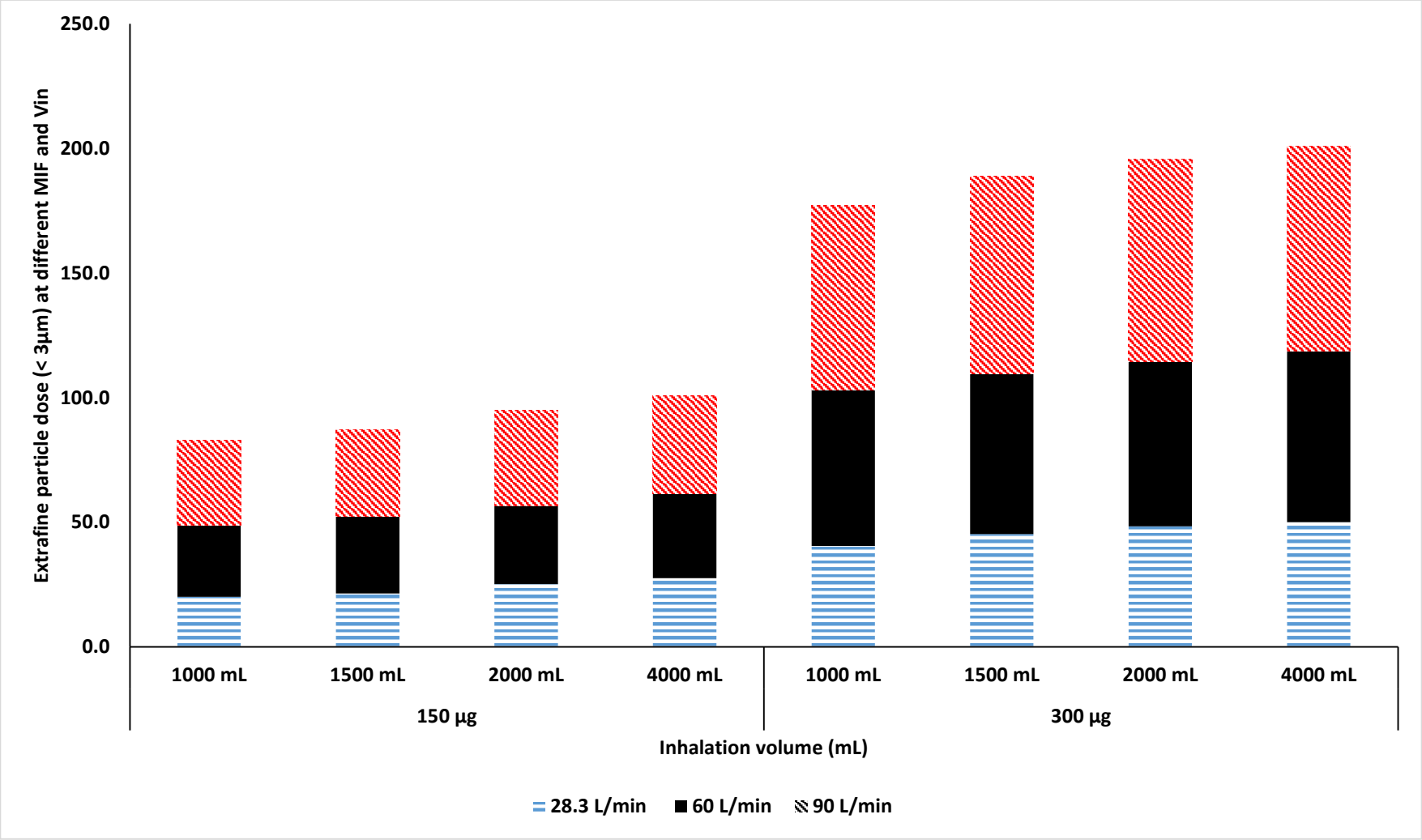

Figure 6.5: The extra fine particle dose (EFPD) of indacaterol emitted from the Onbrez Breezhaler® as a function of the inhalation flow (MIF) and volume (Vin) used for both dose strengths 150 and 300 µg.

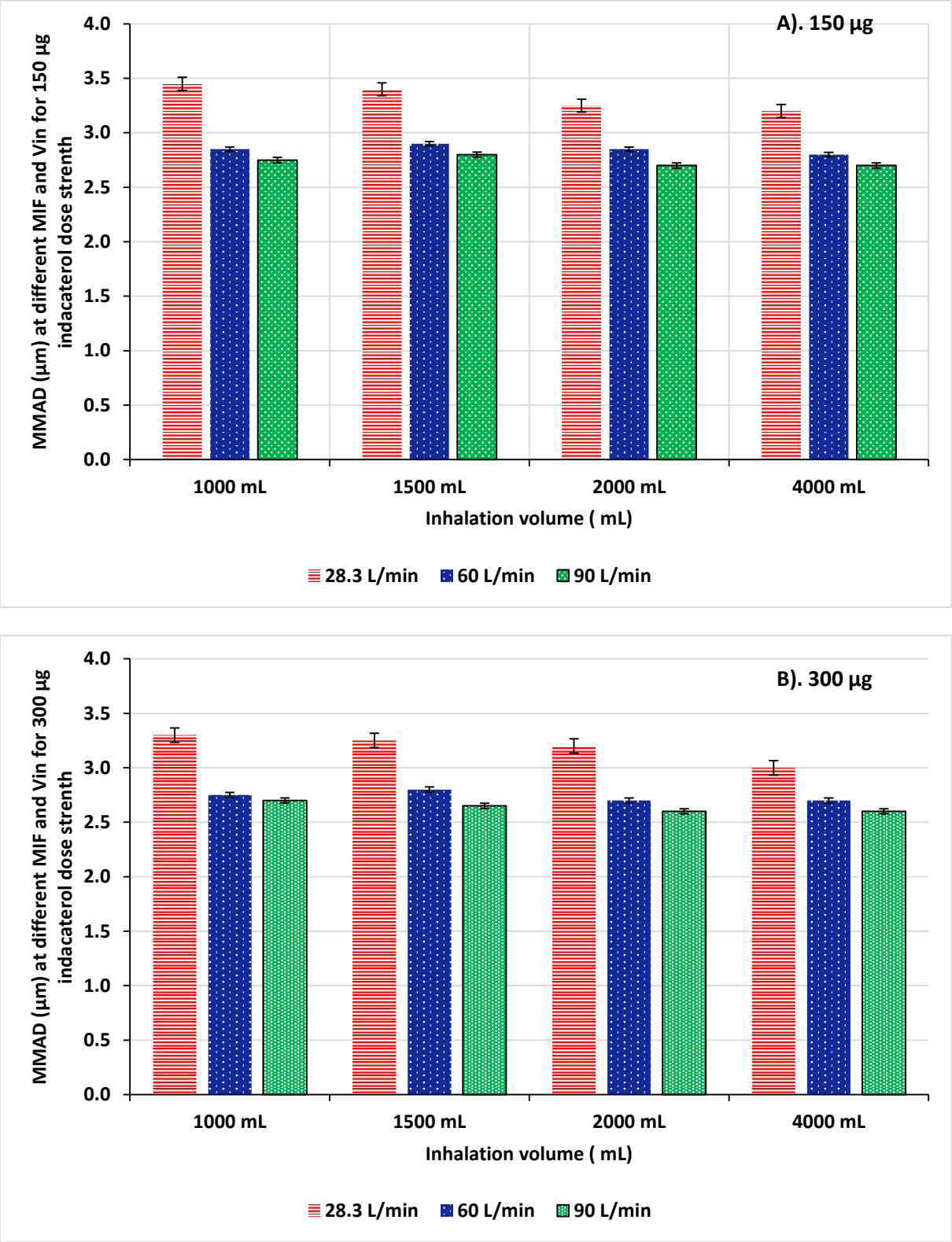

Figure 6.6: The Mass median aerodynamic diameter (MMAD) of indacaterol emitted particles at different inhalation flow (MIF) and inhalation volumes (Vin) for both A). 150 µg indacaterol dose strength. B). 300 µg indacaterol dose strength.

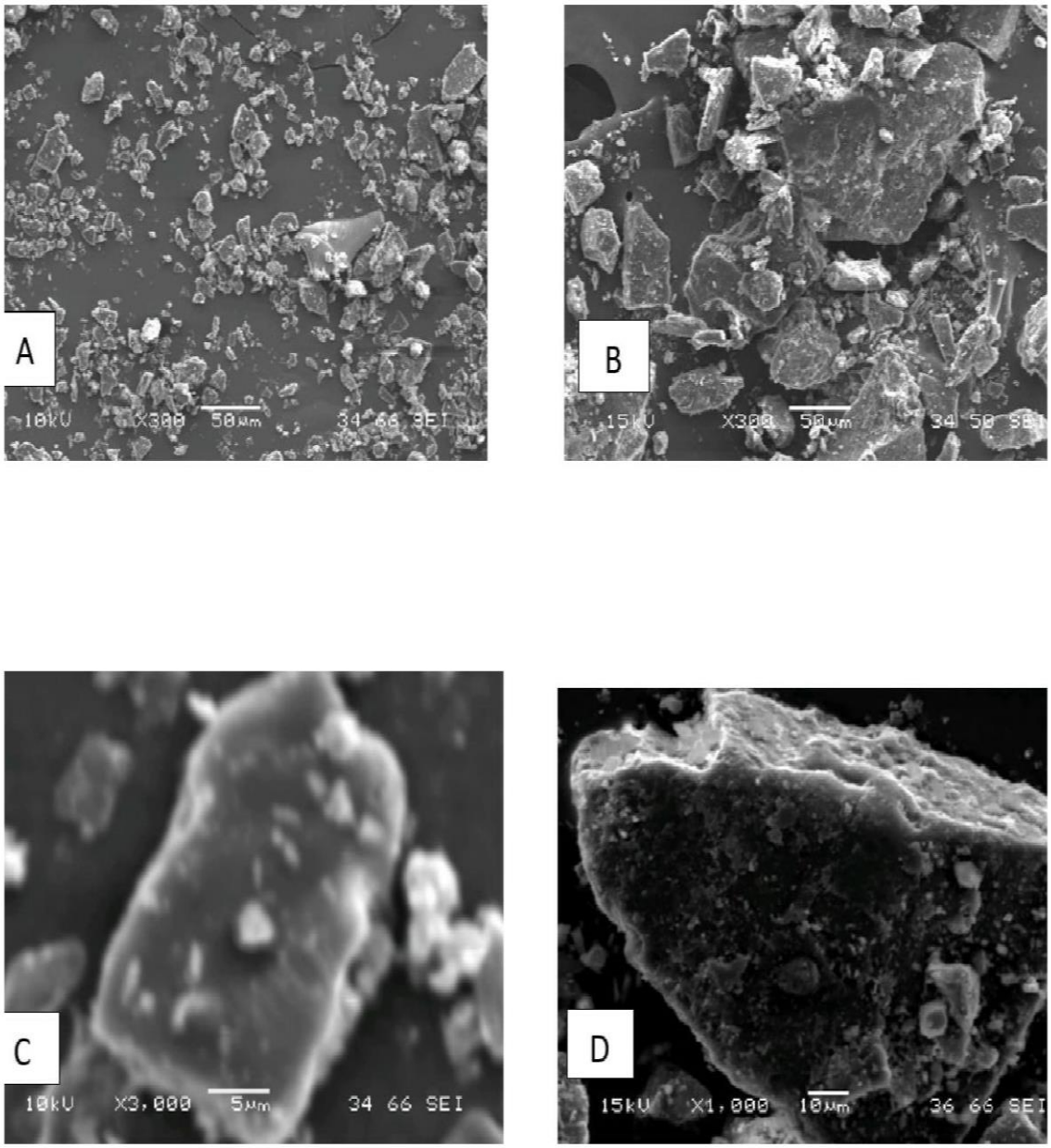

Figure 6.7: Scanning electron micrographs of indacaterol Onbrez Breezhaler 150 µg and 300 µg powder formulations. a) 150 µg general view, b) 300 µg general view, c) 150 µg close view, d) 300 µg close view.

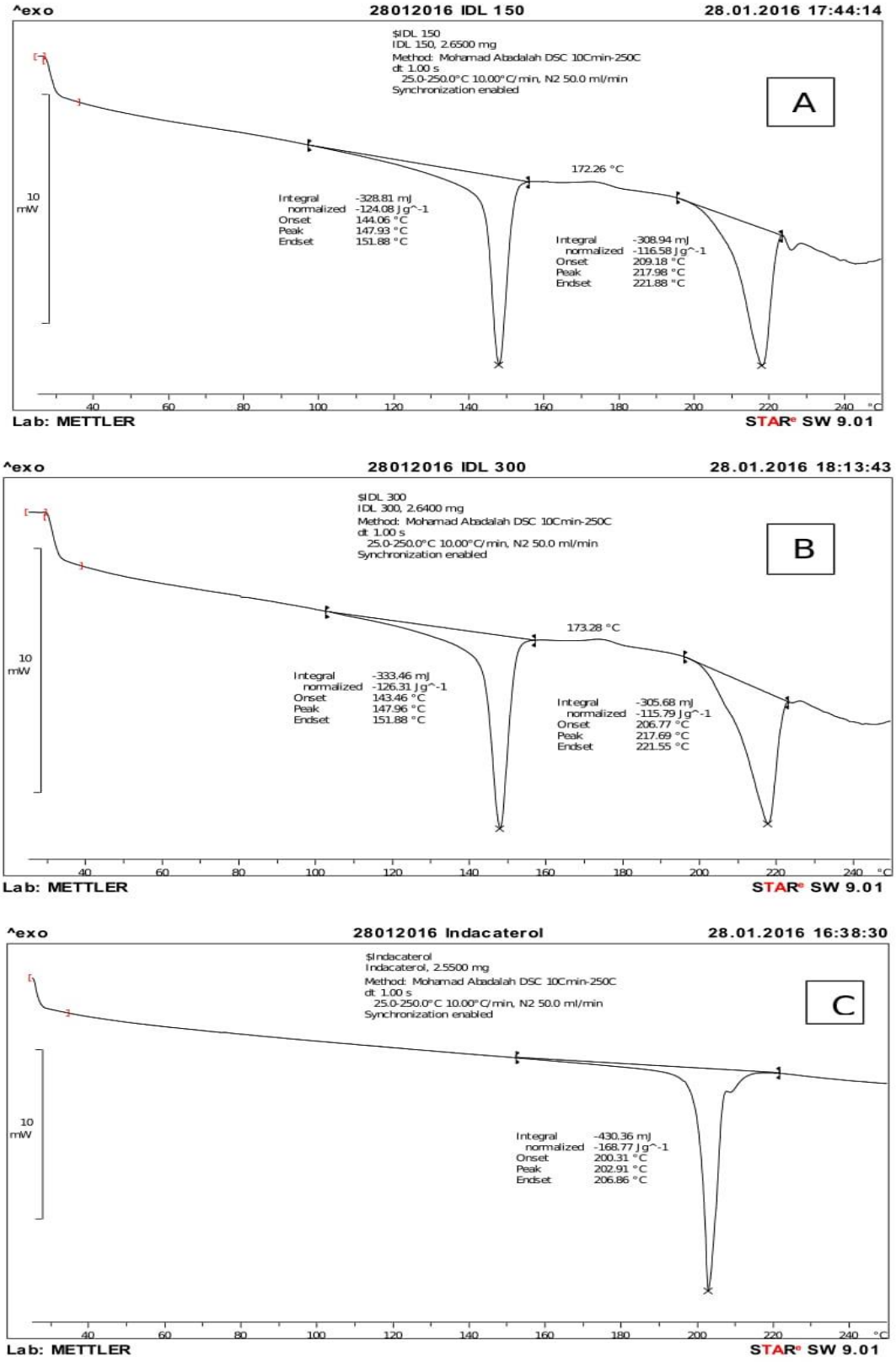

Figure 6.8: Differential scanning calorimetry (DSC) of indacaterol Onbrez Breezhaler: a) 150 µg and b) 300 µg and c) indacaterol powder.

6.4 Discussion

The Onbrez Breezhaler® formulation, like many DPI devices, contains a blend of the micronised drug with tomahawk shaped α-lactose monohydrate carrier (Figure 6.7 C and D). As shown from the DSC thermogram (Figure 6.8 A and B) the endothermic transition peaking at 147 ^{o}C corresponds to the loss of water of crystallisation whilst the endothermic peak at 217 ^{o}C is due to the melting of α- lactose monohydrate (Lerk et al., 1984). A small exothermic transition is observed at around 172 ^{o}C corresponding to the small amount of amorphous content (Larhrib et al., 2003). Indacaterol showed a sharp endothermic melting peak (Figure 6.8 C) which is not shown in the Onbrez Breezhaler formulation either because it was masked by lactose melting peak or it was not detected due to it comprise a smaller amount in the DPI formulation. Each capsule contains indacaterol maleate equivalent to 150 µg or 300 µg Indacaterol. Onbrez Breezhaler® 150 µg and 300 µg are formulated with similar amounts of carrier *i.e.*, 24.8 mg for the 150 µg indacaterol and 24.6 mg for the 300 µg dose (Moen, 2010), corresponding to a 0.6% w/w and 1.2% w/w drug/lactose ratio for 150 µg and 300 µg indacaterol respectively. It can be seen from the scanning electron micrographs (Figure 6.7 A and B) that 150 µg indacaterol formulation has a higher concentration of fine carrier particles in comparison to 300 µg indacaterol formulation. The performance of DPIs is profoundly influenced by the physical properties of the carrier particularly their particle size and surface roughness (Larhrib et al., 1999). These morphological features of lactose carrier play a significant role in determining DPI performance by influencing the flow and hence drug content uniformity and influencing the adhesion and detachment of drug from the surface of the carrier. Kaialy et al., (2012) reported that reducing the particle size of the carrier improves the amount of respirable drug delivered from a DPI but has an adverse effect on drug content uniformity and result in more drug deposited in the oropharyngeal region (Kaialy et al., 2012).

Despite the difference observed in the carrier particle size, size distribution (SEM) and drug to carrier ratio yet both formulations produced a good drug content uniformity (% CV < 1.8%) and a % recovery of 100% (Table 6.5).

Table 6-5: Mean, Percent recovery and % coefficient of variation (% CV) of indacaterol maleate content contained in 150 µg and 300 µg indacaterol Onbrez Breezhaler® (n =6).

Indacaterol maleate dose strength	Mean dose per capsule ± SD	% CV
150 µg	151.32 ± 2.68	1.77
300 µg	301.46 ± 4.74	1.57

Tables 6.1 and 6.2 summarise the dose emission results after the first (ED1) and after the second inhalation (ED2) as well as the total emitted dose (TED) for the 150 µg and 300 µg indacaterol products as a function of MIF and Vin. The ED1 significantly increased ($p < 0.05$) whereas the ED2 decreased with increasing the MIF and VIN. At the low inhalation flow rate (28.3 L/min), the first inhalation was not sufficient to withdraw most of the dose from the capsule and the second inhalation was necessary to empty the capsule (Figures 6.2 and 6.3). The results confirm the recommendation to make two separate inhalations from each dose to successfully empty the capsule (Laube et al., 2011) it is more important to make two separate inhalations when the only low flow rate is achieved by the patient. The Vin contributed to the capsule emptying after the first inhalation. At the higher inhalation flow rate (120 L/min) most of the dose was emitted from the capsule after the first inhalation, and only small amount of the drug was left in the capsule for the second inhalation (Table 6.1 and 6.2). The impact of the inhaled volume with regard to dose emptying was more pronounced at the low MIFs than at high MIFs (Table 6.1 and 6.2). It is widely accepted that breath-activated DPIs are often associated with flow rate-dependent changes in the emitted dose (Hill and Slater, 1998, Taylor and Gustafsson, 2005, Telko and Hickey, 2005, Johal et al., 2013). Increasing the MIF from 28.3 L/min to 120 L/min resulted in a significant ($p < 0.05$) increase in the total

emitted dose. Overall dose emission was reduced below a MIF of 60 L/min and Vin of 1 L (Table 6.1 and 6.2). Since the reported MIF and Vin of some COPD patients were found to be below these values (Pavkov et al., 2010, Chapman et al., 2011, Colthorpe et al., 2013) this would suggest that not all patients will inhale the full prescribed dose when using the Onbrez breezhaler®.

The resistance of a DPI can be classified with respect to the inhalation flow required to produce a pressure drop of 4 kPa. This value was chosen because it is the one recommended by pharmacopoeia (USP, 2014) for the *in-vitro* characterisation of the dose emitted from a DPI. A device that is characterised as having a low resistance requires an inspiratory flow of 90 L/min to produce this pressure drop (Laube et al., 2011). We found that the flow rate required to achieve a 4 kPa pressure drop through the Breezhaler® was around 100 L/min which is in line with other authors results (Pavkov et al., 2010, Dederichs et al., 2015). This confirms that the Breezhaler® is a low resistance device.

In the case of the Indacaterol Breezhaler®, irrespective of the sampling apparatus used (DUSA or ACI) the TED is almost doubled with increasing indacaterol dose from 150 µg to 300 µg (Tables 6.1- 6.2 DUSA and 6.3- 6.4 ACI), suggesting a linear relationship between inhaled dose and the delivered dose *in-vitro*. The TED_{ACI} is equivalent to ED1 recovered from DUSA1. At the same MIF (90 L/min) and Vin (2 L), the TED_{ACI} was generally smaller than ED1 (Table 6.1 and 6.3). The ACI is composed of many stages and its internal volume (1.155 L) comfortably exceeds the volume of DUSA (0.1 L). As a result the washing of ACI for drug collection is time consuming, and the probability of losing some drug during the washing could be more for ACI than for DUSA. The washing procedure of either DUSA or ACI was carefully conducted as shown from the total recovered dose (TRD) which was around 100% for DUSA (Tables 6.1 and 6.2) and > 92% for ACI (Table 6.3 and 6.4).

Capsule-based DPIs can be limited by powder retention in the capsule and device, which leads to a reduction in the emitted dose (Vidgren et al., 1988). The drug retention could be minimised by coating the drug capsule and delivery device with pharmaceutically acceptable force-control agents (Heng et al., 2013). Irrespective of the dose strength, the residual amount in the capsule after two separate inhalations (RA_{cap}), residual amount in the device (RA_{Dev}) and the total residual amount in capsule and device (TRA is equal RA_{cap} + RA_{Dev}) were decreased with increasing the MIF and Vin (Table 6.1 and 6.2). The RA (Cap) after two separate inhalations is much smaller than the RA (Dev). Thus, inhaling twice from the capsule, inhaling forcefully and prolonging the inhalation time contribute all to the reduction in the TRA. The RA is about two-fold for 300 µg Indacaterol (Table 6.2) in comparison to 150 µg using DUSA (Table 6.1) or ACI (Table 6.3 and 6.4).

The inhaled volumes used to quantify the aerodynamic particle size distribution of inhaled indacaterol exceed the internal volume of the ACI (1.155 L) (Copley et al., 2005) to allow sufficient sampling time for the aerosol bolus transfer from the inhaler device to distal stages of the ACI. The ACI data illustrate the impact of the Vin and MIF increase on the aerodynamic characteristics of indacaterol emitted dose. The results of the FPD as a function of the Vin at different MIFs for both dose strengths 150 µg and 300 µg are shown in Figures 6.4 A and B respectively. The FPD significantly ($p < 0.05$) increased with both the Vin and the MIF irrespective of the dose strength. The FPD was almost doubled when the dose strength increased from 150 µg to 300 µg (Figures 6.4 A and B) across all MIFs and Vins, however as a percentage of the nominal dose, the FPD would be almost the same for both dose strengths suggesting the reliability of the Breezhaler in delivering a consistent drug dosing *in-vitro*. Prolonging the Vin is more critical especially when low MIF is used. For example using an inhaled volume of 4 L at 28.3 L/min would provide similar FPD to 90 L/min and 1 L inhaled volume (Figure 6.4). Thus, prolonging the Vin is essential when high

MIF cannot be achieved. The increase in FPD is more pronounced between 28.3 and 60 L/min, and the rate of increase in the FPD between 60 and 90 L/min is generally reduced. Thus, 60 L/min could be considered as a threshold MIF to operate the Breezhaler, which can be achieved easily achieved in practice due to the low resistance of this device. For example, using the same patients, a mean peak inspiratory flow (PIF) of 72 L/min was generated with the Breezhaler® device compared with a mean PIF of only 36 L/min with the Handihaler® (Price and Chrystyn, 2015). The results of the EFPD (< 3um) for both dose strengths (150 µg and 300 µg) are shown in Figure 6.5, and a similar trend was observed for EFPD (< 3 µm) for both dose strengths with increasing the MIFs and Vins. The high proportion of EFPDs would improve the deposition in the peripheral airways in the lungs, and this is considered beneficial for the management of COPD (Heyder, 2004, De Boer et al., 2015). Our study showed that both the MIF and Vins are important in maximising the EFPD of indacaterol (Figure 6.5). GSD values are > 1.2 µm (Tables 6.3 and 6.4) suggesting polydispersity of the aerosol; this polydispersity is more likely to influence drug distribution in the airways. This polydispersity contains substantial mass fractions of particles smaller than 5 µm (Figures 6.4 A and B). The greatest decrease in MMAD was observed between 28.3 L/min and 60 L/min across all Vins used and increasing the MIF above 60 L/min did not make much difference in the MMAD (Figure 6.6 A and B), suggesting that 60 L/min is the minimum MIF to operate the Breezhaler. An MMAD < 5 µm is considered to be necessary for sufficient airway deposition (Roche et al., 2013). The difference in FPD < 5 µm between different flow rates and volumes are also strongly reflected in the differences in the EFPF (< 3 µm). The turbulent generated at high flow rates 60 and 90 L/min was able to generate sufficient shear forces between drug-drug particles. Such phenomenon would be able to enhance particles dispersion leading to the formation of smaller particle size (Abadelah et al., 2017). Prolonging inhaled volume may have also provided particles with sufficient time of flight allowing drug-drug particles

detachment. The performance of Breezhaler is dependent on both inhalation flow rate and volume. The increase in FPD is more pronounced between 28.3 and 60 L/min. Prolonging the Vin is more critical especially when low MIF is used. Breezhaler was effective in producing respirable particles with an MMAD < 5 µm irrespective of the inhalation flow rate, but the mass fraction of particles with an aerodynamic diameter < 3 µm is more pronounced between 60 and 90 L/min as shown in Figure 6.5. The difference in the drug particle size distribution as a function of the flow rate and volume is more likely to influence drug distribution in the airways.

6.5 Conclusions

Although the difference in particle size of lactose carrier both indacaterol formulations 150 µg and 300 µg provided an excellent drug content uniformity with a % CV < 1.8. The indacaterol dose emission from the capsule based Onbrez Breezhaler® for both dose strengths 150 and 300 µg demonstrated inhalation flow dependent dose emission. Furthermore, the inhaled volume (Vin) was important when low MIF was used. The aerodynamic characteristics of indacaterol showed that Breezhaler® was effective in producing respirable particles with an MMAD < 5 µm irrespective of the inhalation flow rate, but the mass fraction of particles with an aerodynamic diameter <3 µm is more pronounced between 60 and 90 L/min. The difference in the drug particle size distribution as a function of the flow rate and volume is more likely to influence drug distribution in the airways.

This chapter results showed that a minimum MIF of 60 L/min and inhaled volume of 1L is required when using the indacaterol Breezhaler®. Finally, the results confirm the recommendation of using two separate inhalations for each dose when high MIF is not achieved through Onbrez Breezhaler®.

Chapter 7. *Ex-vivo* study of Indacaterol Breezhaler® using real life recorded and simulated COPD patients' inhalation profiles

7.1 Chapter overview

It is obvious that the square wave profiles generated by the vacuum pump are by no mean a precise simulation to the patients' IP. It has been argued that this method fails to reliably assess how a DPI would perform for weak patients such as those with severe impaired lung function and very severe COPD (Al-Showair et al., 2007b, Copley et al., 2014). Patient IP is bell shaped with a gradual increase in the ACIM to reach the maximum inspiratory flow (Chrystyn and Price, 2009). In real life COPD patients' IP, the inspiratory parameters MIF, Vin and ACIM act all together during the inhalation manoeuvre, but the extent of each parameter in the overall dose emission and aerodynamic characteristics of indacaterol Breezhaler® is not known in the literature. The present chapter assess the performace of indacaterol Breezhaler® (150 µg) using real life COPD patients' IP, in addition to the impact of each parameter of the inhalation manouever on the aerodynamic dose emission characterisitcs of indacaterol Breezhaler using simulated COPD patients' IP.

The patient inability to achieve the required optimum inhalation effort will result in poor drug delivery to the lungs (Haidl et al., 2016). The patient IP comprises of different parameters namely MIF, Vin and ACIM (Olsson and Asking, 1994, Chrystyn et al., 2015, Bagherisadeghi et al., 2017).The IP characteristics depend on the patient body mass index (BMI), gender, age and disease status (Chrystyn and Price, 2009), which makes the inhalation manoeuvre parameters differ from one patient to another when inhaling through the same DPI device. The difference in the inhalation manoeuvre parameters between patients results in a different dose delivered to the lungs (Pasquali et al., 2015, Buttini et al., 2016a). The square wave IP generated by the vacuum pump applies a constant flow rate which is maintained for a specific period of time during aerosolisation *in-vitro* testing to represent the inhalation time. Furthermore, results in a rapid ACIM through the DPI beyond the capability of a typical patient (Copley et al., 2014).

Recently, researchers have adapted a new methodology to replace the use of the vacuum pump with *in-vivo* generated patients' IPs using breath simulator (BRS) (Olsson et al., 2013). This methodology is more representative than the electronic lung methodology (Brindley et al., 1994). The *ex-vivo* methodology has been extensively used to determine the difference in aerodynamic characteristics when patient IPs are used instead of compendial vacuum pump (Chrystyn et al., 2015, Bagherisadeghi et al., 2017). Breath simulator has opened up the opportunity to improve the clinical relevance of *in-vitro* orally inhaled product (OIP) testing techniques (Copley et al., 2014). Rather than using a BRS to generate and apply inhalation /exhalation profile that mimics human subject researcher have started to work with real patients' IPs to scope product performance.

The present chapter was designed to study the effect using *in-vivo* generated COPD patients' IP on the dose emission of indacaterol Breezhaler®. The only difference between our study and the above-mentioned study (Chrystyn et al., 2015) was that the ACI was used instead of NGI to determine inhaled indacaterol aerodynamic particle distribution. Furthermore, effect of each inspiratory parameter (MIF, Vin and ACIM) was determined using simulated COPD patients' IPs. These simulated IPs were produced by altering the raw profiles to change only one parameter at a time. The ranges of MIF (40 L/min, 65 L/min and 85 L/min), Vin (1 L, 2 L and 3 L), ACIM (2 L/s^2, 4 L/s^2 and 8 L/s^2) were chosen to be of clinical relevance based on previous in-vivo studies (Pavkov et al., 2010, Chapman et al., 2011, Colthorpe et al., 2013), and also to be within the specifications of both ACI and BRS.

7.2 Inhalation profiles

7.2.1 Calculation of the inhalation profiles parameters

The MIF for each profile was the maximum flow point achieved by the patient. The Vin was calculated from the area under the curve of each profile where the inhalation MIF is represented in y-axis versus time in x-axis. The IP time was calculated from the zero starting point of the profile, then reaching the maximum flow and finally returned to zero.

The ACIM was calculated from the patient profile data. Whereas linear regression was calculated for each 0.1 second interval between the inhalation flow and time, then highest slope with Square regression close to 1 was chosen as the ACIM for each profile.

The flow rates values were then changed into (mL/s) to be replayed using the breath simulator. Only TXT files format are accepted to be used with the simulator.

Table 7-1: illustrates the calculation of the inhalation profile parameters.

T(s)	Q (L/min)	ACC (L/s^2)	R^2	IV(L)	Q (mL/s)
0	0	0.722684	1	0.001	0.000
0.05	2.168052	0.639054	1	0.003	36.134
0.1	4.085213	1.918357	1	0.006	68.087
0.15	9.840283	2.512637	1	0.011	164.005
0.2	17.378193	**2.938076**	1	0.018	289.637
0.25	26.192422	1.909365	1	0.024	436.540
0.3	31.920518	1.699543	1	0.029	532.009
0.35	37.019147	1.445895	1	0.033	616.986
0.4	41.356833	1.209125	1	0.036	689.281
0.45	44.984207	0.992193	1	0.039	749.737

7.2.2 Recorded COPD inhalation profiles

The IPs of 38 patients with different COPD severity aged 55-79 with a mean age of 66 were recorded using an inhalation profile recorder. The profiles were recorded using an empty (Placebo) Onbrez Breezhaler® inhaler device, and the patients were able to generate an average MIF of 88 L/min. The patients were given the patient information leaflet (PIL) to read and trained to inhale as hard and fast as they can from the start of the inhalation manoeuvre and for as long as they could.(COPD profiles were generated in previous in-vivo study (Azouz et al., 2015b). [see appendix for profiles' figures]

Table 7-2: Patient's demographic details and inhalation parameters.[Patients' inspiratory parameters were highlighted]

Volunteer code	Age (yrs)	BMI (kg/m^2)	SEX (M/F)	PEF	FEV1	MIF (L/min)	Vin (ml)	ACIM (L/sec^2)	Time(s)
1	71	48.6	F	180	1	**28.3**	**0.7**	**1.9**	2.4
2	61	29.4	M	160	1.1	**36.0**	**1.2**	**2.1**	4.1
3	73	39.5	M	305	1.8	**39.2**	**2.0**	**5.1**	4.4
4	62	32.1	F	205	1	**43.8**	**1.6**	**2.2**	3.5
5	55	19.8	M	220	1.65	**51.8**	**1.6**	**3.9**	3.7
6	74	22.1	M	255	1.6	**53.5**	**2.6**	**3.8**	4.6
7	69	30	M	200	1.15	**54.8**	**2.0**	**3.6**	3.3
8	53	26.4	F	235	1.75	**54.9**	**2.5**	**2.9**	5.3
9	74	18.4	F	65	0.8	**66.0**	**1.0**	**4.7**	1.7
10	73	24.2	M	185	1.2	**71.0**	**2.4**	**4.4**	3.4
11	63	20.2	M	160	1.25	**76.3**	**2.6**	**6.3**	4.3
12	69	21.5	F	160	1.3	**78.4**	**1.9**	**4.1**	2.5
13	73	32.3	M	130	0.5	**80.9**	**3.0**	**5.2**	3.3
14	67	24.8	M	190	1.15	**81.3**	**2.5**	**2.8**	3.1
15	69	33.2	M	170	0.85	**83.7**	**1.8**	**5.2**	2.3
16	78	21.2	M	290	1.5	**87.8**	**3.0**	**5.1**	4.0

BMI= body mass index, PEF= peak expiratory flow, FEV1= forced expiratory volume in 1 seconds, MIF= maximum inspiratory flow, Vin= Inhaled volume, ACIM= initial acceleration of the inhalation manoeuvre, Ti=inhalation time in seconds.

7.2.3 Simulated COPD patients' inhalation profiles

The inspiratory parameters (*e.g.,* MIF, ACIM and Vin) were measured for each profile; the MIF for each profile is the highest flow the patient can achieve from the start to the finish of the inhalation time. The Vin was calculated from the area under the curve of the inhalation flow against the time profile. While, ACIM was determined by firstly calculating the slope of the flow values against the inhalation time, then the value with a high linear regression (r^2) was chosen. In the case of Onbrez Breezhaler the linear regression was almost $r^2=1$ for all the values. Therefore ACIM was determined as the values with high slope.

The IPs were classified into three main groups depending on the MIF that was generated by the COPD patient's low (40 L/min) medium (65L/min) and high (85L/min). Then, both ACIM and Vin of these profiles were altered to obtain Vin values of 1, 2 and 3 L and ACIM values of 2, 4 and 8 L/s^2. The Vin was altered by increasing or decreasing the inhalation time (Ti) after the MIF of the profile. The ACIM was altered for chosen profiles by increasing or decreasing the steepness of the initial slope of the profile. The patients were able to achieve a MIF of 88.93 L/min (29.48), Vin of 2.13 L (0.60) and ACIM of 6.18 L/s^2 (4.23) when inhaling through Onbrez Breezhaler®. Therefore, the parameters for simulated IPs were designed to mimic these values. [Summary of the simulated patients' IP (n=27) used in the study are provided in the appendix]

7.3 Materials and methods

7.3.1 Materials and HPLC method

The materials used for the determination of aerodynamic dose emission of indacaterol Breezhaler® were discussed in Chapter 3 (section 3.1.3). A validated HPLC method for the quantification of the indacaterol was explained in Chapter 3 (section 3.2.5).

7.3.2 Measurement of the dose emission aerodynamic characteristics using inhalation profiles.

The *ex-vivo* methodology was used to replay the patients' IPs using breath simulator; a mixing inlet was used to enable the measurement of the aerosolised dose with breath simulator (Nadarassan, 2010; Olsson, 2013; Chrystyn et al. 2015). The principle of using the breath simulator (BRS) with Andersen cascade impactor (ACI) was achieved by introducing a mixing inlet in between the pre-separator of the cascade impactor and the Alberta Idealised Throat (IAT) see Figure 7.1. The role of the mixing inlet is enabling the ACI to work at constant flow rate (*e.g.,* 90 L/min) and therefore, not affecting the principle of ACI mode of work. An inhalation flow generated by the breath simulator will pass through the inhaler device, and it is the one responsible for dose de-aggregation. The ACI was connected to the vacuum pump by the mean of a critical flow controller; then the mixing inlet was connected to the breath simulator. The supplementary air cylinder was connected to the breath simulator and mixing inlet via controlled valve connection. The controlled valve was opened to introduce the supplementary air to the mixing inlet. The flow rate was monitored to ensure that flow rate at the mouthpiece is constant at 0 L/min before dose actuation.

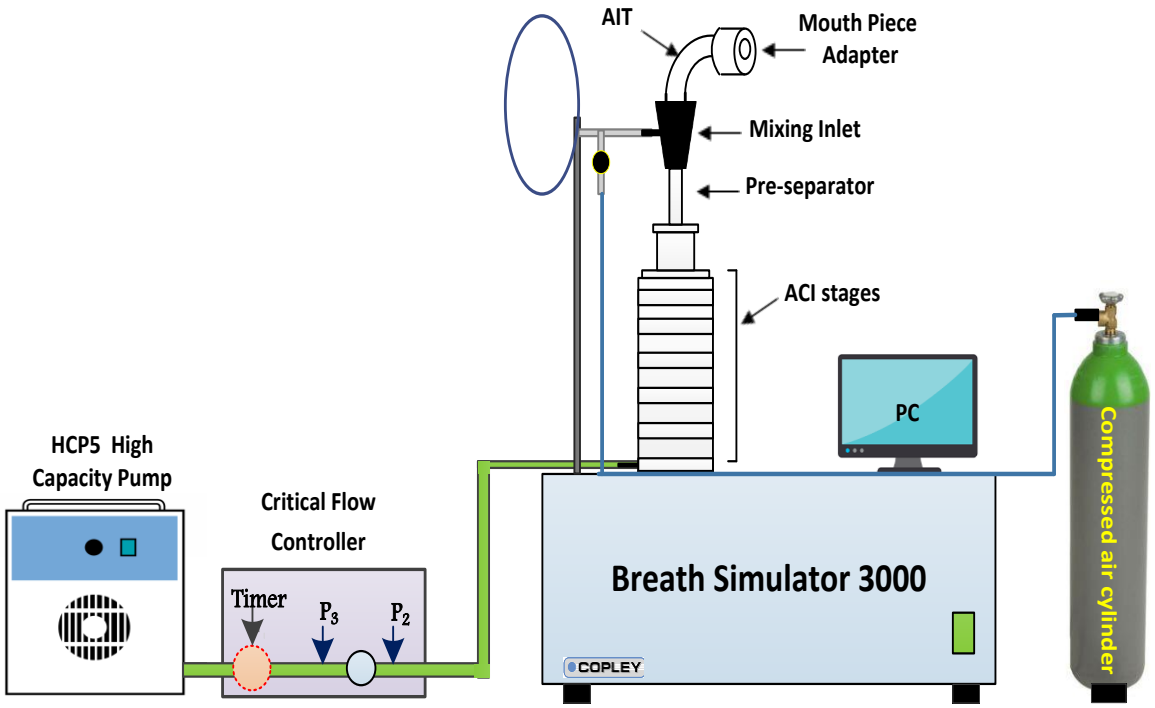

Figure 7.1: Breath simulator, ACI and AIT experimental set-up

7.3.3 Measurement of dose content uniformity using dose unit sampling apparatus DUSA.

For DPIs, the dose emptying as well as the aerodynamic particle size distribution (APSD) is defined by the inhalation manoeuvre. Therefore, the IPs should be applied using both DUSA and ACI in order to scope performance of the inhaler device using breath simulator fully. The investigation of dose delivery uniformity (DDU) is usually carried out using DUSA, which capture the entire dose delivered by the orally inhaled product (OIP).

The experimental set-up is similar to that applied with ACI (Section 7.3.2) instead of using ACI; DUSA was used to measure dose emission uniformity of the inhaler device. All profiles (Table 7.2) were used to aerosolise the dose of 150 µg indacaterol into the DUSA. Three determination of each profile was carried out using 3 capsules, then total emitted dose (TED_{DUSA}) and total residual amount (TRA_{DUSA}) were determined after recovering the dose emitted into the DUSA and amount retained in the capsule and device.

7.4 Experimental set-up

7.4.1 Andersen cascade impactor set-up

The manufacturing protocol was carried out to ensure that all ACI stages, the pre-separator, the mixing inlet and the Alberta idealised throat (AIT) are washed, cleaned and dried before use. An oven (Memmert Ltd, Germany) was used for drying. However, appropriate time was given for all the washed parts to cool at room temperature in order to ensure that no electrostatic charge would affect the aerosolised drug powder. The collection plates were cleaned with acetone, and thereafter sprayed with a silicone lubricant and left to dry for 20 mins to avoid particle bouncing when hitting the plate during the experiment. The inner face of the AIT was sprayed with a silicone lubricant to maintain a condition which would be a realistic representation to the patient's throat and to prevent particles bouncing off the surface (Zhou et al., 2011).

The ACI was set to operate at either 60 or 90 L/min. When 60 L/min was used stages 0 and 7 of the ACI were replaced by stages -1 and -0, while stages -1 and 6 were replaced by the stages -2A and -1A when 90 L/min was used. The pre-separator was chosen for each operating flow rate. The pre-separator was filled with 10 mL of a washing solution (MeOH: water 60:40 % v/v) to prevent large particles from bouncing into the stages of the ACI and thereby affecting the deposition results (USP, 2014, Copley, 2015). The ACI stages were assembled in order, and a 82mm filter (Whatman®, UK) was then placed in the filter stage to collect the remaining particles in the air stream. The mixing inlet was positioned between the pre-separator and the AIT to introduce supplementary air through the side arm (Figure 7.1).

7.4.2 Breath Simulator- ACI set-up

Figure 7.2 shows the experimental set-up of the breath simulator (BRS 3000) with ACI. The breath simulator warming profile was carried out (20 cycles) prior to the experiment run.The system warm- up procedure was recommended by the manufacturer (Copley, 2015).

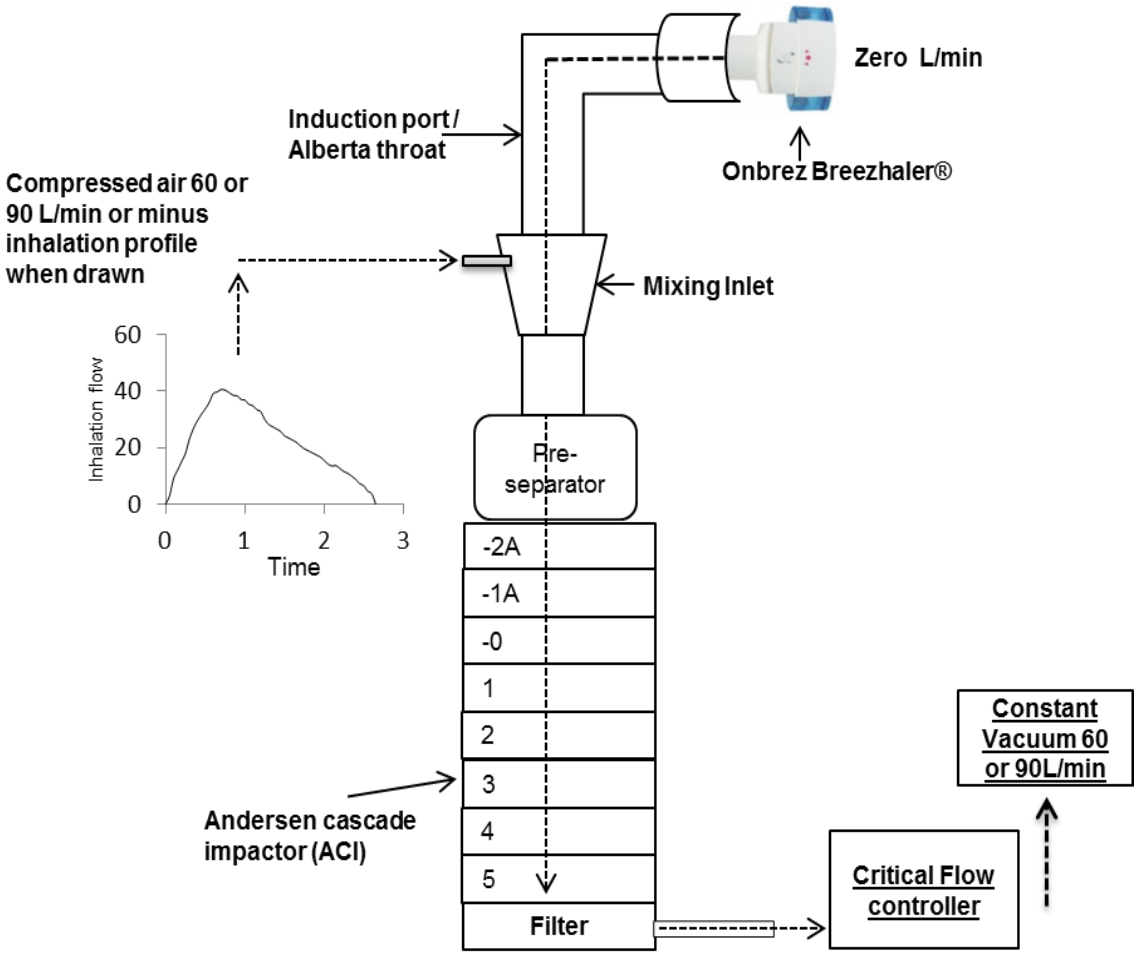

Figure 7.2: Schematic diagram of the methodology set-up.

The ACI was connected to the vacuum pump by using the critical flow controller (TPK 2000, Copley Scientific Ltd, UK). Then, the ACI was connected to the breath simulator using the mixing inlet side arm. The supplementary air cylinder was connected to the breath simulator using a regulatory flow valve. The vacuum pump was switched on 20 minutes before the first determination to ensure flow stability. The airflow through the ACI was set either at 60L/min

or 90L/min in accordance with the chosen IPs. The flow from the vacuum pump was set while the regulatory flow valve remained closed.The flow meter (DFM 2000, Copley Scientific Ltd, UK) was used to measure the flow through ACI (flow stability P3/P2 $\leq$ 0.5). When the required flow was displayed on the meter's screen and set, the regulatory valve was then opened to introduce the supplementary air through the mixing inlet side arm. The flow meter screen was then monitored to ensure that the supplementary air introduced is equal to that introduced by the vacuum pump, and the flow at the mouthpiece is 0 L/min.

Then, the run button on the screen of the BRS was pressed in order to replay the IPs which are programmed to be used with BRS 3000. The IP flow condition was withdrawn from the supplementary air. Therefore, the inhalation profiles' MIF should be 5 L/min below the operating flow rate.The flow rate was kept constant through the ACI using the vacuum pump. For IPs below 60 L/min, an operating flow rate of 60 L/min was used, while an operation flow rate of 90 L/min was used for IPs above 60 L/min. ACI stages and collection plates (including the holo plates) were assembles depending on the flow rate of set-up (Discussed in Chapter 3- section 3.2.1).

The indacaterol dose emission was carried out by using three capsules for each IP following the recommendations and the dose preparation instructions in patient information leaflet (PIL) of the Onbrez Breezhaler® (Novartis Pharmaceuticals Ltd, UK). Once the three capsules were aerosolised into the impactor, the residual amount (RA) left in the capsules and device was recovered. For each IP, three determinations were made in order to ensure that dose emission procedure is reproducible.

After dose aerosolisation and before starting the washing procedure the AIT, the mouthpiece and the mixing inlet side arm were sealed with parafilm to ensure that no drug powder was wasted during the ACI disassemble procedure.

The washing procedure was undertaken once the run was finished and the three capsules of 150 µg indacaterol were aerosolised into the ACI. The washing solution was MeOH:water 60:40 % v/v with dexamethasone (1µg/mL) as an internal standard. Appropriate volumes were added to the mouthpiece, the AIT, the pre-separator, the ACI stages, the capsule, the device and the filter to recover the drug dose from each part of the experiment. The samples were transferred into 20 mL vials and then sonicated for 15 minutes to facilitate drug particle solubility. Thereafter, all samples were transferred into 2 mL HPLC amber vials by using a plastic syringe and 0.45 filter tips (Thermo Scientific Ltd, UK) and sealed properly with a vial cap. The indacaterol amounts in each sample were then quantified using a validated HPLC method (HPLC method validation discussed in Chapter 4 – Section 4.4).

Table 7-3: Volumes of washing solution used to collect the emitted dose from different experiment parts.

Experiment part	Washing solution volume
Idealised Alberta Throat (AIT)	10 mL
Mouthpiece (MP)	5 mL
Pre-separator (PS)	20 mL
ACI stages	10 mL
Filter	10 mL
Capsules (n=3) (Cap)	10 mL
Device (Dev)	10 mL

7.4.3 Data analysis

The Copley Inhaler Testing Data Analysis Software (CITDAS version 2.0, Copley Scientific Ltd, UK) was used to calculate the aerodynamic dose emission parameters.

The aerodynamic particle size distribution (APSD) characteristics of the emitted dose were calculated. The total emitted dose (TED) was obtained from the cumulative amounts of indacaterol maleate deposited in the Alberta idealised throat (IAT), the pre-separator (PS) and all the stages of the ACI. The fine particle dose (FPD) was the mass associated with particles < 5 µm, and the extra-fine particle dose (EFPD) was the mass of powder associated with particles < 3 µm.The fine particle fraction (FPF) was the FPD divided by the TED. The mass median aerodynamic diameter (MMAD) was the size corresponding to the 50th percentile of the cumulative mass-weighed distribution of the amount deposited in the ACI. The geometric standard deviation (GSD) is the square root of the size corresponding to 15.87 % divided by square root of the size for 84.13%. The residual amount (RA) was obtained as the sum of the dose retained in the capsule and device after aerosolisation. The total recovered dose (TRD) was calculated as the sum of the total emitted dose (TED) and the residual amount (RA).

A one-way and Two-way ANOVA were used to determine the difference in the TED, FPD, RA, *etc*. Tuckey test was used to determine the difference between the different parameters used. The hypothesis of ($p < 0.05$) was used to determine the significant difference.

7.5 Results

7.5.1 Dose emission aerodynamic characteristics of indacaterol Breezhaler® using COPD patients' IP

The mean inhalation parameters of the selected profiles MIF, Vin, ACIM and Ti were 64.9 L/min, 2.1 L, 4.2 L/s^2 and 3.4 s respectively (Table 7.2). A summary of indacaterol aerodynamic dose emission characteristics results of all IPs selected is shown in Table 7.4. The results were arranged according to the profiles' MIF, and the results of TED, FPD and MMAD of 150 µg indacaterol emitted from Onbrez Breezhaler® for all the profiles are shown in Figures 7.3, 7.4 and 7.6.

Table 7-4: Mean (SD) of Indacaterol aerodynamic dose emission characteristics of 18 COPD patient profiles from Onbrez Breezhaler® [3 Capsules of 150 µg indacaterol dose per determination, 3 determinations for each profile]

Profile Number	FPD (µg)	TED (µg)	% FPF	RA(µg)	TRD	MMAD (µm)	GSD
1	28.7 (1.6)	92.0 (4.2)	31.2 (0.3)	45.3 (3.8)	137.3 (1.3)	3.7 (0.1)	1.8 (0.0)
2	34.5 (1.7)	103.6 (5.2)	33.3 (0.1)	33.2 (2.6)	136.8 (0.8)	3.4 (0.1)	1.7 (0.1)
3	31.7 (0.8)	100.1 (0.6)	31.6 (0.8)	34.4 (2.7)	134.5 (4.3)	3.2 (0.1)	1.7 (0.1)
4	37.1 (0.8)	105.4 (3.2)	35.2 (0.3)	28.6 (3.2)	134.0 (2.5)	3.3 (0.1)	1.7 (0.0)
5	39.7 (1.4)	109.2 (6.5)	36.4 (0.9)	29.0 (1.3)	138.2 (5.2)	3.1 (0.1)	1.7 (0.1)
6	40.4 (2.1)	111.1 (2.3)	36.4 (0.2)	29.6 (2.2)	140.6 (1.1)	2.9 (0.1)	1.7 (0.1)
7	39.2 (2.5)	108.1 (6.1)	36.3 (0.3)	29.6 (2.1)	137.7 (3.9)	3.0 (0.0)	1.7 (0.0)
8	43.2 (1.1)	116.1 (2.5)	37.2 (0.7)	27.4 (0.6)	143.5 (2.3)	3.1 (0.0)	1.7 (0.0)
9	37.6 (1.2)	113.8 (1.2)	33.0 (0.5)	22.3 (3.2)	136.1 (1.2)	2.5 (0.1)	2.0 (0.1)
10	39.8 (0.9)	116.5 (1.0)	34.2 (0.3)	19.9 (2.8)	136.4 (1.0)	2.4 (0.0)	2.0 (0.1)
11	40.6 (1.2)	120.6 (2.7)	33.7 (0.2)	15.4 (1.4)	136.1 (2.7)	2.2 (0.0)	2.0 (0.1)
12	41.1 (0.4)	114.0 (1.0)	36.0 (0.1)	15.7 (1.12)	129.7 (5.3)	2.4 (0.0)	2.0 (0.0)
13	43.5 (0.7)	125.2 (1.1)	34.8 (0.2)	16.2 (0.35)	141.4 (1.1)	2.2 (0.0)	2.1 (0.1)
14	37.8 (1.8)	119.7 (1.5)	31.6 (0.1)	17.3 (2.1)	137.0 (1.1)	2.6 (0.0)	2.0 (0.1)
15	37.4 (0.6)	114.5 (1.2)	32.7 (0.3)	18.9 (1.5)	133.4 (1.2)	2.4 (0.1)	2.0 (0.1)
16	45.6 (0.8)	125.1 (1.4)	36.5 (0.7)	11.4 (1.1)	136.5 (0.4)	2.3 (0.1)	2.0 (0.0)
Mean(SD)	**39.7 (5.0)**	**113.4 (8.9)**	**34.9 (2.4)**	**23.4 (8.9)**	**136.8 (3.1)**	**2.7 (0.5)**	**1.9 (0.2)**

FPD = fine particle dose, **TED** = Total emitted dose, **%FPF** = fine particle dose as % of the emitted dose, **RA** = Residual amount, **TRD** =Total recovered dose, **MMAD** = Mass median aerodynamic diameter, **GSD** = Geometric standard deviation.

The results of dose emission uniformity of Breezhaler® using DUSA are presented in Table 7.5. In contrast to the dose emitted with ACI more indacaterol dose was found to be recovered using DUSA.

Table 7-5: Mean (SD) of the DUSA results of all IPs used in the study.

Profile number	TED	TRA	TRD	%TRD
1	96.35 (2.16)	52.17 (1.96)	148.52 (2.06)	99.01 (1.37)
2	107.46 (2.79)	43.60 (4.17)	151.05 (3.38)	100.70 (2.25)
3	121.99 (4.17)	28.63 (2.26)	150.63 (6.43)	100.42 (4.29)
4	123.55 (2.66)	26.80 (2.80)	150.35 (1.14)	100.23 (0.76)
5	135.79 (4.93)	17.44 (1.29)	153.23 (3.64)	102.15 (2.43)
6	120.14 (1.50)	30.04 (1.01)	150.18 (2.51)	100.12 (1.67)
7	134.01(1.22)	19.08 (2.97)	153.08 (1.75)	102.05 (1.17)
8	127.22 (3.88)	24.02 (0.73)	151.24 (4.62)	100.83 (3.08)
9	111.13 (3.28)	39.98 (3.39)	151.12 (6.68)	100.74 (4.45)
10	133.18 (2.12)	18.41(1.71)	151.58 (1.59)	101.06 (1.06)
11	132.95 (5.09)	20.93 (1.41)	153.87 (5.50)	102.58 (3.67)
12	122.46 (3.69)	28.25 (2.36)	150.71 (2.33)	100.47 (1.56)
13	128.99 (4.33)	19.17 (3.70)	148.15 (2.63)	98.77 (1.76)
14	124.34 (1.29)	25.78 (1.14)	150.12 (1.85)	100.08 (1.24)
15	127.33 (5.28)	25.54 (2.03)	152.87 (3.25)	101.91 (2.16)
16	120.53 (6.35)	28.67 (4.07)	149.20 (2.28)	99.47 (1.52)
Mean (SD)	**123.07 (1.49)**	**28.05 (1.17)**	**151.13 (1.65)**	**100.75 (1.10)**

TED = Total emitted dose; dose trapped inside the sampling unit; **TRA** = Total residual amount; dose retained in the capsule and device; **TRD** = Total recovered dose; delivered dose in relation to the nominal dose 150 µg

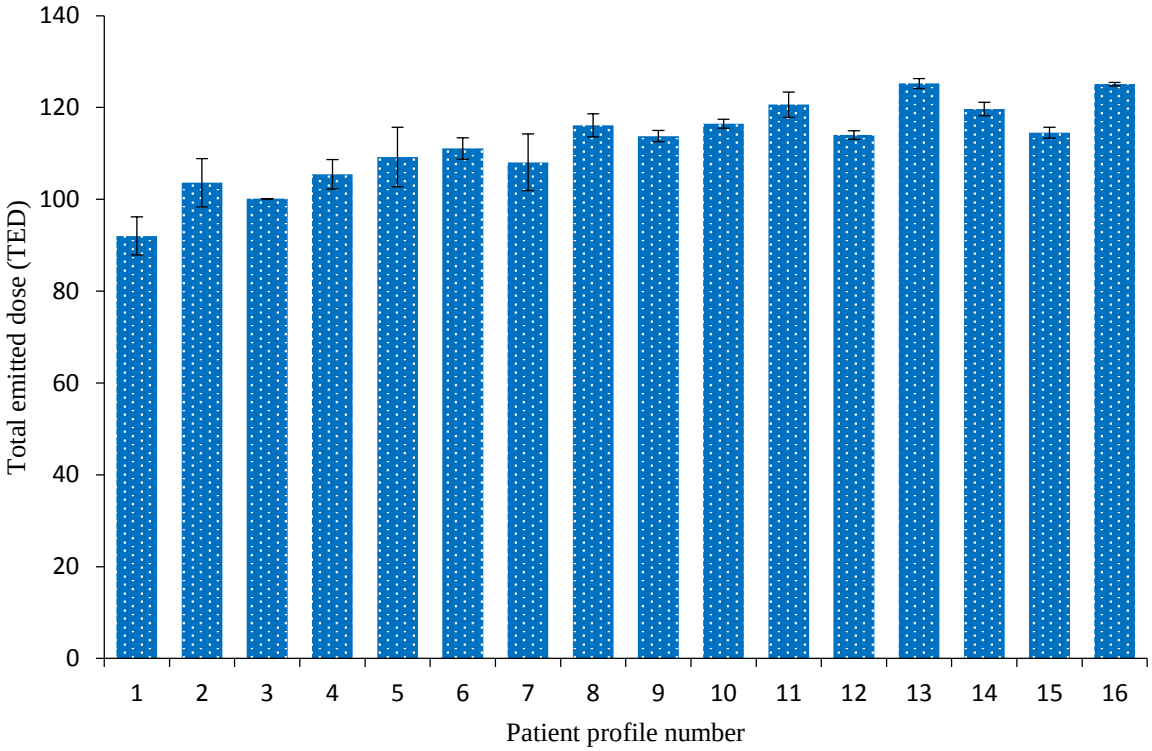

Figure 7.3: Mean (SD) of the Total emitted dose (TED) for 16 COPD inhalation profiles.

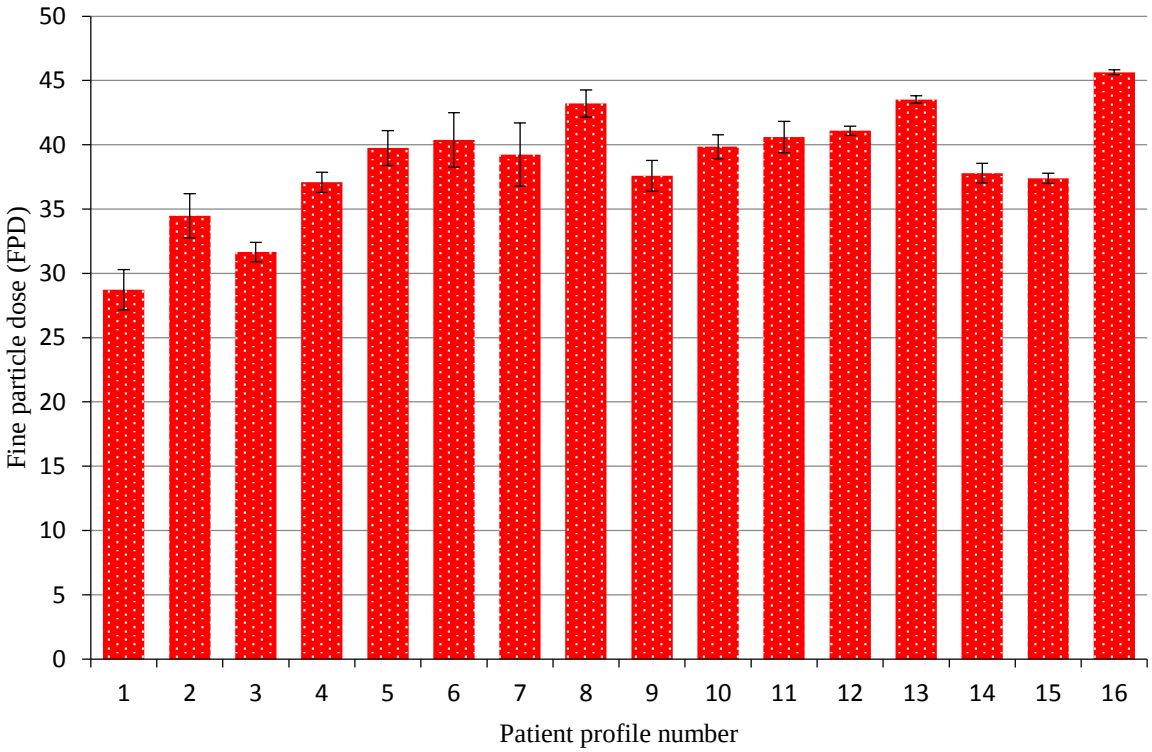

Figure 7.4: Mean (SD) of the fine particle dose (FPD) for 16 COPD inhalation profiles.

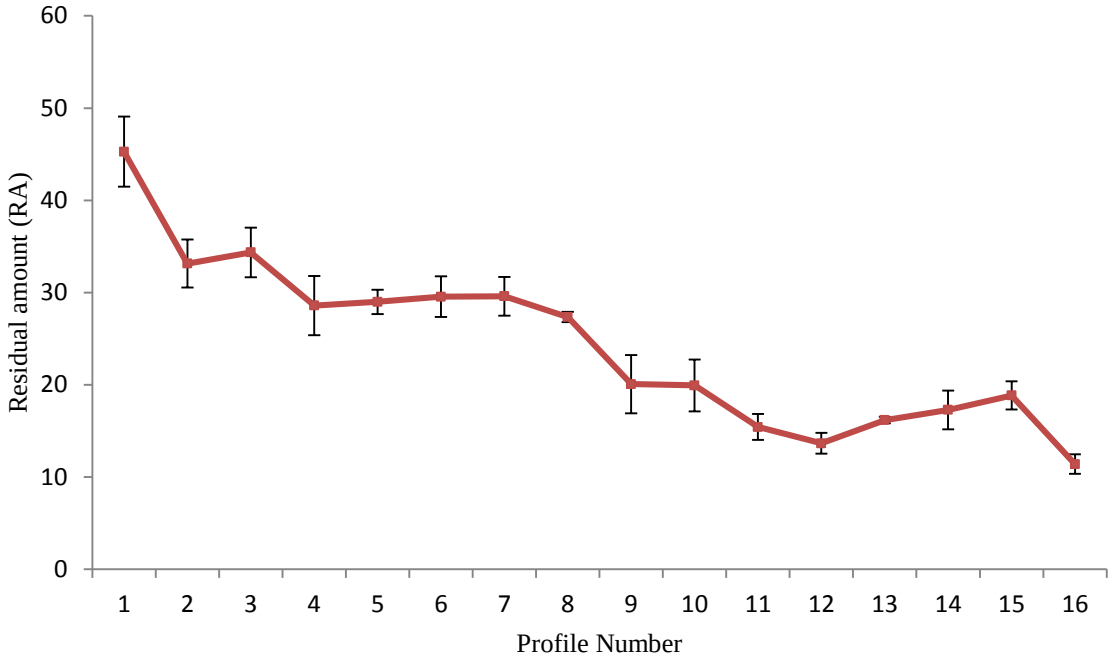

Figure 7.5: Mean (SD) of the residual amount (RA) retained in both device and capsule for 16 COPD inhalation profiles.

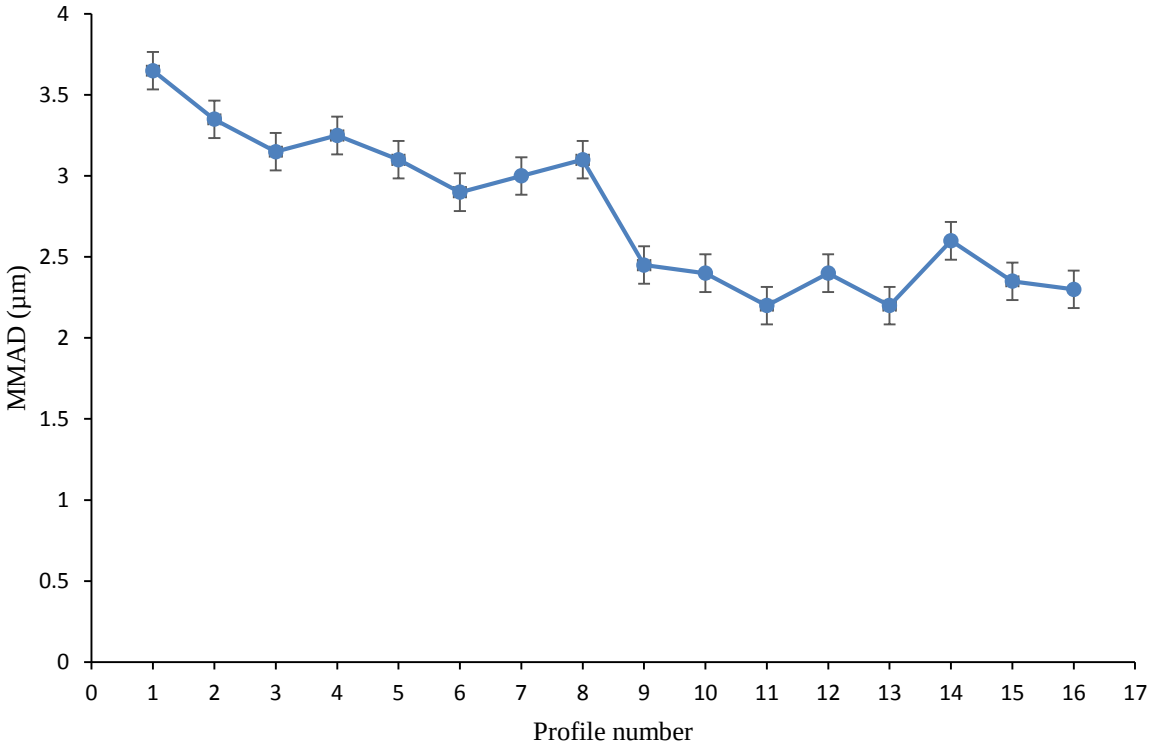

Figure 7.6: Mean (SD) of the MMAD results for 16 COPD inhalation profiles.

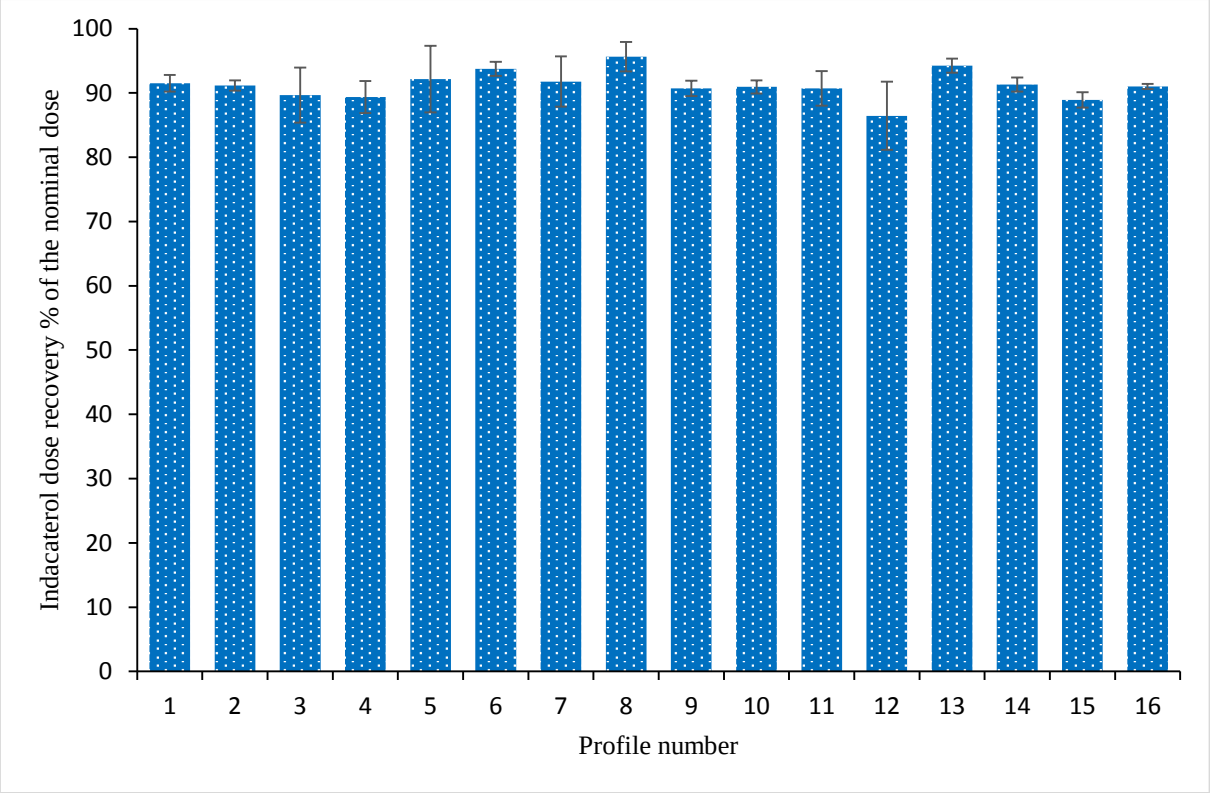

Figure 7.7: Total recovered dose (TRD) of 16 COPD patient profiles.

The results of the aerodynamic dose emission of indacaterol Breezhaler® using recorded COPD patients' IP showed that TED (as % of 150 µg indacaterol nominal dose) was ranging from 61% to 83% with a mean (SD) 113.4 µg (8.9) (Figure 7.3). The fine particle dose (FPD) of indacaterol was ranging from 19% and 30% of with a mean (SD) of 39.7 µg (5.0) (Figure 7.4). The MMAD results show that marked reduction in the particle size with the increase of profiles MIF from 3.7 µm to 2.3 µm (Figure 7.6). The dose emission from Breezhaler was consistent with almost 93% dose recovery with ACI (Figure 7.7) and 99% with DUSA (Table 7.5). The present performance study results demonstrate that TED, FPD increased significantly ($p < 0.05$) when the MIF of the IPs increased (Figures 7.3 and 7.4). In contrary, TRA an MMAD decreased significantly (Figures 7.5 and 7.6).

7.5.2 Dose emission aerodynamic characteristics of indacaterol Breezhaler® using simulated COPD patients' IP

The results of the aerodynamic characteristics of profiles divided into three categories depending on the MIF value (Low 40 L/min – Medium 65 L/min and high 85 L/min). A summary of the data is presented in Tables 7.6, 7.7 and 7.8 respectively. Furthermore, DUSA results of the dose emission of all the profiles using breath simulator are also shown in Table 7.9

Table 7-6: Mean (SD) Indacaterol dose emission aerodynamic characteristics (150µg nominal dose) for low MIF profiles (n= 3) using 3 doses for each determination.

	Vin ~ 1L		
40 L/min	**ACIM ~ 2.0**	**ACIM ~ 4.0**	**ACIM ~ 8.0**
FPD	29.4 (0.7)	31.6 (0.2)	34.7 (0.7)
TED	113.5 (2.8)	114.8 (1.2)	120.4 (1.1)
FPF%	25.9 (3.2)	27.6 (1.9)	28.8 (2.3)
LPM	70.2 (1.6)	68.3 (1.2)	72.8 (1.4)
%EFPD	13.1(1.1)	15.9 (1.0)	16.8 (0.3)
RA	24.8 (2.4)	22.6 (2.6)	22.4 (3.4)
TRD	138.3 (1.2)	137.5 (2.4)	142.8 (3.0)
MMAD	3.4 (0.1)	3.2 (0.1)	3.1 (0.0)
GSD	1.8 (0.0)	1.8 (0.0)	1.9 (0.0)
	Vin ~ 2L		
	2.0	**4.0**	**8.0**
FPD	33.3 (1.8)	35.8 (1.9)	38.3 (0.5)
TED	117.7 (1.2)	118.1(4.6)	122.1 (2.9)
FPF%	28.3 (0.8)	30.3 (3.0)	31.4 (1.2)
LPM	70.4 (1.9)	69.7 (2.8)	70.5 (2.5)
%EFPD	14.1 (0.1)	14.5 (0.9)	15.7 (0.4)
RA	22.9 (1.8)	21.1 (1.0)	18.9 (0.9)
TRD	140.5 (2.2)	139.2 (1.0)	141.0 (3.7)
MMAD	3.3 (0.1)	3.3 (0.1)	3.4 (0.1)
GSD	1.8 (0.1)	1.8 (0.1)	1.8 (0.0)
	Vin ~ 3L		
	2.0	**4.0**	**8.0**
FPD	36.5 (0.7)	38.8 (1.1)	40.3 (0.7)
TED	120.4 (1.8)	121.7 (1.4)	123.0 (0.6)
FPF%	30.3 (1.2)	31.9 (3.0)	32.7 (0.9)
LPM	70.7 (2.8)	71.4 (2.5)	71.8 (3.2)
%EFPD	17.1 (0.2)	17.7 (0.3)	17.9 (0.5)
RA	21.7 (1.0)	19.9 (0.7)	17.2 (0.9)
TRD	142.1 (3.8)	141.6 (2.7)	140.2 (2.3)
MMAD	3.1 (0.0)	3.1 (0.1)	3.2 (0.1)
GSD	1.9 (0.0)	2.0 (0.0)	1.9 (0.1)

Table 7-7: Mean (SD) Indacaterol dose emission aerodynamic characteristics (150µg nominal dose) for medium MIF profiles (n= 3) using 3 doses for each determination.

	Vin ~ 1L		
65L/min	**ACIM ~ 2.0**	**ACIM ~ 4.0**	**ACIM ~ 8.0**
FPD	37.9 (1.3)	38.1 (0.6)	40.5 (0.4)
TED	115.9 (2.4)	116.0 (1.4)	123.2 (3.1)
FPF%	32.7 (3.0)	32.9 (1.2)	32.9 (3.2)
LPM	67.9 (4.4)	66.8 (0.9)	69.4 (2.2)
%EFPD	20.7 (0.8)	20.3 (0.2)	21.9 (0.7)
RA	21.9 (0.9)	21.3 (1.7)	19.9 (2.2)
TRD	137.8 (1.4)	137.2 (0.9)	143.0 (2.5)
MMAD	2.9 (0.1)	2.9 (0.1)	2.8 (0.0)
GSD	2.0 (0.0)	1.9 (0.1)	2.1 (0.0)
	Vin ~ 2L		
	2.0	**4.0**	**8.0**
FPD	39.0 (0.4)	39.9 (0.5)	42.5 (1.6)
TED	118.4 (2.0)	119.0 (2.0)	124.2 (1.6)
FPF%	33.0 (2.2)	33.5 (0.8)	34.2 (1.7)
LPM	73.6 (0.9)	68.7 (1.6)	70.7 (2.3)
%EFPD	18.6 (1.3)	21.4 (0.6)	23.2 (1.0)
RA	20.9 (0.7)	19.0 (3.3)	17.9 (0.6)
TRD	139.4 (4.1)	138.0 (3.8)	142.1 (2.4)
MMAD	3.0 (0.1)	2.9 (0.1)	2.9 (0.1)
GSD	2.2 (0.1)	2.0 (0.0)	2.0 (0.0)
	Vin ~ 3L		
	2.0	**4.0**	**8.0**
FPD	40.3 (0.3)	41.7 (0.8)	43 (0.3)
TED	121.6 (2.2)	122.8 (3.8)	125.9 (1.6)
FPF%	33.2 (2.3)	34.0 (1.9)	34.2 (3.2)
LPM	66.1 (1.2)	70.6 (2.8)	72.4 (2.2)
%EFPD	22.0 (0.9)	22.6 (0.6)	24.1 (0.9)
RA	18.5 (0.9)	16.2 (1.1)	15.2 (0.7)
TRD	140.1 (0.7)	139.1 (1.1)	141.0 (2.8)
MMAD	2.9 (0.0)	2.8 (0.1)	2.7 (0.0)
GSD	2.0 (0.0)	1.9 (0.0)	2.1 (0.1)

Table 7-8: Mean (SD) Indacaterol dose emission aerodynamic characteristics (150µg nominal dose) for high MIF profiles (n= 3) using 3 doses for each determination.

	Vin ~ 1L		
85 L/min	**ACIM ~ 2.0**	**ACIM ~ 4.0**	**ACIM ~ 8.0**
FPD	40.7 (1.1)	42.0 (1.0)	43.7 (0.2)
TED	118.8 (1.7)	119.4 (1.0)	124.0 (1.2)
FPF%	34.3 (1.1)	35.4 (0.6)	35.8 (1.9)
LPM	67.9 (2.7)	71.0 (1.9)	73.1 (2.9)
%EFPD	22.2 (0.5)	20.6 (0.3)	21.9 (1.2)
RA	18.9 (3.0)	18.7 (2.1)	17.3 (0.7)
TRD	137.7 (0.8)	138.1(1.3)	141.2 (3.9)
MMAD	3.0 (0.1)	2.9 (0.0)	2.8 (0.1)
GSD	2.0 (0.1)	2.0 (0.0)	2.1 (0.1)
	Vin ~ 2L		
	2.0	**4.0**	**8.0**
FPD	41.8 (0.2)	43.4 (0.3)	44.8 (1.5)
TED	120.4 (0.8)	121.0 (0.4)	125.9 (0.8)
FPF%	34.7 (0.5)	35.8 (1.8)	36.2 (0.5)
LPM	72.4 (1.6)	72.6 (1.0)	74.1 (3.5)
%EFPD	20.2 (0.8)	21.5 (0.4)	23.0 (0.2)
RA	18.2 (0.6)	17.4 (1.2)	14.8 (0.7)
TRD	138.6 (0.9)	138.4 (1.3)	140.7 (0.6)
MMAD	2.9 (0.0)	2.8 (0.0)	2.7 (0.0)
GSD	2.1 (0.0)	2.0 (0.0)	2.0 (0.0)
	Vin ~ 3L		
	2.0	**4.0**	**8.0**
FPD	44.1 (1.0)	46.8 (0.5)	49.8 (1.0)
TED	123.1 (1.9)	123.9 (1.0)	127.5(1.0)
FPF%	35.9 (1.7)	37.8 (1.3)	38.7 (2.0)
LPM	73.5 (2.4)	71.8 (3.0)	73.0 (2.6)
%EFPD	21.6 (0.8)	23.4 (0.2)	26.1 (0.2)
RA	17.6 (0.8)	14.0 (1.6)	12.2 (0.6)
TRD	140.6 (0.9)	137.9 (2.1)	139.7 (3.1)
MMAD	2.9 (0.0)	2.8 (0.1)	2.6 (0.0)
GSD	2.0 (0.0)	2.0 (0.0)	2.1 (0.0)

Table 7-9: Mean (SD) Indacaterol dose emission data using DUSA (150µg nominal dose) for all profiles (n= 3) using 1 dose for each determination.

	40 L/min		65 L/min		85 L/min	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
	1L -2ACIM					
TED	116.80 (1.25)		121.84 (0.11)		125.41 (0.73)	
RA	33.51 (1.00)		25.36 (1.04)		22.76 (0.54)	
TRD	150.32 (0.25)		147.21 (0.93)		148.17 (0.19)	
	1L -4ACIM					
TED	117.96 (2.08)		122.99 (3.50)		126.27 (3.50)	
RA	30.58 (0.73)		24.92 (3.18)		22.32 (3.71)	
TRD	148.54 (1.35)		147.92 (0.32)		148.59 (0.21)	
	1L-8ACIM					
TED	121.31 (0.16)		125.39 (1.06)		129.40 (1.68)	
RA	27.98 (2.77)		21.19 (1.49)		19.70 (0.62)	
TRD	149.29 (2.93)		146.58 (0.43)		149.11 (1.06)	
	2L/-2ACIM					
TED	118.32 (1.26)		125.50 (0.05)		129.37 (2.99)	
RA	29.38 (4.48)		21.84 (1.90)		20.24 (1.74)	
TRD	147.70 (1.22)		147.34 (1.85)		149.61(1.25)	
	2L-4ACIM					
TED	119.48 (0.28)		126.19 (2.38)		130.06 (1.12)	
RA	27.74 (0.53)		21.16 (0.35)		21.16 (0.35)	
TRD	147.22 (0.81)		147.35 (2.73)		151.22 (1.47)	
	2L-8ACIM					
TED	122.81 (0.79)		128.26 (1.11)		131.83 (1.95)	
RA	24.75 (1.39)		20.42 (1.19)		20.42 (1.19)	
TRD	147.56 (0.61)		148.68 (0.08)		152.25 (0.76)	
	3L-2ACIM					
TED	123.98 (0.44)		126.83 (1.47)		131.29 (0.64)	
RA	24.05 (1.01)		19.31 (0.77)		18.20 (0.80)	
TRD	148.03 (0.57)		146.14 (0.69)		149.49 (0.17)	
	3L-4ACIM					
TED	124.61 (2.65)		128.73 (1.36)		132.30 (1.78)	
RA	23.53 (2.00)		19.49 (0.55)		16.51 (1.56)	
TRD	148.14 (0.65)		148.22 (1.91)		148.82 (0.22)	
	3L-8ACIM					
TED	127.30 (3.35)		129.47 (0.31)		134.83 (1.58)	
RA	20.43 (1.42)		17.93 (0.20)		14.58 (2.44)	
TRD	147.73 (4.77)		147.40 (0.51)		149.41 (0.86)	

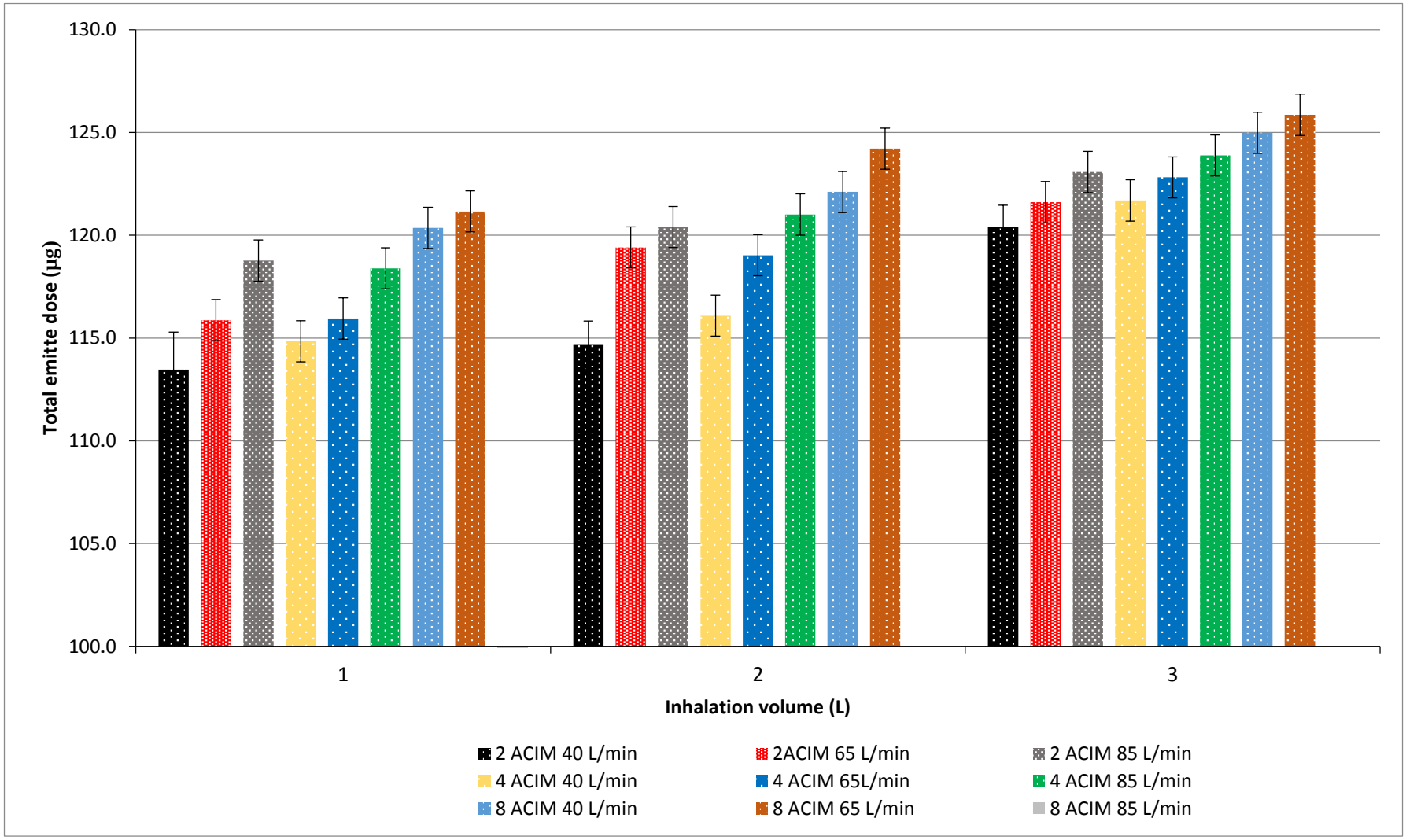

Figure 7.8: Mean (SD) of indacaterol TED for all inhalation profiles at different MIF, Vin and ACIM (n=3).

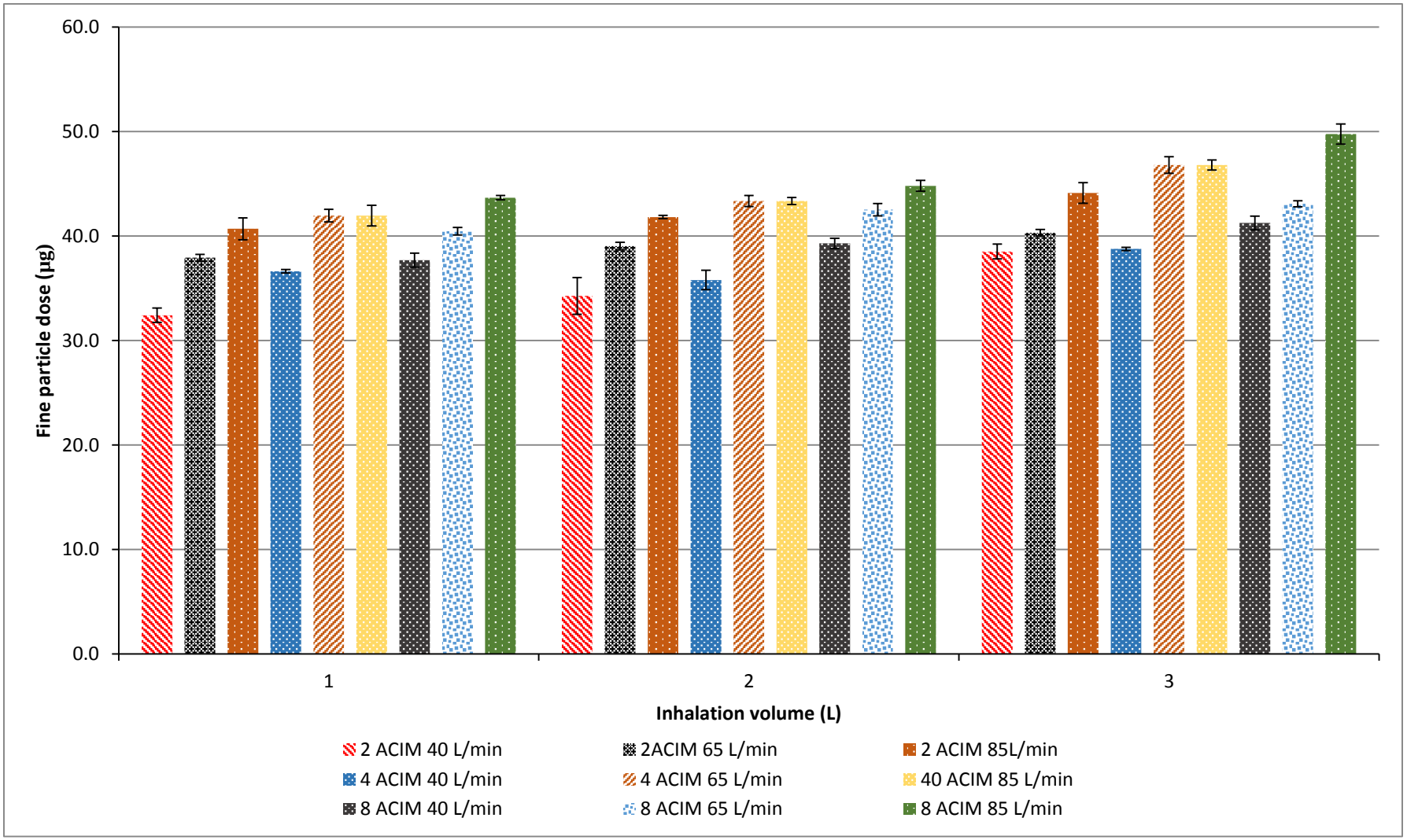

Figure 7.9: Mean (SD) of indacaterol FPD for all inhalation profiles at different MIF, Vin and ACIM (n=3).

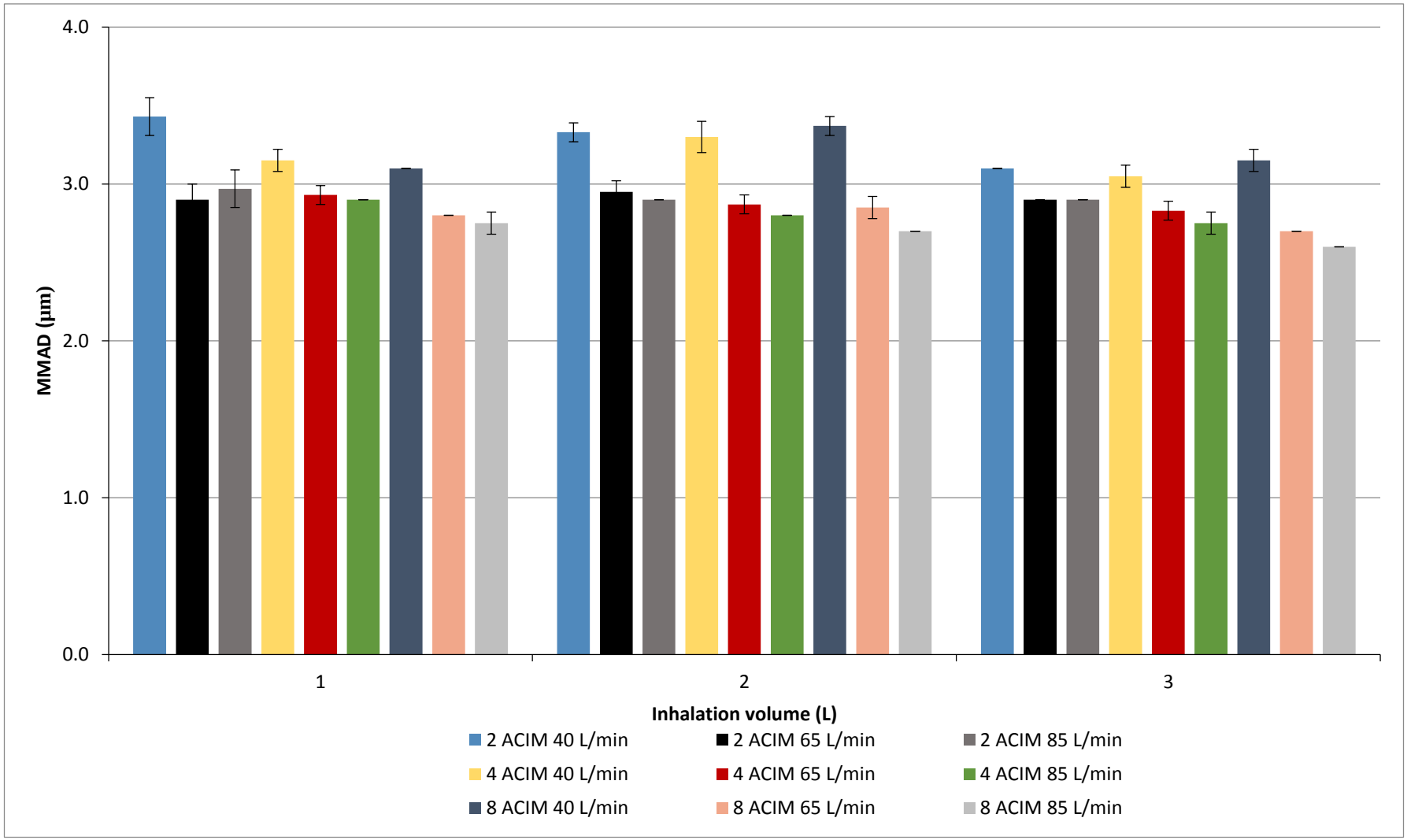

Figure 7.10: Mean (SD) of indacaterol MMAD for all inhalation profiles at different MIF, Vin and ACIM (n=3).

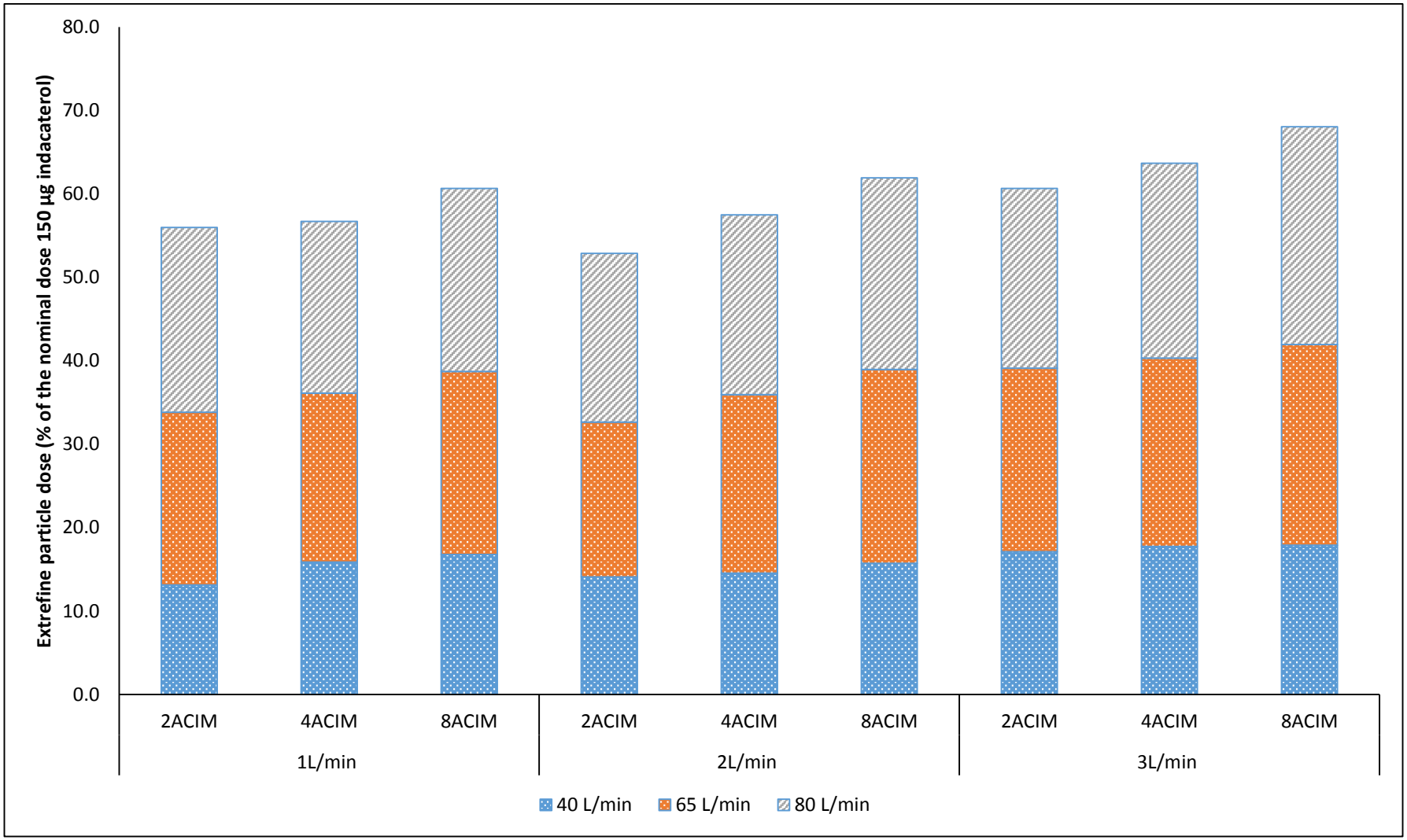

Figure 7.11: EFPD for all inhalation profiles used at different MIF, Vin and ACIM (n=3).

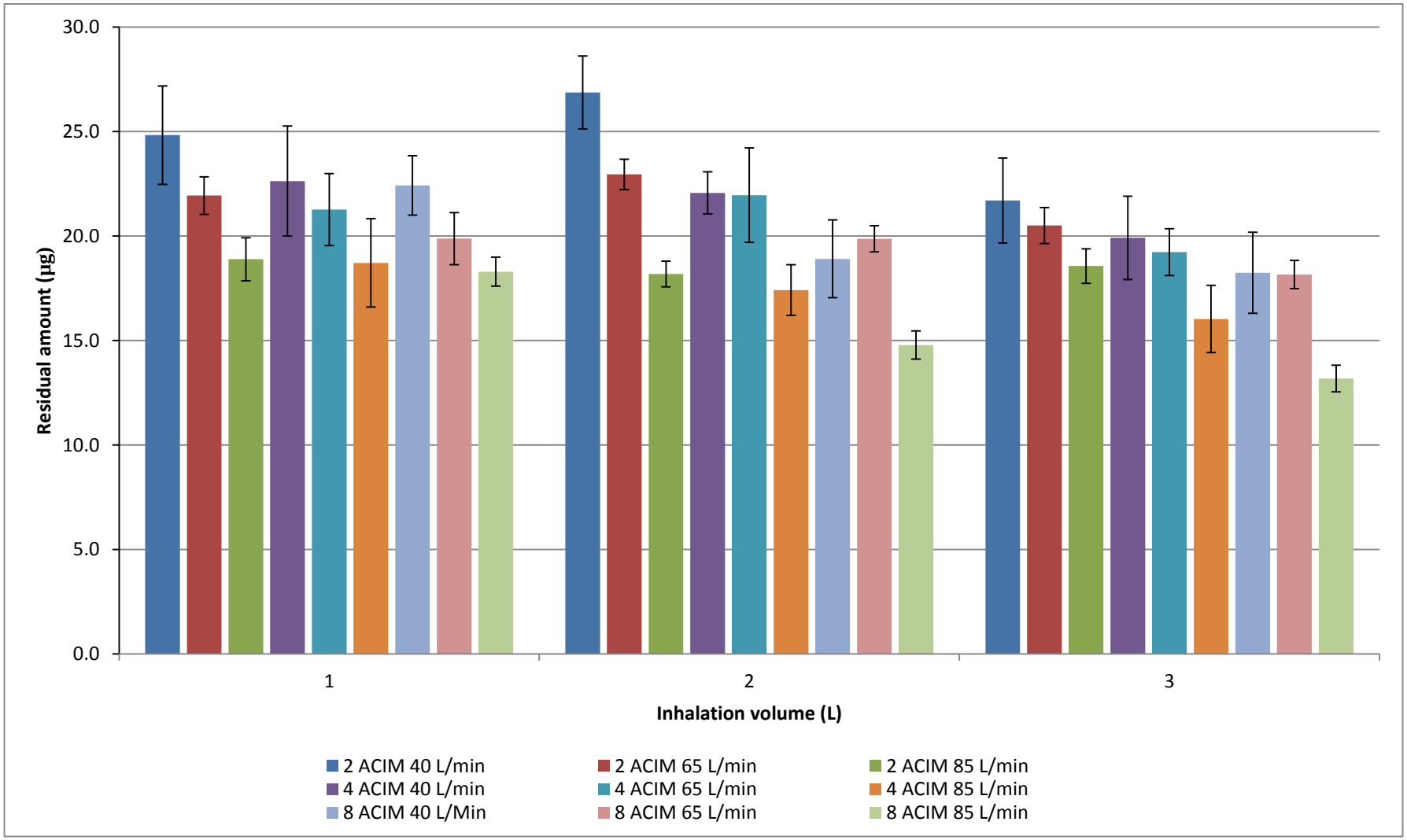

Figure 7.12: Mean (SD) of indacaterol RA for all inhalation profiles at different MIF, Vin and ACIM (n=3).

The profiles were classified into three different categories depending on the MIF value of the profiles (40, 65 and 85 L/min). For each group the profiles were simulated to changes one parameter at a time, therefore ACIM and Vin effect on the aerodynamic dose emission characteristics of indacaterol can be studied [see appendix for simulated IPs figures].

The results show the both FPD and TED increased significantly ($p < 0.05$) with the increase of the Vin and ACIM at each category of the MIF values low, medium and high (Tables 7.6 7.7 and 7.8). The FPD and TED were increased from 29.4µg to 49.9µg and 113.5µg to 127.5µg respectively. The MMAD decreased significantly ($p < 0.05$) with increasing the MIF from 40 L/min to 85 L/min; furthermore, ACIM and Vin have shown an impact on the MMAD especially when combined with high flow rate (Figure 7.10). The EFPD results showed no significant change at low and medium MIF when ACIM and Vin increased, However at high MIF the increase of Vin and ACIM show a significant ($p <0.05$) on the EFPD of indacaterol dose emitted from Breezhaler (Figure 7.11). The residual amount (RA) left in the device and capsule showed a significant decrease ($p <0.05$) with increasing the Vin, especially at low and medium MIF profiles, at high profiles small change was observed. The ACIM also shows a slight effect on the RA (7.12).

7.6 Discussion

The performance of 150µg indacaterol Breezhaler® was determined using real life recorded patients' IPs (7.2.2). Furthermore, the effect of each inspiratory parameter was assessed using simulated patients' IPs.

The *ex-vivo* methodology used is describing the process of generating dose emission data for inhaler devices by replaying *in-vivo* recorded patients' IPs using analytical technique coupled with breath simulator as shown in Figure 7.1. This technique is widely used to determine the APSD as well as the dose delivered uniformity providing an approximation to the dose patient would receive when using the inhaler device (Nadarassan et al., 2010, Olsson et al., 2013, Chrystyn et al., 2015). The method set-up used in this study was similar to that reported by (Bagherisadeghi et al., 2017).The benefits of using ACI over NGI when studying the aerodynamic characteristics of inhaler devices are that the ACI stages and pre-separator can be changed when different inhalation flow used (28.3, 60 and 90 L/min). While with NGI changes in the applied flow rate is accompanied with an adjustment to stages cut-off diameter. Therefore, using ACI enables direct comparison between the amounts of inhaled drug particles deposited on each stage (Copley, 2015). The internal dead volume of ACI (1.155 L) is smaller when compared to NGI (2.025 L), the use of ACI enables the determination of low volume profiles (Mohammed et al., 2012). For these reasons, our study was conducted using ACI rather than NGI to investigate the performance of Onbrez Breezhaler® using *in-vivo* recorded COPD patients' IPs. The recorded patients' IPs (Table 7.2) show that most of the patient with COPD are not able to generate a pressure drop corresponding 4kPa and inhaled volume of 4 L when recommended by the pharmacopoeia (USP, 2014) when they have inhaled through Breezhaler®. The Breezhaler® is a low resistance (0.017 $kPa^{0.5}$ min/L) and an inhalation effort exceeding 100 L/min is required to generate the 4 kPa inside the inhalers (Explained in Chapter 2, section 2.6.4.1).

DPI formulation de-aggregation, dispersion and emission are dependent on the inhalation manoeuvre characteristics MIF, Vin and ACIM (Chrystyn et al., 2015, Bagherisadeghi et al., 2017). The inspiratory effort generated by the patient during the inhalation manoeuvre creates a pressure drop inside the device that releases the dose from the DPI. The latter pressure drops is directly related to the inspiratory effort generated by the patient (Clark and Hollingworth, 1993). DPIs are breath actuated devices and it has been reported that MIF has an effect on the dose emission as well as lung deposition of all DPIs, especially those with low resistance devices such as Aerolizer® and Breezhaler® where dose emission is mainly dependent on the generated MIF (Nielsen et al., 1997, Chew and Chan, 2001, Weuthen et al., 2002, Meyer et al., 2004, Alaboud et al., 2010, Colthorpe et al., 2013).

7.6.1 COPD patients' IPs

The inspiratory parameters of inhalation manoeuvre MIF, Vin and ACIM have shown an effect on the dose emission as well as aerodynamic particle size distribution of indacaterol Breezhaler®. However, the impact of the MIF was more observable when compared to the effect of ACIM and Vin, due to that all the parameters act together and it was difficult to differentiate the effect of ACIM and Vin. The results show that increasing the MIF result in a significant ($p < 0.05$) increase in TED (92.0 µg to 125.1 µg) and FPD (28.7 µg to 45.6 µg) of indacaterol 150 µg dose (Figures 7.3 and 7.4). In contrast, the MMAD and TRA decreased significantly ($p < 0.05$) with increasing of MIF (Figures 7.5 and 7.6). Suggesting that increasing the MIF of the profiles lead to a subsequent increase in the turbulent energy inside the device, which therefore results in more dose being delivered from Breezhaler®. The results again demonstrate the flow rate dependency of Onbrez Breezhaler® as shown in previous chapters (4 and 6). Furthermore, the particle size distribution of the inhaled bolus is obvious from MMAD of all profiles ranging from 3.7 µm to 2.3 µm (Figure 7.6).

Drug retention inside the inhaler continues to be a factor plaguing the performance of novel inhalers (Tajber et al., 2009). Drug retention varies between inhaler devices in that some studies have reported between 30 and 50% of the nominal dose is retained within the device. It is important that the complete dose is released from the inhaler so as to maximise the therapeutic effect, minimising drug wastage and avoiding potential dosage errors during the next inhalation (Abadelah et al., 2017). Irrespective of the MIF for all profiles used there was always some indacaterol retained in the capsule/ device (Table 7.4). However, the TRA is reduced with the MIF from 45% to 11% of the total nominal dose for profile 1 and 16 respectively. The length of device inhalation channel, as well as coating material, affect the residual amount of drug retained in the capsule and device. For instance, Easyhaler has short inhalation channel and reported to have less residual amount comparing to a device with longer inhalation channel such as Aerolizer® (Coates et al., 2004, Azouz and Chrystyn, 2012).

The dose emission uniformity of all profiles chosen in this study was determined using DUSA instead of ACI in the experimental – setup (Figure 7.1). The recovered dose from the DUSA representing the TED, while dose retained in the capsule/device represent TRA. A good correlation was observed between both ACI and DUSA results. The increase of MIF leads to an increase in TED_{DUSA} and a reduction in total residual amount TRA_{DUSA} (Tables 7.4 and 7.5). The TED_{DUSA} was higher than TED using ACI and the reasons for that difference are higher dead volume and inter-stage particle loss of ACI (Byron et al., 2004, Kamiya et al., 2009). Therefore, the washing procedure for DUSA is simple comparing to that for ACI and less chance of losing drug during the washing procedure. The consistency of washing procedure can be seen from the TRD for ACI, which was almost 92 % of the nominal dose (Figure 7.7), and for DUSA was almost 99% (Table 7.5).

7.6.2 Simulated patients' IPs

The effect of each inspiratory parameter MIF, Vin and ACIM was determined using simulated patients' IPs where only one parameter was changed at a time. when we varied the MIF we maintained both the ACIM and Vin fixed using 3 levels, *i.e.*, Vin (1 L, 2 L and 3L), ACIM (2 L/s^2, 4 L/s^2, 8 L/s^2) (Tables 7.6, 7.7 and 7.8). For all the three levels, increasing the MIF from 40 L/min to 85 L/min resulted in a significant ($p < 0.05$) increase in both TED and FPD of indacaterol from Onbrez Breezhaler (Figure 7.8 and 7.9). At a fixed high Vin (3 L) and high ACIM (8 L/s^2), the impact of the flow rate on the TED and FPD was the greatest as reported in ERS/ISAM task force report (Laube et al., 2011). The increase of the MIF was associated with both an increase in both TED and FPD, which is in agreement with (Laube et al., 2011, Chrystyn et al., 2015). Particles with an MMAD < 5 µm are considered as the respirable dose and are suitable for targeting the peripheral airways (Heyder, 2004, Roche et al., 2013). Irrespective of the MIF, the Onbrez Breezhaler was able to generate particles of clinical relevance with an MMAD <5µm. Moreover, at medium (65 L/min) and high (85 L/min) MIFs, an increase in the extrafine particles with an MMAD <3 µm were obtained (Figure 7.10). The latter is considered important in targeting smaller airways, and they found to be useful in the management of respiratory diseases (De Boer et al., 2015, Scichilone, 2015).

The Vin is another inhalation parameter to be considered when evaluating the performance of DPIs. The pharmacopeial DPIs testing *in-vitro* suggested that a Vin of 4 L should be drawn through the inhaler. However, most of the patients with asthma and COPD were unable to achieve this value, and only a few patients were able to exceed 2 L Vin with a mean value ≥ 2 L (Broeders et al., 2003, Azouz et al., 2015b). The inhaled volumes used to quantify the aerodynamic particle size distribution of inhaled indacaterol exceeds the internal volume of the ACI (1.155 L) (Copley et al., 2005) to allow sufficient sampling time for the aerosol

bolus transfer from the inhaler device to distal stages of the ACI (Mohammed et al., 2012, Mohammed et al., 2014). In the previous section (7.2.2), the patient using Breezhaler® achieved a Vin ranging from 0.7 L to 3.3 L.

The present chapter shows that TED increased significantly ($p < 0.05$) with increasing the Vin especially when low MIF and ACIM were used (Figure 7.8). For instance, at a fixed MIF of (40 L/min) and ACIM (2 L/s^2) the increase of the Vin from 1 L to 3 L resulted in an increase in the TED from 113.5 (2.8) µg to 120.4 (1.8) µg (Table 7.6) suggesting that when the MIF is not sufficient to de-aggregate the dose the Vin can play an important role to promote the de-aggregation of the powder formulation. (Kamin et al., 2002) reported the effect of the Vin on the total mass output and FPD which is in agreement with the present chapter. At low MIF (40 L/min) and ACIM (2 L/s^2) increasing the Vin from 1 L to 3 L subsequently increase FPD of indacaterol from 29.4 (0.7) µg at 1L Vin to 36.5 (0.7) µg at 3L Vin (Table 7.6) suggesting that more aerosol was pulled to distal part of the impactor. At medium and high MIF values 65 and 85 L/min, increasing the Vin resulted in a less pronounced effect on the TED and FPD of indacaterol Breezhaler® (Figures 7.8 and 7.9). The β-receptors are abundant in the alveolar wall a region where no smooth muscle exists and whose functional configuration is unknown. The location of β-receptors in the lungs suggests that drug targeting is important for maximum therapeutics effect for example salbutamol require a more peripheral delivery to the medium and small airways to produce a therapeutic effect (Labiris and Dolovich, 2003). Thus particle size affects the lung deposition of an aerosol and influence the clinical effectiveness Zanen et al., (1996) reported that "in patients with mild to moderate asthma, particles with an MMAD of approximately 3 µm were more effective than larger particle size aerosol in producing bronchodilation since it has the best penetration and retention in the lungs in the presence of airways narrowing" (Zanen et al., 1996). Breezhaler® was effective in producing particles with an MMAD of around 3 µm, and the Vin has less effect on MMAD than MIF

suggesting that Vin is involved more in mass transfer rather than aerosol dispersion to cause a further decrease in the MMAD as shown from the present results (Figure 7.10).

The ACIM has been reported to be very important in dose emission of the inhaled aerosol (Everard et al., 1997, Chrystyn, 2007, Chrystyn et al., 2015, Bagherisadeghi et al., 2017). Patients may achieve the same peak flow but with different accelerations. The one inhaling fast through DPIs would benefit more from the inhaled dose because the latter may leave the device before even the peak flow is reached as shown by the schematic diagram (Figure 7.13) reported by Chrystyn and Price (2009).

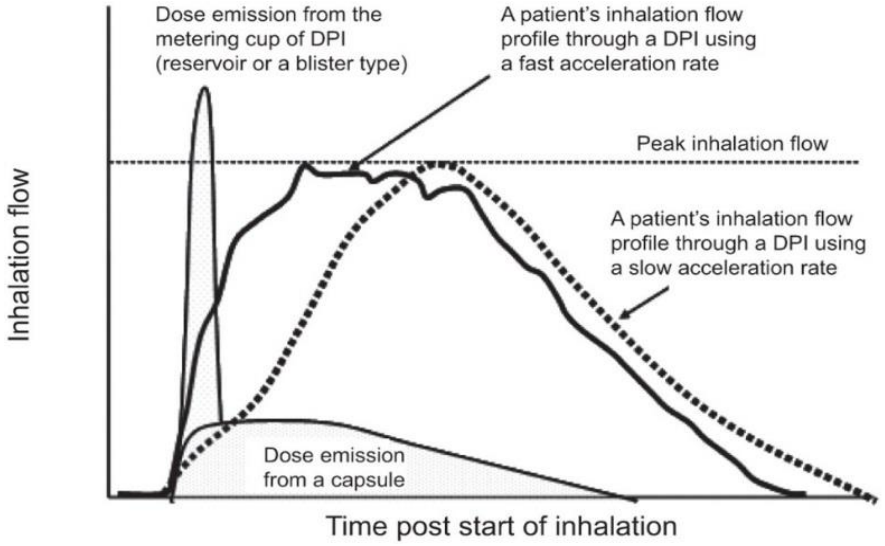

Figure 7.13: The inhalation profiles for different types of DPIs.

ACIM was found to significantly ($p < 0.05$) affect the TED and FPD (Figure 7.8 and 7.9), whereas only a marginal decrease in the MMAD was observed mainly at high MIF (Figure 7.10). The increase of ACIM form 2 L/s^2 to 8 L/s^2 at fixed MIF (40 L/min) and Vin (1 L) resulted in an increase in the TED from 113.5 (2.8) µg to 120.4 (1.1) µg and FPD from 29.4 (0.7) µg to 34.7 (0.7) µg respectively (Table 7.7).

The ACIM had the almost similar effect to the Vin with regards to TED and FPD. Thus, the greater the ACIM, the greater is the TED and the greater the mass transfer to the lower stages

of the impactor. Achieving a high peak flow faster would not only promote high emission of the dose, but also increases the amount of the drug reaching the lower stages of the impactor with more particles in the extrafine range with high tendency for peripheral deposition as shown from the values of the MMAD at high flow rate (85 L/min) and ACIM (8 L/s^2). This study showed that approximately 50% of the emitted dose deposited in the upper parts of the impactor (Alberta throat, mixing inlet and the pre-separator) as a large particle mass (LPM) irrespective of the inhalation manoeuvre.

The RA amount of ACI study of Indacaterol left in the capsule and device was affected by all the inhalation parameters (Tables 7.6, 7.7 and 7.8). Increasing the MIF whilst keeping both the Vin and ACIM fixed, resulted in a significant decrease in the RA. For example, increasing the MIF from 40 L/min to 85 L/min at Vin (1 L) and ACIM (2 L/s^2) resulted in a significant decrease ($p < 0.05$) in the RA from 24.8 (2.4) µg to 18.9 (3.0) µg (Tables 7.6 and 7.8). At a fixed MIF (65 L/min) and ACIM (4 L/s2) and increasing the Vin from 1 to 3 L caused a significant decrease in the RA from 21.3 (1.7) µg to 16.2 (1.1) µg (Table 7.7). At a fixed MIF (40 L/min) and Vin (3 L) but increasing the ACIM from 2 to 8 L/s^2, resulted in a significant decrease in the RA from 21.7 (1.0) µg to 17.2 (0.9) µg (Table 7.6 and Figure 7.12). The residual amount was reduced by 50 % by combining all the inhalation manoeuvre parameters to their optimal values (*i.e.*, increasing the MIF from 40 L/min to 85 L/min; ACIM from 2 L/s^2 to 8 L/s^2; Vin from 1 L to 3 L). It is interesting to note that complete emptying of the capsule and device is still a challenge irrespective of the inhalation manoeuvre. The amount left in the device and capsule could be due to intrinsic and / or extrinsic factors such as the material composing the coat (intrinsic) of the inhaler device and moisture (extrinsic). The EFPD (% nominal dose) increased with increasing the ACIM and Vin, however, the major parameter affecting the EFPD was found to be the MIF. The combined effect of MIF, Vin

and ACIM result in an increase in the EFPD from 13.1% at low level to 26.2% at high level (Figure 7.11).

The dose emission of all profiles using DUSA attached to Breath simulator was determined. For all Vins and ACIMs, increasing the MIF from 40 L/min to 85 L/min resulted in an increase in the TED. For example, at a fixed Vin (1 L) and ACIM (2 L/s^2), increasing the MIF from 40 L/min to 85 L/min resulted in a significant increase in the dose emission from 116.80 (1.25) µg to 125.41 (0.73) µg (Table 7.9) suggesting dose emission flow rate dependency of the Onbrez Breezhaler as discussed in the previous chapters (4 and 6). Similarly, for all MIFs and ACIMs, increasing the Vin resulted in a significant ($p < 0.05$) increase in the TED. The impact of the Vin was not extensively studied, but this study shows the dose emission Vin dependency. For example, at a fixed MIF (40 L/min) and (ACIM 2 L/s^2), increasing the Vin from 1 L to 3 L, the TED increased from 116.80 (1.25) µg to 123.98 (0.44) µg (Table 7.9). It is interesting to note that varying the MIF or the Vin have almost the same impact on the TED suggesting the importance of the Vin in the dose emission from capsule based devices as we reported earlier in chapter 8. Equally, ACIM also showed an impact on the TED but with less effect than the MIF and Vin (Table 7.9). The dose emission study using DUSA follows the same trend to the results obtained with the ACI. However, more dose was recovered from the DUSA (Table 7.9) in comparison to the ACI for the reasons explained in the previous section (7.6.1). The TRD approached the nominal dose of 150 µg irrespective of the MIF, Vin or ACIM and all the TRD values fall in the range of 85%-125% of drug content uniformity for DPIs as described in the Pharmacopeia (CDER, 1998).

7.7 Conclusion

In present *ex-vivo* aerodynamic characteristics of indacaterol Breezhaler®, dose emission was investigated using both recorded real life and simulated COPD patients' IPs. The use of *ex-vivo* methodology provides more realistic representation to the way patient use the inhaler and dose they would receive in real life use. Indacaterol Breezhaler® have demonstrated a flow dependent dose emission, which is in line with previous chapter (4 and 6) finding using real life, generated COPD IPs.

The use of simulated IPs enables us to assess the effect of each parameters (MIF, Vin and ACIM) in the inhalation manoeuvres. All inhalation parameters have shown an impact on the dose emission. However, the impact of the MIF was more pronounced than the Vin and ACIM by affecting all the aerodynamic dose emission characteristics (*i.e.,* TED, FPD, and MMAD). By combining high MIF, Vin and ACIM, the quality of the inhaled particles was improved. The profiles dose delivery uniformity using DUSA have demonstrated a consistent dose delivery of indacatrerol from Onbrez Breezhaler®

Chapter 8. General Discussion and Conclusion

8.1 General discussion

In-vitro studies were introduced to evaluate product performance directly with a reduced cost and offer benefits in terms of ethical consideration when compared to *in-vivo* studies (Meyer et al., 2004). The dose and its particle size distribution emitted from a DPI is determined by the patient, the inhaler device and formulation (Abadelah et al., 2017). Most DPIs are breath actuated and are highly dependent on the patient's inhalation flow. The threshold inhalation flow required to de-aggregate and disperse the drug powder is not determined for each DPI, and more effort is needed to shade the light on the minimum flow required to operate each DPI more efficiently to benefit the patients (Scichilone, 2015, Haidl et al., 2016). The inhalation manoeuvre is affected by a wide variety of factors namely MIF, Vin and ACIM. All of which have shown an impact on the dose emission and aerodynamic characteristics of emitted dose form a DPI (Bagherisadeghi et al., 2017). The pharmacopeial DPIs testing *in-vitro* suggested that a Vin of 4 L should be drawn through the inhaler. However, most of the patients with asthma and COPD were unable to achieve this value, and only a few patients were able to exceed 2 L Vin with a mean value ≥ 2 L (Broeders et al., 2003, Azouz et al., 2015b). The inhaled volumes used to quantify the aerodynamic particle size distribution of inhaled indacaterol exceeds the internal volume of the ACI (1.155 L) (Copley et al., 2005) to allow sufficient sampling time for the aerosol bolus transfer from the inhaler device to distal stages of the ACI (Mohammed et al., 2012, Mohammed et al., 2014). The compendial method of testing the inhalers using the vacuum pump clearly does not reflect the capabilities of a typical patient. The applied inhalation profile is a square wave taking the flow rate from 0 L/min to a pre-determined constant value (X L/min) for short duration of time and then back to 0 L/min. This profile is by no mean achievable by healthy subject never mind an asthmatic patient (Copley, 2015).

The dose emission results of indacaterol and formoterol (Chapter -4) showed that the impact or Vin on the dose emptying become less significant if the patient achieve high MIF. Suggesting that proper dose de-aggregation taken place when high MIF was achieved due to the increase in the turbulence force generated inside the device, which in turn depend on the intrinsic resistance of the DPI. The dose emission is flow dependent on the MIF generated by the patients however, patients who are unable to achieve minimum flow of 60 L/min when using Foradil Aerolizer® or Onbrez Breezhaler® should be encouraged to inhale twice for each single dose. The present chapter showed that MIF impact is enhanced with increasing the Vin. In case of high resistance device such as Easyhaler® used in the present work (Chapter -5) low volume of 750 mL was found to be efficient to empty the dose, the reason for that is the small metering chamber in the design of the Easyhaler®. The results showed that increasing the Vin above 750 mL does not bring much improvement in the dose emission characteristics of formoterol from an Easyhaler®. It is a high resistance device, therefore, the force generated at MIF of 30 L/min is corresponding to the same turbulent force when 60 L/min is generated through low resistance devices such as Aerolizer® and Breezhaler®, because the turbulence energy generated upon inhalation is a function of both device resistance (R) and MIF (Q) (Clark and Hollingworth, 1993). The increase in MIF from 30 L/min to 60 L/min and finally 90 L/min was accompanied with an increase in the dose emission characteristics of formoterol Easyhaler®, suggesting that more force was generated inside the device when the MIF was increased. However, that increase was less pronounced when MIF value of 60 L/min or above was achieved. The dose emission of indacaterol Breezhaler® was found to be not affected by difference in the dose strength 150µg and 300µg. It has been seen from scanning electron micrographs that 150 µg indacaterol formulation has a higher concentration of fine carrier particles in comparison to 300 µg indacaterol formulation. Despite the difference observed in the carrier particle size, size distribution

(SEM) and drug to carrier ratio yet both formulations produced a good drug content uniformity (% CV < 1.8%) and a % recovery of 100%. A similar dose emission pattern to that seen in Chapter 4 was observed for both dose strengths. The increase in the MIF resulted in a significant increase ($p < 0.05$) in the ED1 and TED, while reverse trend was seen with ED2 and TRA, as discussed before the resulted turbulent force was increased when the MIF increased and therefore, an adequate dose de-aggregation has occurred inside the device. The Vin also shown an important effect in dose emptying especially when low MIF 28.3 L/min and 60 L/min were achieved, suggesting that at low MIF dose de-aggregation was incomplete and consequently second inhalation was needed to successfully empty the dose. Breath simulator-based methods (BRS) have been recently introduced to study the aerodynamic characteristics of the inhaled products using previously recorded patient inhalation profile. They enable replay of patient-generated or standardised inhalation profiles thereby replacing the pharmacopeial method using the vacuum pump.This *ex-vivo* methodology, using patients' IPs instead of the vacuum pump, provided a more realistic assessment of the aerodynamic properties of the inhaled medication that a patient would likely inhale compared with corresponding data obtained using compendial methodology. The inspiratory parameters namely MIF, Vin and ACIM of the COPD patients' IP act together, therefore, the use of simulated IPs where one parameter was changed at time was important to assess the effect of each parameter on its own. The use of both COPD recorded and simulated profiles showed that indacaterol dose emission from Onbrez Breezhaler® was mainly affected by the MIF, but when the high MIF was not achieved then, ACIM and Vin showed an important role to maximise dose emission. In other words, MIF can be considered as the main factor, but ACIM and Vin are also important to maximise both dose emptying and the aerodynamic characteristics of indacaterol Breezhaler®. In conclusion, the results of the dose emission showed that a minimum MIF and Vin is required for an adequate dose de-aggregation from

formoterol Aerolizer® (60 L/min with 1L), Easyhaler® (30 L/min with 0.75 L) and the indacaterol Breezhaler® (60 L/min with 1L). Patients with asthma and COPD are not able to generate the pharmacopeial recommendations inhalation parameters for DPIs *in-vitro* testing, *i.e.*, Vin of 4 L and MIF corresponding to 4kPa pressure drop. Therefore, when testing DPIs *in-vitro* a range of MIF and Vin of clinical relevance to the inhalation parameters achieved the inhaler device should be used. The square wave profile generated by the vacuum pump is not representative to the typical inhalation profiles, the profile generated by the pump exacerbating the ACIM where no patient or even health volunteers were able to achieve maximum MIF at the start of the inhalation manoeuvre. Consequently, recently introduced ex-vivo methodology is recommended to the performance of the DPIs and provides a more realistic data on the dose patient would inhale in real life when using the inhaler device. The *ex-vivo* methodology provides a realistic representation of the patient's inhalation manoeuvre parameters MIF, Vin and ACIM. The ex-vivo methodology has been used to study the dose emission aerodynamic characteristics of the indacaterol Breezhaler® using both COPD patients' IPs and simulated patients' IPs. The effect of MIF was more pronounced on the aerodynamic dose emission characteristics of indacaterol Breezhaler® than Vin and ACIM using *ex-vivo* methodology with both unaltered and simulated COPD patients' IPs. The minimum inspiratory conditions to operate DPIs should be considered taking into account the maximum MIF, Vin and ACIM patients could generate in real life.

8.2 Summary and future work

In the present work, the effect of the inspiratory parameters MIF, Vin and ACIM on the dose emission and the aerodynamic characteristics of the dose emitted from three different DPIs Foradil Aerolizer®, Easyhaler® and Onbrez Breezhaler® was determined using both *in-vitro* and *in-vivo* techniques.

The pharmacopoeia methods for the *in-vitro* dose emission testing of DPIs involve using a vacuum pump to generate an inhalation with a constant MIF which corresponds to a 4 kPa pressure drop with an inhaled volume of either 2 L (FDA, 1998) or 4 L (EP, 2013, USP, 2014). Several studies have shown that most patients with asthma and COPD are unable to produce a pressure drop of 4 kPa when using DPIs neither they can generate a Vin of 4 L (Nielsen et al., 1997, Pavkov et al., 2010, Azouz et al., 2015b).The airflow square waved profile generated by the vacuum pump cannot be replicated by a human and is not representative of the bell-shaped patient IPs in real life use (Copley et al., 2014).

Recently, researchers have developed an *ex-vivo* methodology using patients' IPs rather than the compendial vacuum pump square wave profiles.The *ex-vivo* methodology set-up was adapted by connecting an inertial impactor (*i.e.*, ACI or NGI) with BRS using the mixing inlet side arm. The BRS was used in the procedure to replay the *in-vivo* recorded IPs through the inhaler device. The use of the mixing inlet allows a constant airflow to be drawn through the cascade impactor. This is achieved by balancing the air drawn through the impactor with a supplementary flow into the mixing inlet such that the airflow through the inhaler *in-situ* at the induction port is maintained at zero before dose aerosolisation. Therefore, the dose emission from the inhaler device occurs when a programmed IP is withdrawn from the supplementary flow and replayed at the mouthpiece of the inhaler using BRS.

In the present work, instead of using the pharmacopoeia recommended MIF which corresponding to a 4 kPa pressure drop with a Vin of 4L. The dose emission and

aerodynamic characteristics were determined using a wide range of MIFs and Vins to mimic the inspiratory parameters that the patient had achieved when they inhaled through the inhaler during the routine use. Furthermore, an *ex-vivo* methodology similar to that reported by (Bagherisadeghi et al., 2017) was used to determine the effect of the IP characteristics MIF, Vin and ACIM on the aerodynamic dose emission characteristics of the indacaterol Breezhaler® using previously recorded COPD patients' IPs (Azouz et al., 2015b).

Chapter 2 provides a review on respiratory system physiological and pathological features, in addition to a review of the previous studies carried out using different inspiratory conditions and techniques. The development and validation of two HPLC methods for the quantification of indacaterol and formoterol was explained in details in chapter 3 with the material and laboratory instrumentation used to study the *in-vitro* and *ex-vivo* dose emission of indacaterol and formoterol from three marketed inhaler products Easyhaler®, Onbrez Breezhaler® and Foradil Aerolizer®.

The dose emission performance of indacaterol Breezhaler® and formoterol Aeroliser® was determined using wide range of MIF (28.3 L/min to 120 L/min) and Vin (0.5 L to 2 L) after two separate inhalations in Chapter 4. Equally data form both devices showed that ED1 significantly ($p < 0.05$) increased with increasing the MIF for all Vin used, while ED2 and TRA showed a reverse trend. The impact of the Vin on the dose emission of was more pronounced at low MIFs 28.3 L/min and 60 L/min, suggesting that dose de-aggregation was incomplete at low MIF 28.3 L/min and 60 L/min and in this case, a high volume was required to empty the capsule. While at high MIF 90 L/min and 120 L/min, most of the dose was emitted after one inhalation. The results of this chapter suggest that a minimum MIF of 60 L/min and Vin of 1 L would be required to efficiently operate both devices.

The effect of the MIF and Vin on the dose emission and aerodynamic dose emission characteristics of the formoterol Easyhaler® using a wide range of MIF and Vin was

determined in Chapter 5. The results showed that TED, FPD and % FPF significantly increased ($p < 0.05$) with the increasing the MIF from 28.3 L/min to 90 L/min. In contrast, MMAD significantly decreased ($p < 0.05$) with increasing MIF, suggesting increase in the turbulent energy with increasing the MIF and therefore, more efficient dose de-aggregation and dose dispersion. The increase in the Vin above 750 mL does not bring much difference in the aerodynamic characteristics of formoterol from Easyhaler®. The *in-vitro* ACI and DUSA results suggest that although the Easyhaler® demonstrated some flow dependent dose emission, it was effective at low inhalation MIF and Vin and thereby can be used by patients with different degree of airway's obstruction.

The effect of the dose strength on the dose emission of indacaterol Breezhaler® 150 µg and 300 µg in-*vitro* using different MIFs and Vins was studied in Chapter 6. The results showed that despite the difference in particle size of lactose carrier both indacaterol formulations 150 µg and 300 µg provided an excellent drug content uniformity with a % CV < 1.8. The results indacaterol dose emission from the capsule based Onbrez Breezhaler® for both dose strengths (Tables 6.1 and 6.2) for DUSA and (Tables 6.3 and 6.4) for ACI demonstrated inhalation flow dependent dose emission as discussed in the previous chapter, the inhaled volume (Vin) was important when low MIFs 28.3 and 60 L/min were used. The aerodynamic characteristics of indacaterol showed that Breezhaler® was effective in producing respirable particles with an MMAD < 5 µm over the range of the MIFs used in the study. However, the mass fraction of particles with an aerodynamic diameter < 3 µm is more pronounced between 60 and 90 L/min. The difference in the drug particle size distribution as a function of the flow rate and volume was noticed, and it is more likely to influence drug distribution in the airways. It is clear from the results that a minimum MIF of 60L/min and a Vin of 1L were required when using the indacaterol Breezhaler®. Finally, the results confirm the recommendation of using

two separate inhalations for each dose when a high MIF through an Onbrez Breezhaler® is not achieved.

The aerodynamic dose emission characteristics of indacaterol Breezhaler using real life recorded patients' IPs and simulated IPs have been investigated (Chapter – 7). The results showed flow rate dependent dose emission of indacaterol Breezhaler®, the aerodynamic parameters TED, FPD increased and MMAD decreased significantly ($P < 0.05$) with increasing the profile's MIF. The inspiratory parameters in the IPs acting together and the extent of each parameter effect was not determined using real life recorded COPD IPs. Therefore, simulated IPs were used to assess the effect of each inspiratory parameters on the dose emission of 150µg indacaterol Breezhaler®. The results showed that all inhalation parameters have shown an impact on the dose emission. However, the impact of the MIF was more pronounced than the Vin and ACIM by affecting all the aerodynamic dose emission characteristics (*i.e.*, TED, FPD, and MMAD). By combining high MIF, Vin and ACIM, the quality of the inhaled particles was improved. The use of *ex-vivo* methodology provides more realistic representation to the way patient use their inhaler and the dose they would receive in real life.

To sum up, depending on the intrinsic resistance of the inhaler devices, a minimum MIF and Vin was required to operate the device and aerosolise the dose to the patient's lungs. The present work highlighted the minimum MIF and Vin to operate Easyhaler® (30 L/min – 750 mL) while higher MIF and Vin were founded for low resistance Aerolizer® and Breezhaler® (60 L/min – 1L). Furthermore, patients using DPIs should inhale forcefully from the start of the inhalation manoeuvre for as long as they can, however, patients who cannot meet these conditions should be encouraged to inhale twice to ensure dose emptying for each single dose. The use of simulated inhalation profiles along with real life recorded COPD patients' IP enable better understanding and evaluation to the extent of each inspiratory parameter MIF,

Vin and ACIM on the overall dose emission aerodynamic characteristics of 150µg indacaterol Breezhaler® when *ex-vivo* methodology was used.

The *in-vitro* and *ex-vivo* dose emission of indacaterol and formoterol from three marketed DPIs have been investigated in the present work. The *in-vitro* studies highlight the importance of using a wide range of MIF and Vin to study the dose emission using DUSA, while COPD patients' IPs were used to study the performance of indacaterol Breezhaler®. To study the effect of the inspiratory parameters individually simulated profiles were used (*i.e.*, changing one parameter at a time). The present work can be extended in future to cover all the possibilities and conditions for the use of DPIs as follows:

- The range of MIFs and Vins for DUSA and ACI work could be extended.
- The threshold MIF and Vin required for dose de-aggregation is missing in the PIL and it is worth to determine these values for all marketed DPIs.
- The use of both children and adult asthmatic and COPD patients' IPs with different throat geometries (*i.e.*, USP throat, Alberta throats for both adult and children)
- Dose emission using DUSA for all the profiles after one and two inhalations.
- Comparison of the results with the pharmacopeial conditions of MIF (corresponding to 4kPa, with a Vin of 4 L) for in-vitro testing of DPIs.
- The use of a wide range of ACIM and Vin IPs depending on the inhaler device.
- The *ex-vivo* methodology used in this thesis can be extended to other DPIs with different intrinsic resistances and engineered formulations.
- Investigate if there is any possible correlation between the in-vitro, ex-vivo and *in-vivo* data.

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Appendix

Appendix (A)

The robustness data of indacaterol and formoterol HPLC method

The two methods were found to be robust, as slight but deliberate changes in chromatographic conditions, parameters have shown no significance change in the method performance. The acceptance criteria for tailing factor ($tf \leq 2.0$) and for resolution Rs >1.5 (ICH, 2005)

Table 1. Robustness results of indacaterol HPLC method

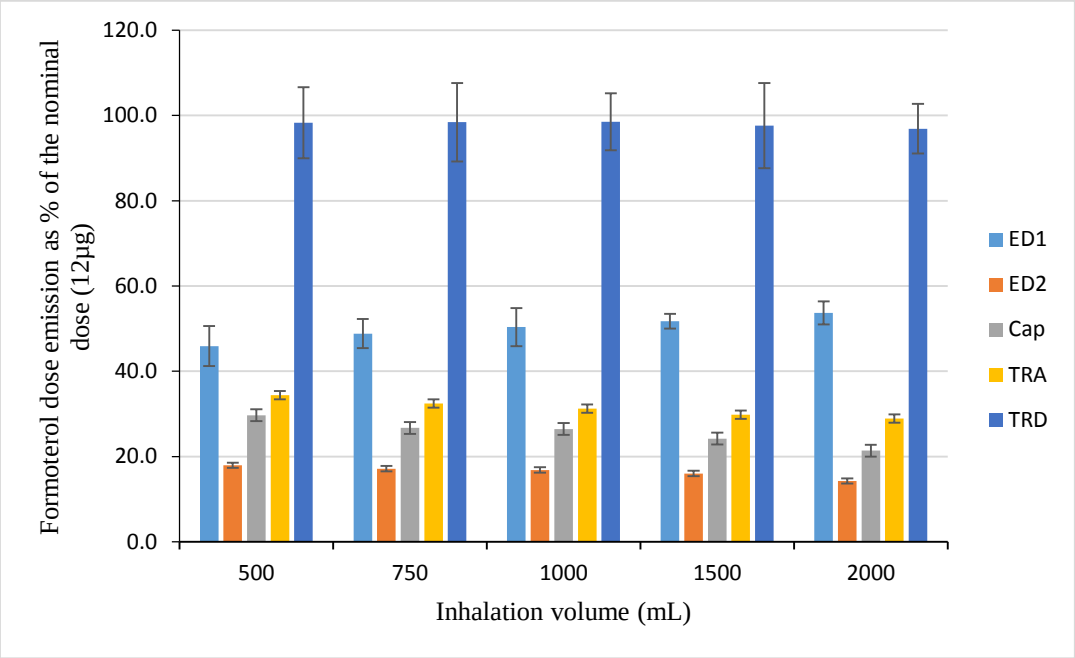
a). Indacaterol method			
Parameter	Variation	Resolution	Tailing factor
Flow rate (mL/min	0.8	5.41	1.5
	1.2	5.97	1.4
Buffer pH	2.3	5.13	1.4
	2.7	6.89	1.6
Column oven Temperature(^{o}C)	35	5.33	1.5
	45	5.84	1.4
Methanol (%)	58%	6.92	1.4
	62%	5.38	1.4

Table 2. Robustness results of formoterol HPLC method

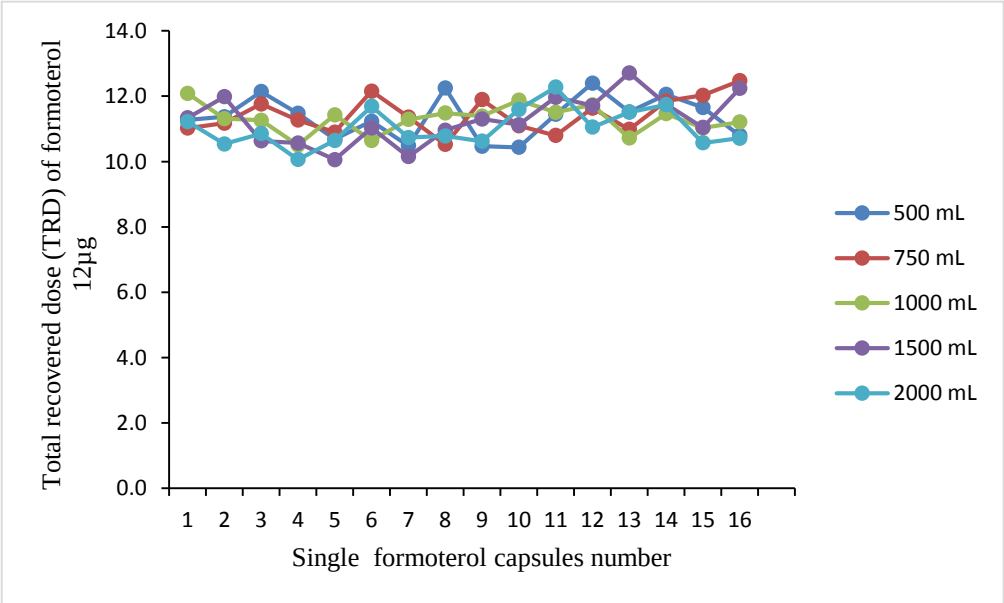
b). Formoterol method			
Parameter	Variation	Resolution	Tailing factor
Flow rate (mL/min	0.5	8.86	1.8
	0.9	6.94	1.7
Buffer pH	2.8	7.01	1.7
	3.2	6.41	1.7
Column oven Temperature (^{o}C)	25	6.56	1.7
	35	5.84	1.6
Acetonitrile (%)	58%	5.12	1.7
	62%	4.55	1.7

Appendix (B)

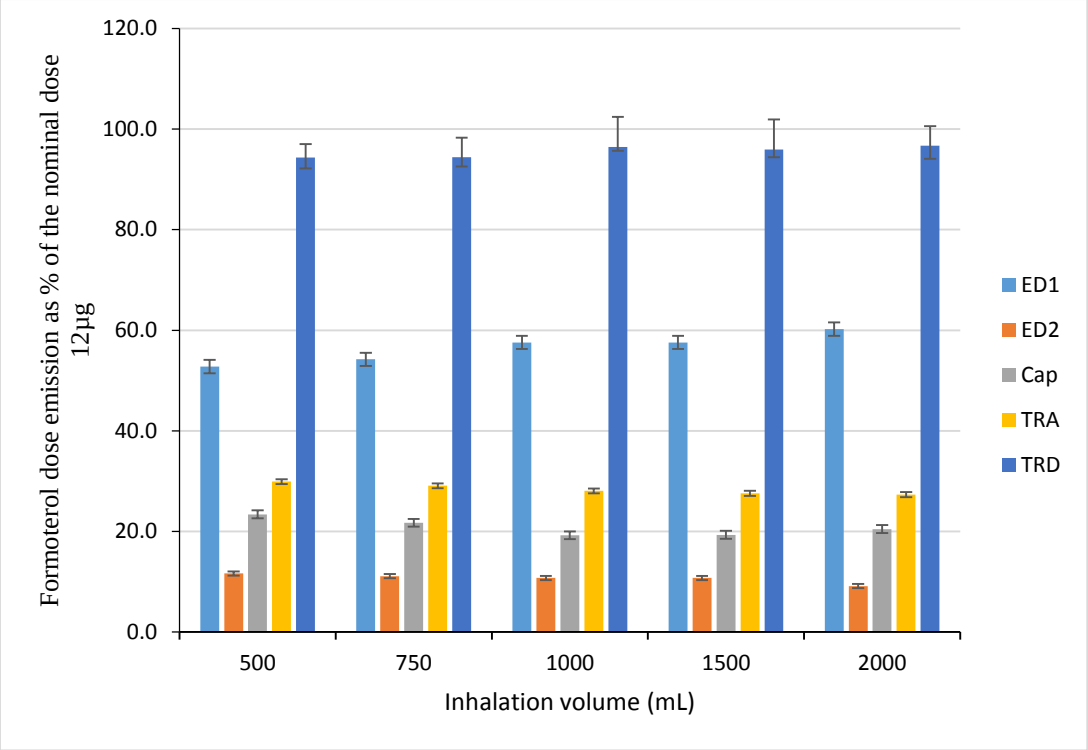
- **Results of formoterol dose emission using two separate inhalations at different MIFs and Vins [n=16]**



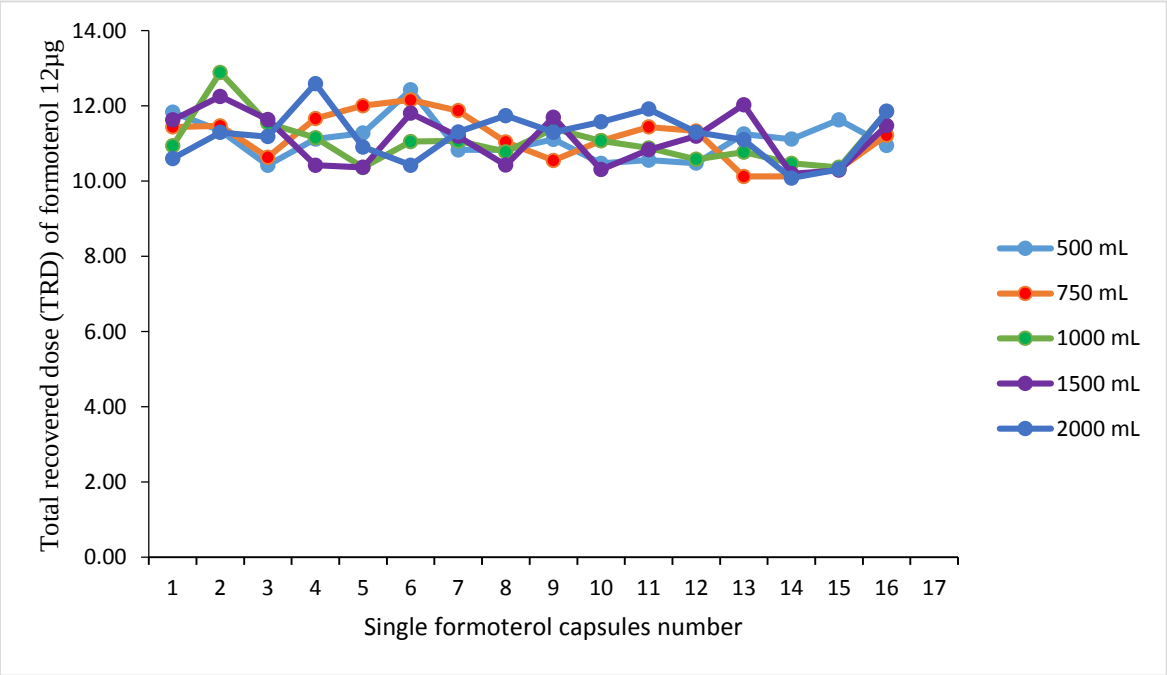
Mean (SD) of the emitted dose after first (ED1) second(ED2) inhalations, Capsule, Total residual amount and total recovered dose from Foradil Aerolizer® using different Vins at MIF of 28.3 L/min. [Results presented as % of the nominal dose].



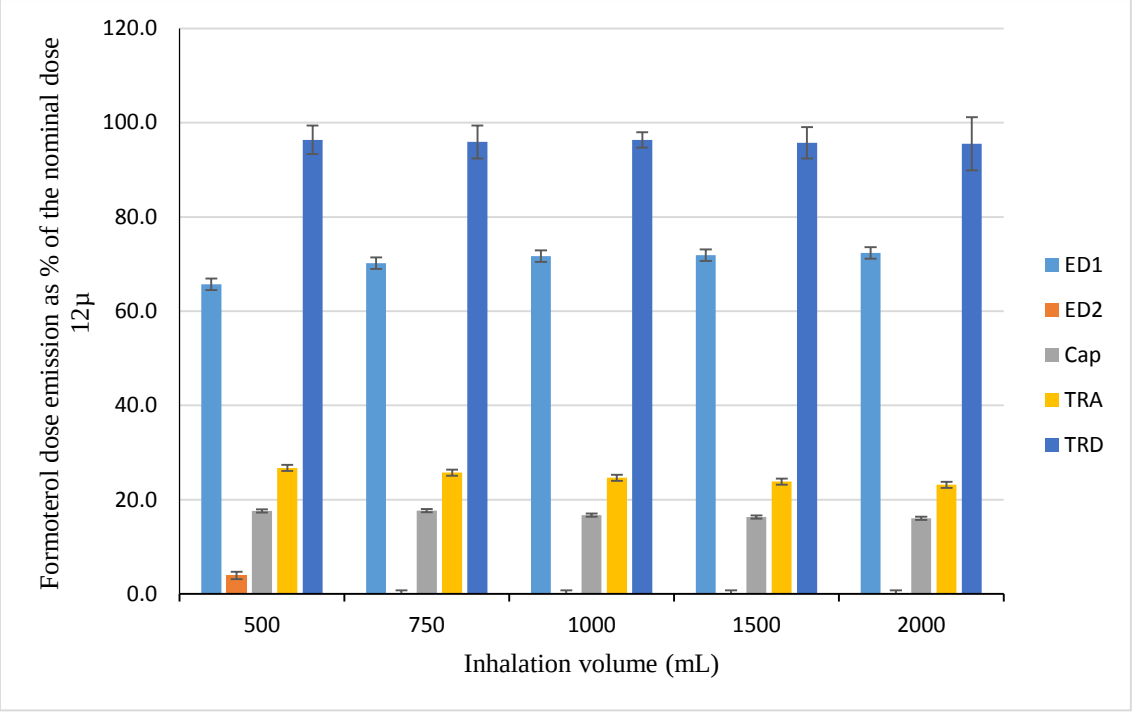
Total recovered dose (µg) of Formoterol emitted from Foradil Aerolizer for 16 separate capsules at different Vins and MIF of 28.3 L/min.



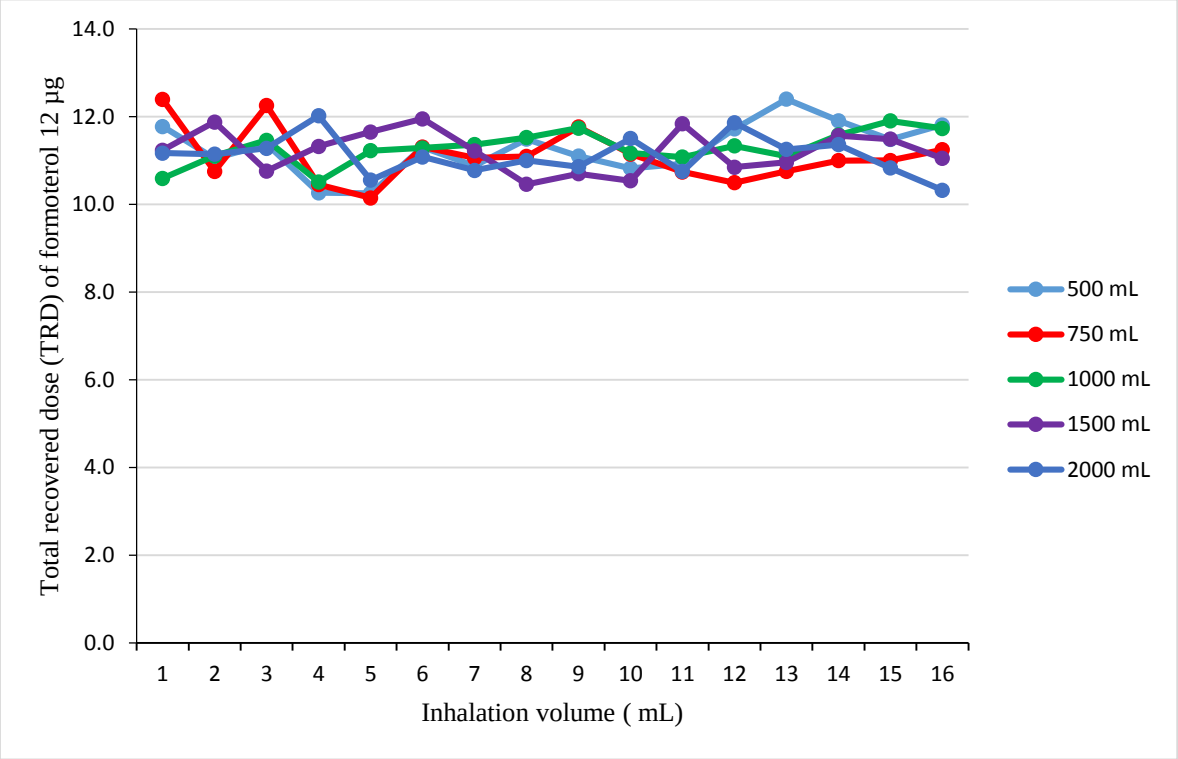
Mean (SD) of the emitted dose after first (ED1) second(ED2) inhalations, Capsule, Total residual amount and total recovered dose from Foradil Aerolizer® using different Vins at MIF of 60 L/min. [Results presented as % of the nominal dose].



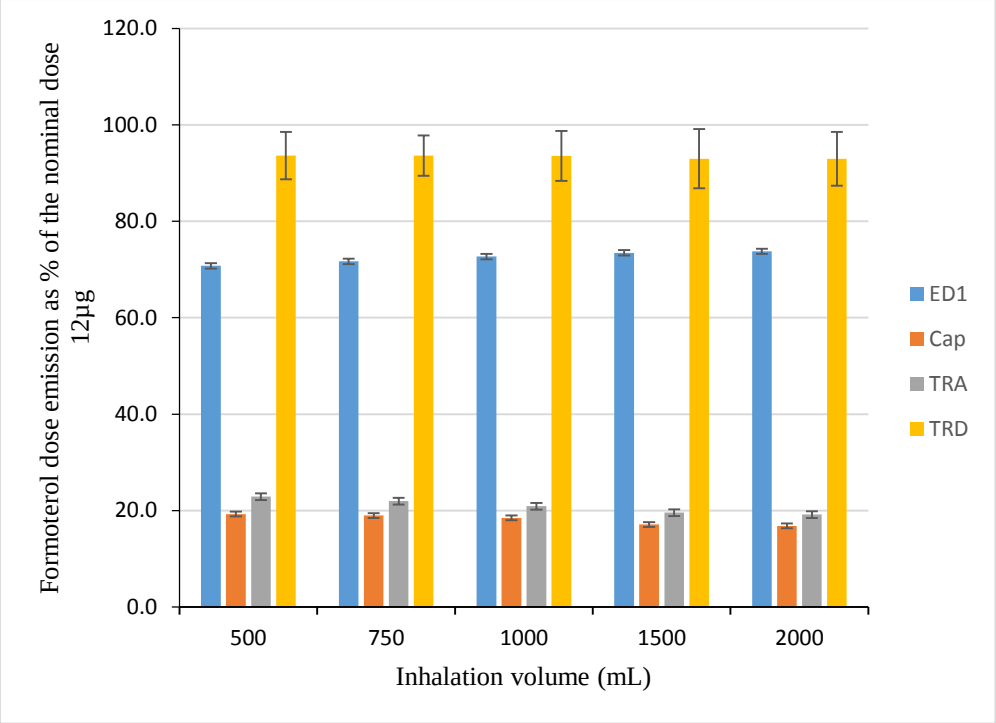
Total recovered dose (µg) of Formoterol emitted from Foradil Aerolizer for 16 separate capsules at different Vins and MIF of 60 L/min.



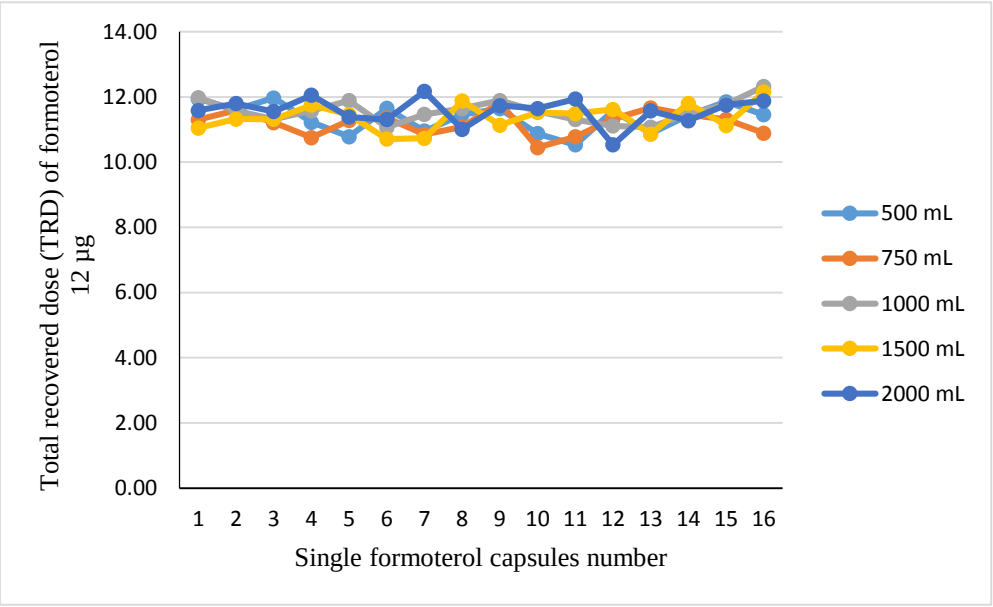
Mean (SD) of the emitted dose after first (ED1), second (ED2) inhalation, Capsule, Total residual amount and total recovered dose from Foradil Aerolizer® using Vins at MIF of 90 L/min. [Results presented as % of the nominal dose].



Total recovered dose (µg) of Formoterol emitted from Foradil Aerolizer for 16 separate capsules at different Vins and MIF of 90 L/min.

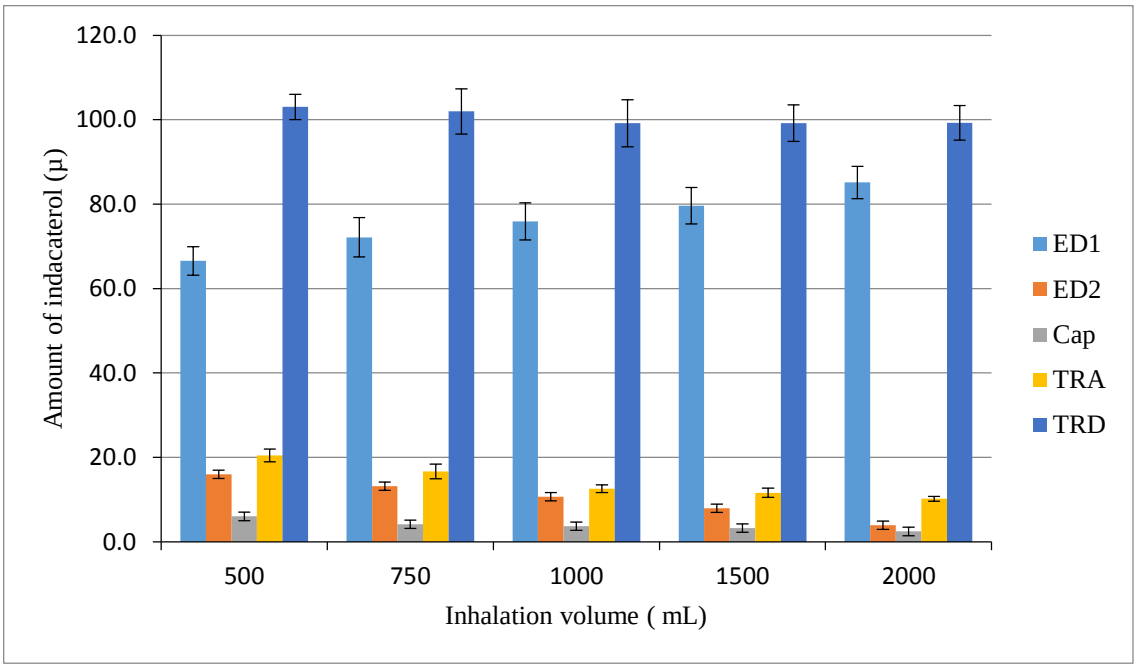


Mean (SD) of the emitted dose after first (ED1) second(ED2) inhalations, Capsule, Total residual amount and total recovered dose from Foradil Aerolizer® using Vins at MIF of 120 L/min. [Results presented as % of the nominal dose].

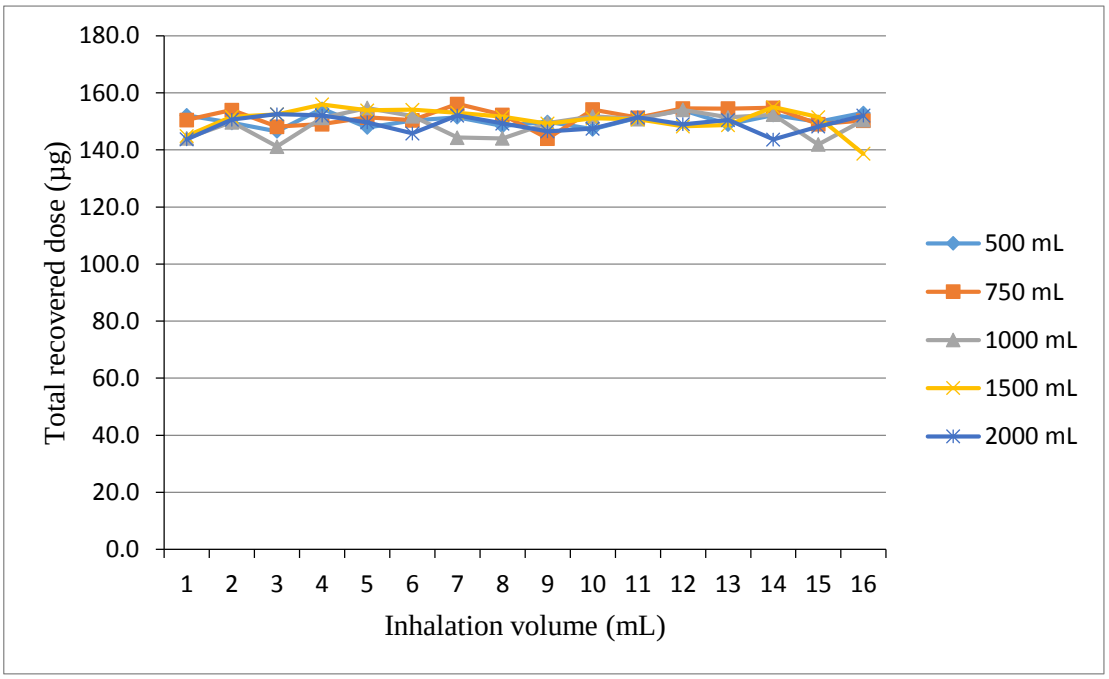


Total recovered dose (µg) of Formoterol emitted from Foradil Aerolizer® for 16 separate capsules at Vins and MIF of 120 L/min

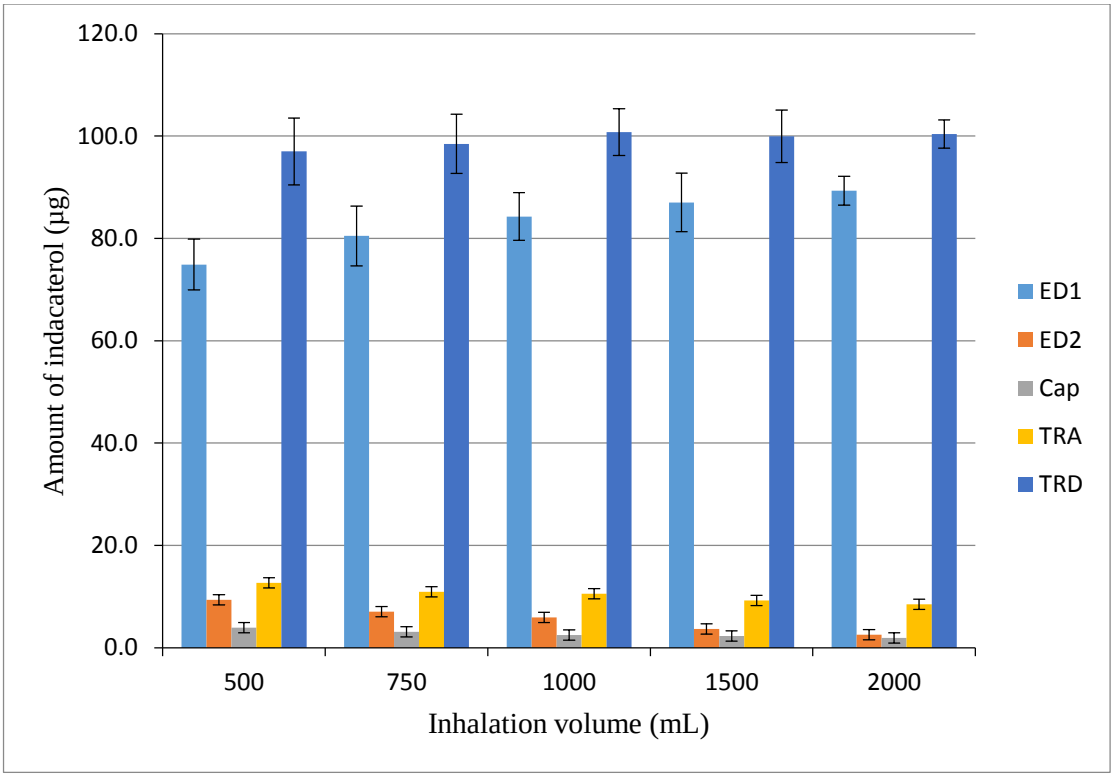
- **Results of indacaterol dose emission using two separate inhalations at different MIFs and Vins. [n=16]**



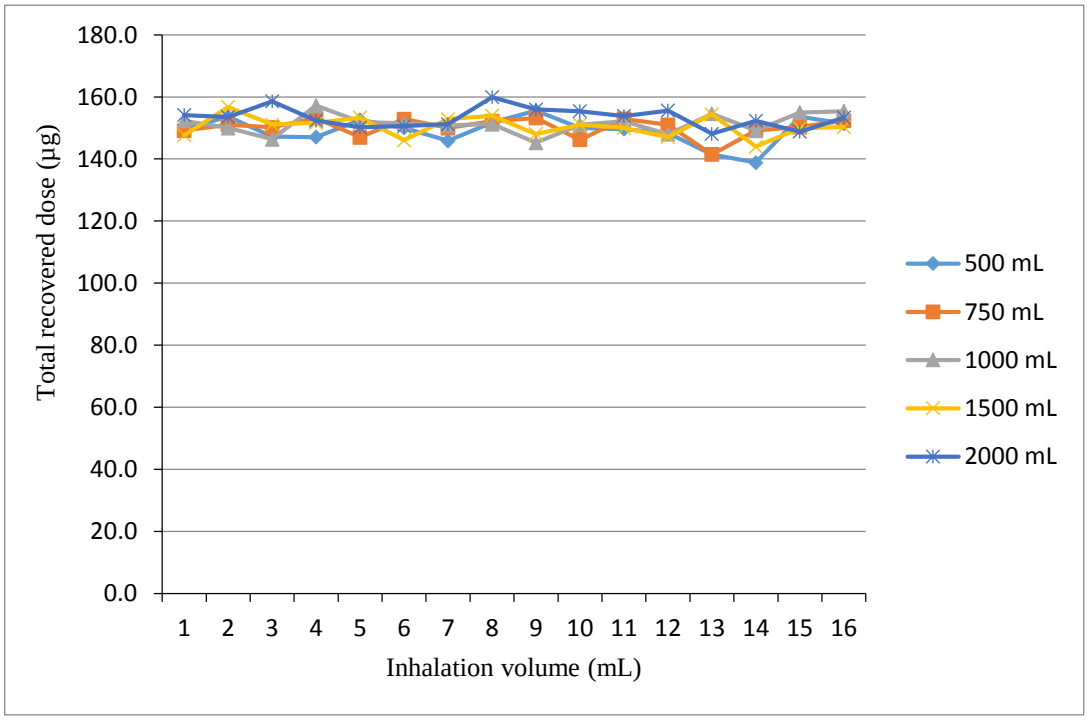
Mean (SD) of the emitted dose after first (ED1) second(ED2) inhalations, Capsule, Total residual amount and total recovered dose from Onbrez Breezhaler® using Vins at MIF of 28.3 L/min. [Results presented as % of the nominal dose].



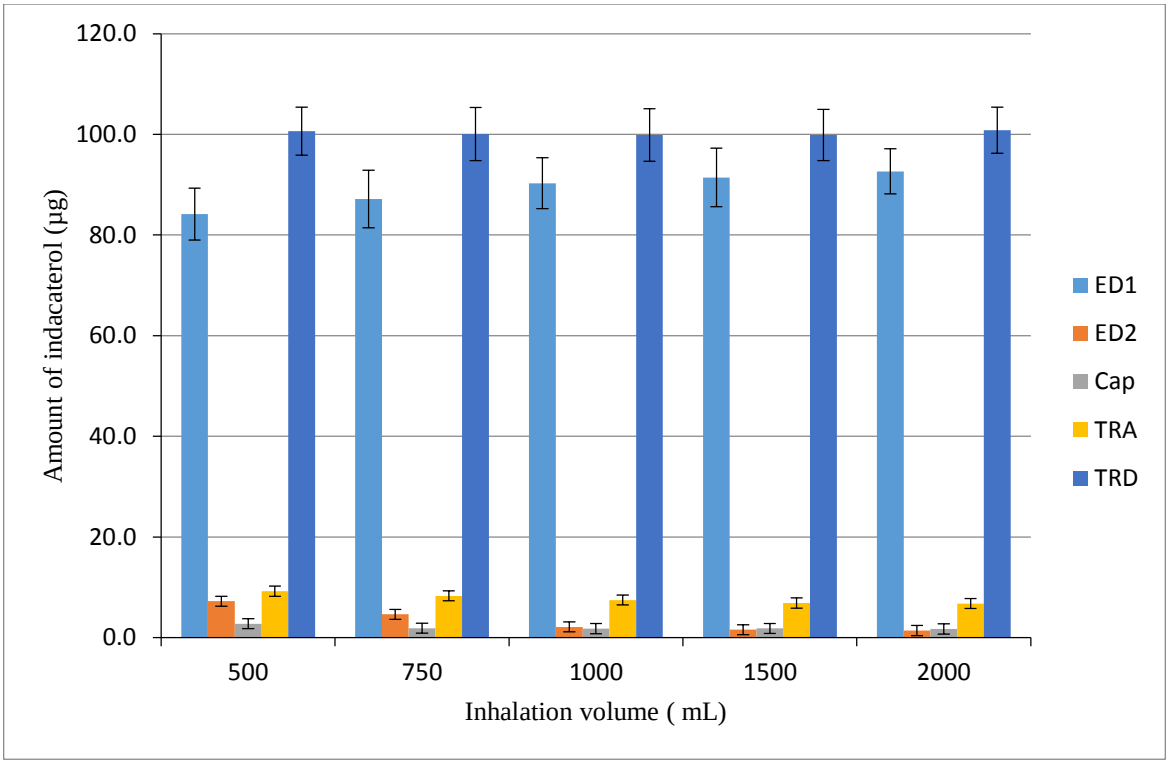
Total recovered dose (µg) of indacaterol emitted from Onbrez Breezhaler® for 16 separate capsules at Vins and MIF of 28.3 L/min.



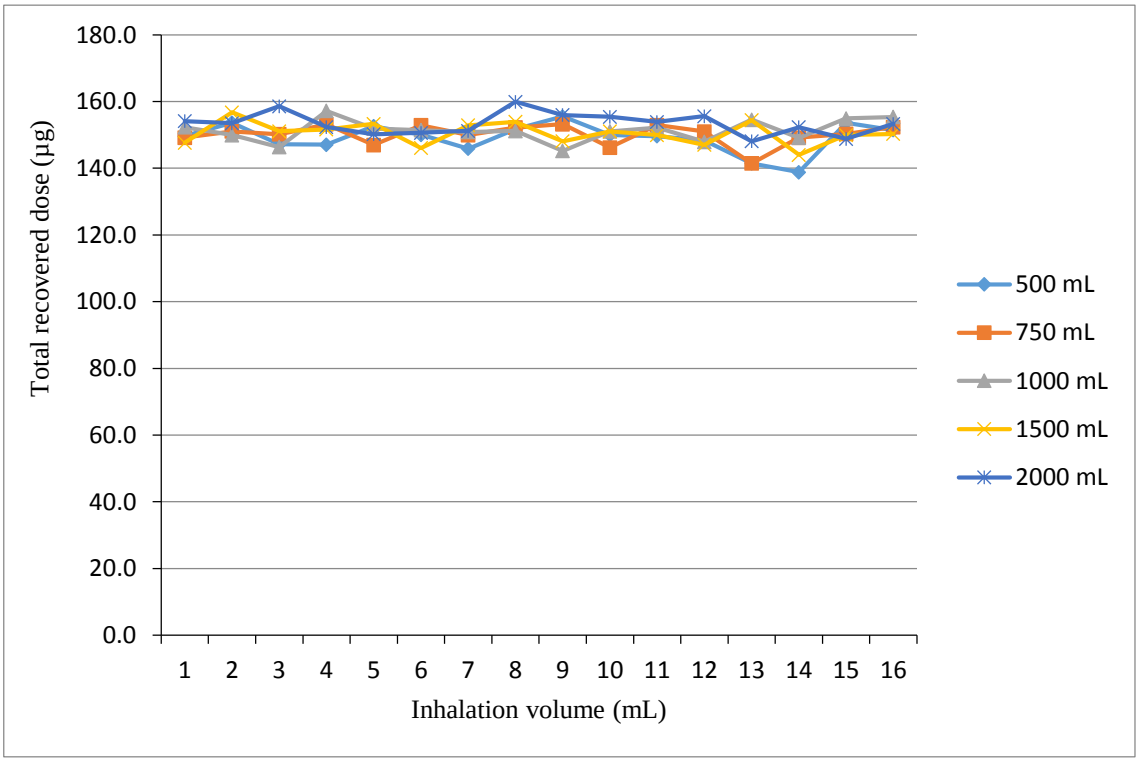
Mean (SD) of the emitted dose after first (ED1) second(ED2) inhalations, Capsule, Total residual amount and total recovered dose from Onbrez Breezhaler® using Vins at MIF of 60 L/min. [Results presented as % of the nominal dose].



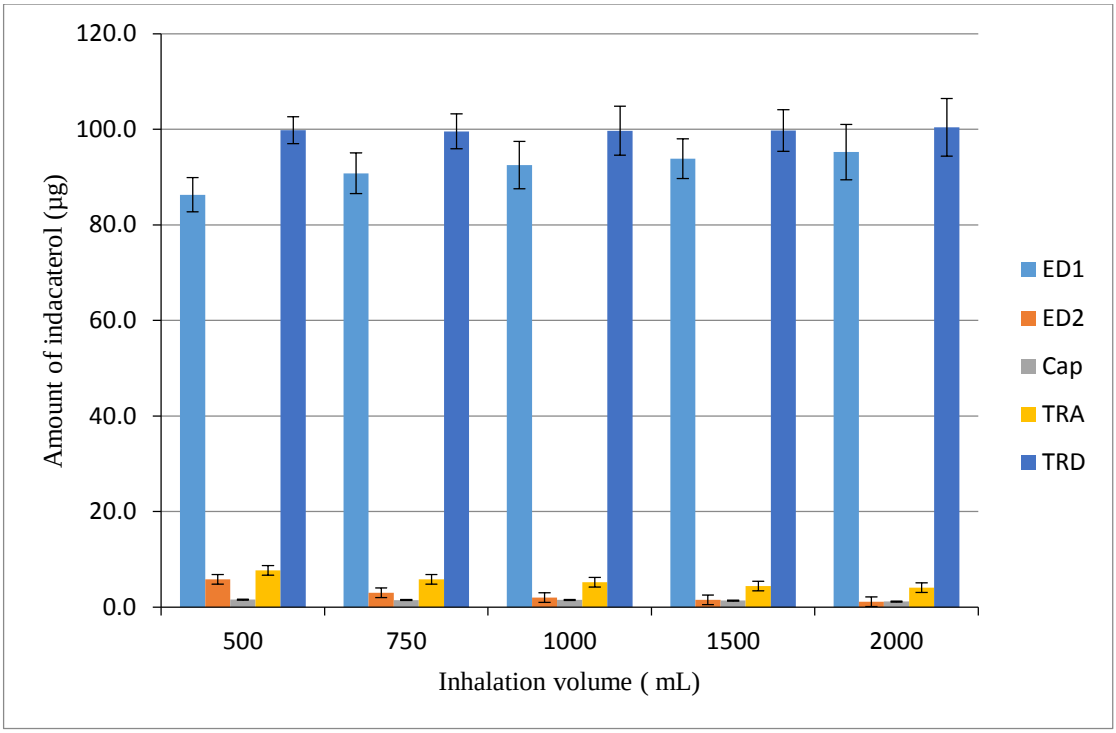
Total recovered dose (µg) of indacaterol emitted from Onbrez Breezhaler® for 16 separate capsules at different Vins and MIF of 60 L/min.



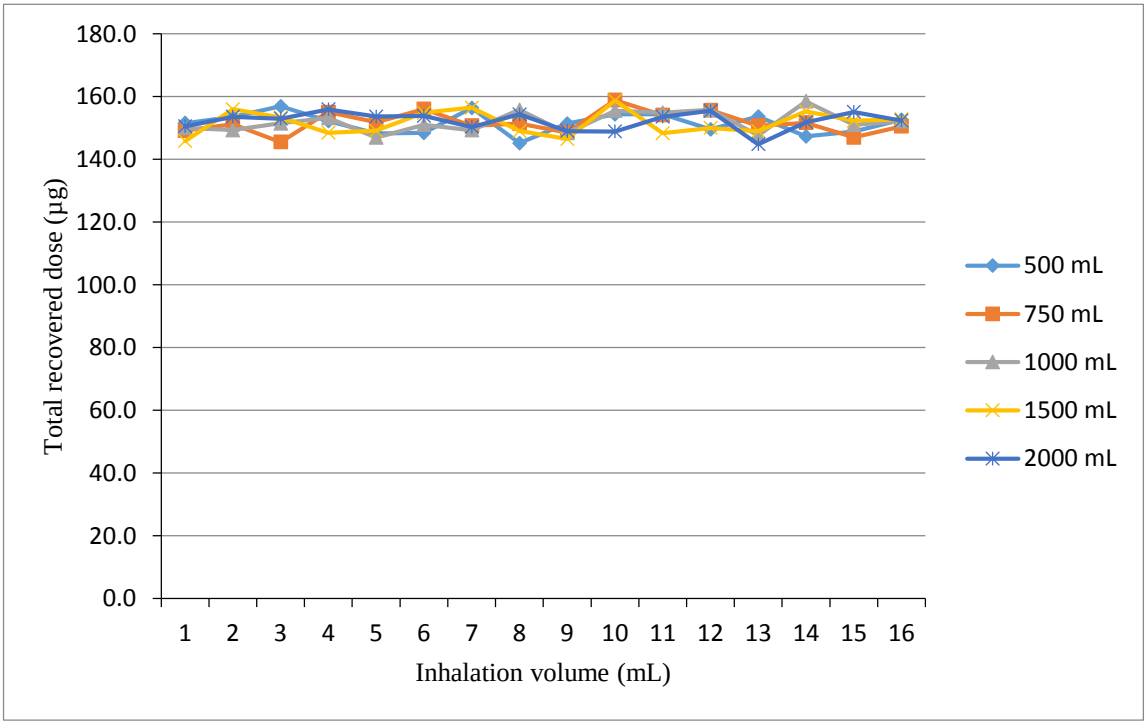
Mean (SD) of the emitted dose after first (ED1), second (ED2) inhalations, Capsule, Total residual amount and total recovered dose from Onbrez Breezhaler® using Vins at MIF of 90 L/min. [% of the nominal dose].



Total recovered dose (µg) of indacaterol emitted from Onbrez Breezhaler® for 16 separate capsules at different Vins and MIF of 90 L/min.



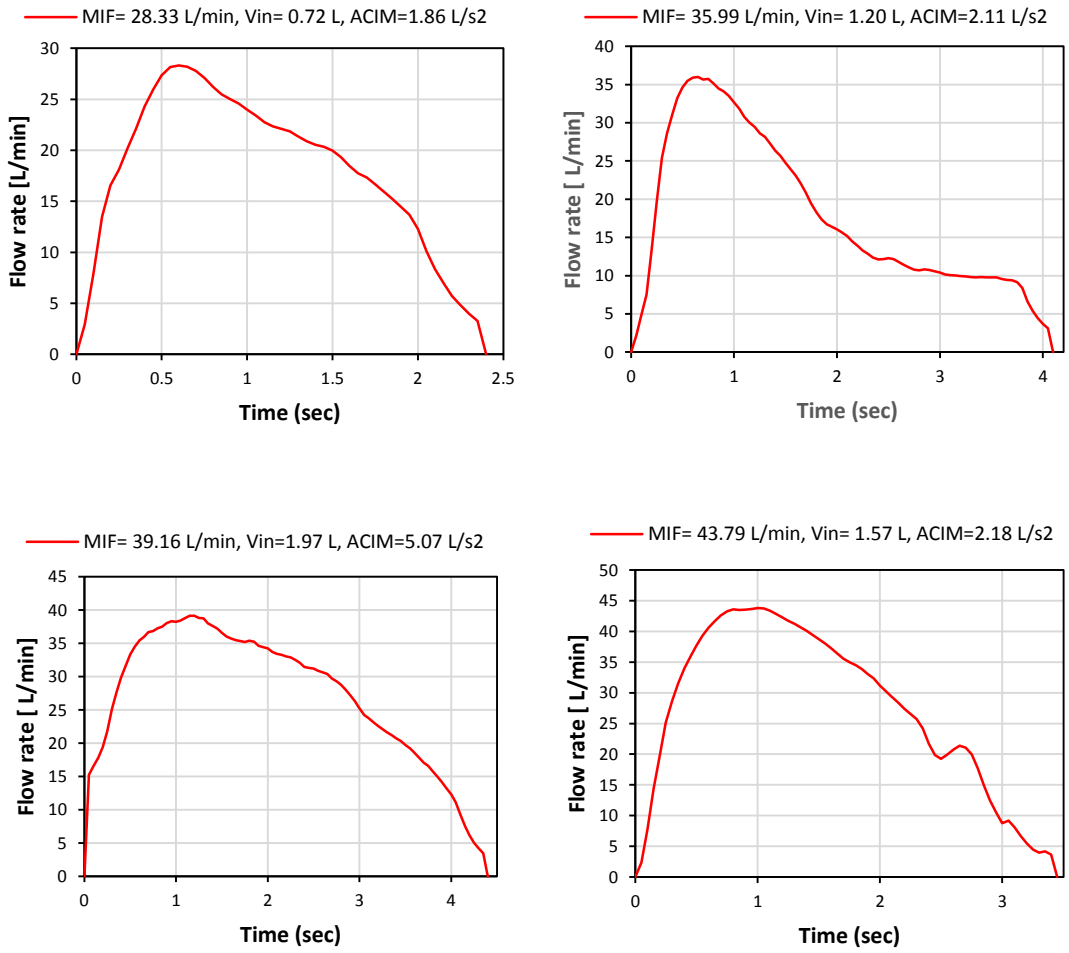
Mean (SD) of the emitted dose after first (ED1), second (ED2) inhalations, Capsule, Total residual amount and total recovered dose from Onbrez Breezhaler® using Vins at MIF of 120 L/min. [% of the nominal dose.

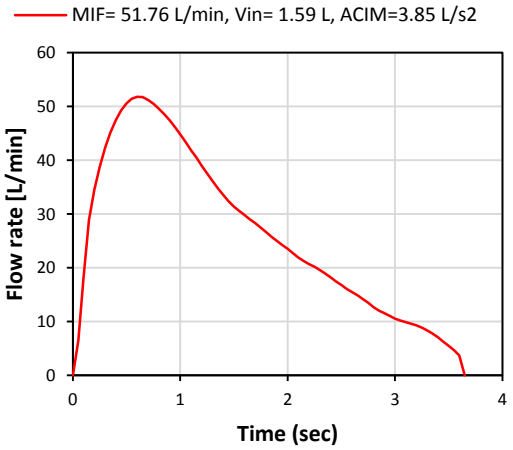


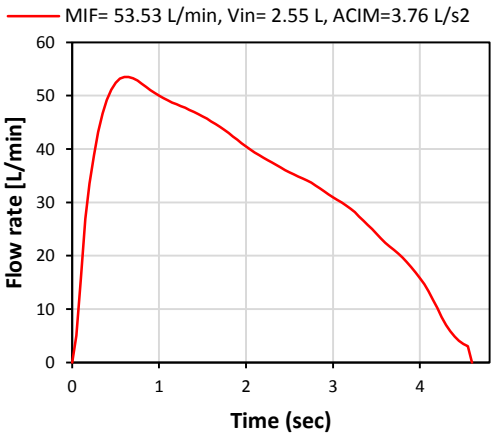
Total recovered dose (µg) of indacaterol emitted from Onbrez Breezhaler® for 16 separate capsules at different Vins and MIF of 120 L/min.

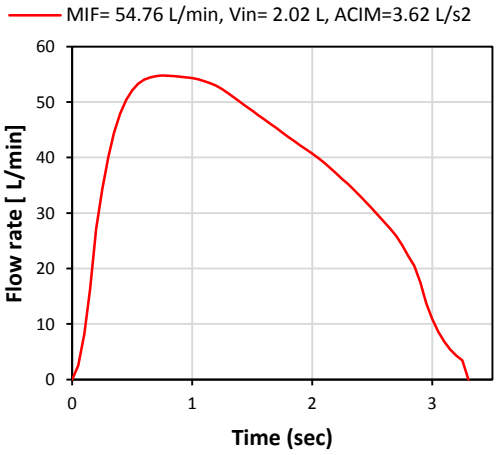
Appendix (C)

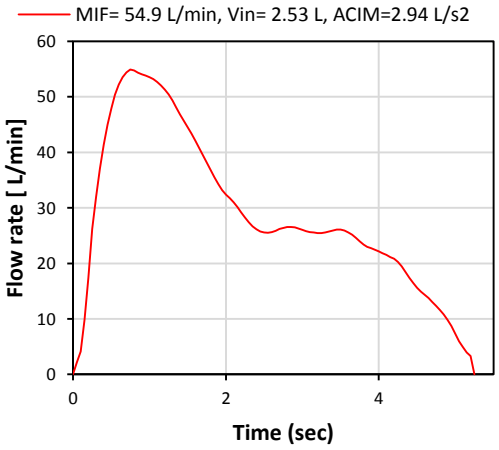
- COPD patients' inhalation profiles generated when patients inhale through an empty Onbrez Breezhaler® (Azouz et al., 2015). [used with permission]

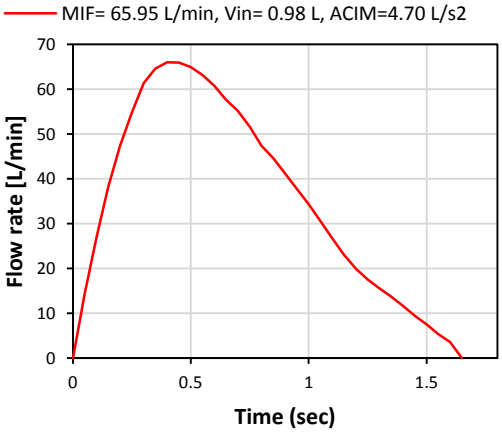


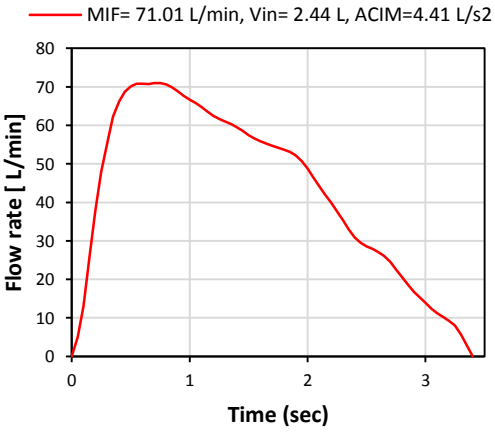

MIF= 51.76 L/min, Vin= 1.59 L, ACIM=3.85 L/s2
Flow rate [L/min]
Time (sec)

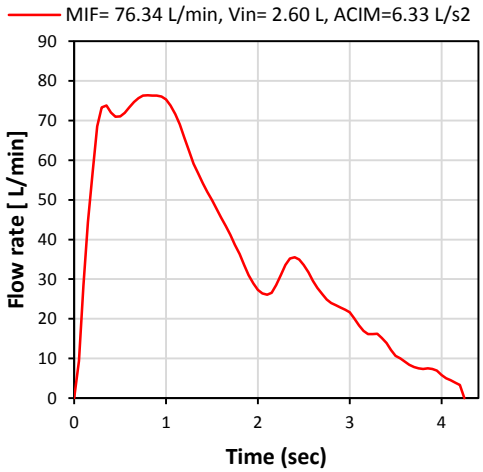

MIF= 53.53 L/min, Vin= 2.55 L, ACIM=3.76 L/s2
Flow rate [L/min]
Time (sec)

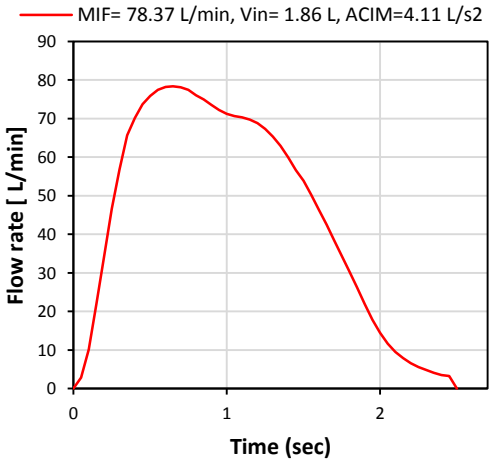

MIF= 54.76 L/min, Vin= 2.02 L, ACIM=3.62 L/s2
Flow rate [L/min]
Time (sec)

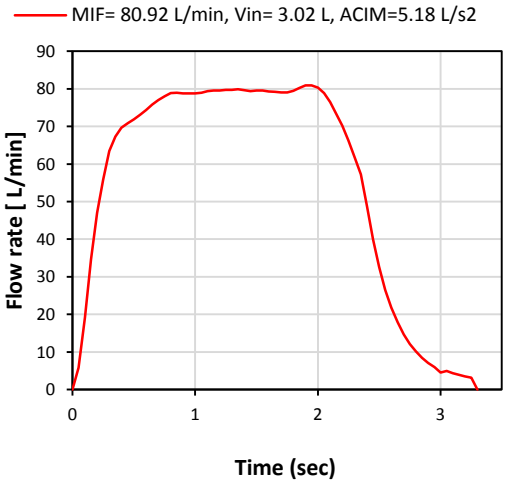

MIF= 54.9 L/min, Vin= 2.53 L, ACIM=2.94 L/s2
Flow rate [L/min]
Time (sec)

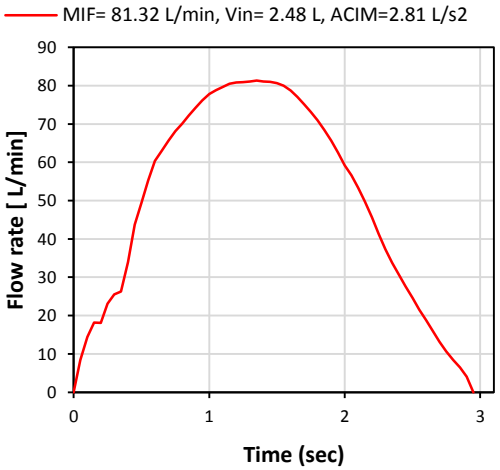

MIF= 65.95 L/min, Vin= 0.98 L, ACIM=4.70 L/s2
Flow rate [L/min]
Time (sec)

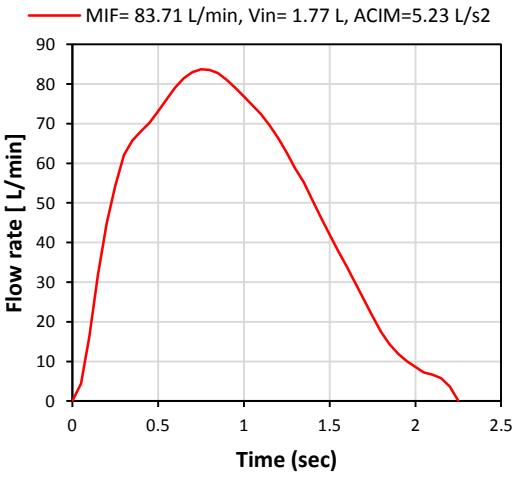

MIF= 71.01 L/min, Vin= 2.44 L, ACIM=4.41 L/s2
Flow rate [L/min]
Time (sec)

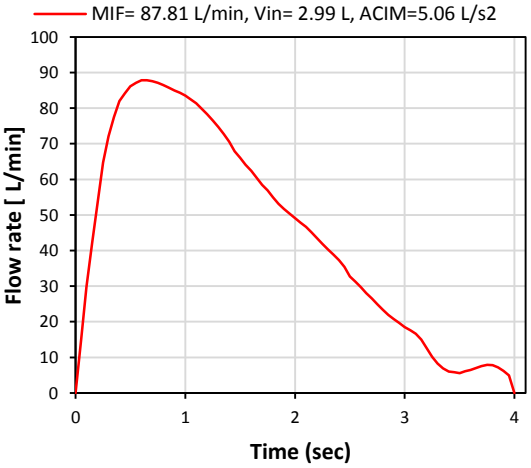

MIF= 76.34 L/min, Vin= 2.60 L, ACIM=6.33 L/s2
Flow rate [L/min]
Time (sec)


MIF= 78.37 L/min, Vin= 1.86 L, ACIM=4.11 L/s2
Flow rate [L/min]
Time (sec)


MIF= 80.92 L/min, Vin= 3.02 L, ACIM=5.18 L/s2
Flow rate [L/min]
Time (sec)


MIF= 81.32 L/min, Vin= 2.48 L, ACIM=2.81 L/s2
Flow rate [L/min]
Time (sec)


MIF= 83.71 L/min, Vin= 1.77 L, ACIM=5.23 L/s2
Flow rate [L/min]
Time (sec)


MIF= 87.81 L/min, Vin= 2.99 L, ACIM=5.06 L/s2
Flow rate [L/min]
Time (sec)

- Simulated COPD patients' inhalation profiles, where one parameter was changed at a time to study the effect of each parameter of the inhalation manoeuvre (MIF, Vin and ACIM) separately. The profiles MIF was chosen from the raw inhalation profiles, while Vin was increased or decreased by altering the points beyond the peak inspiratory flow of the profiles. Furthermore, the ACIM was altered accordingly by increasing or decreasing the initial slope of the inhalation profile [ACIM value with higher R^2 was chosen as the profile main ACIM].
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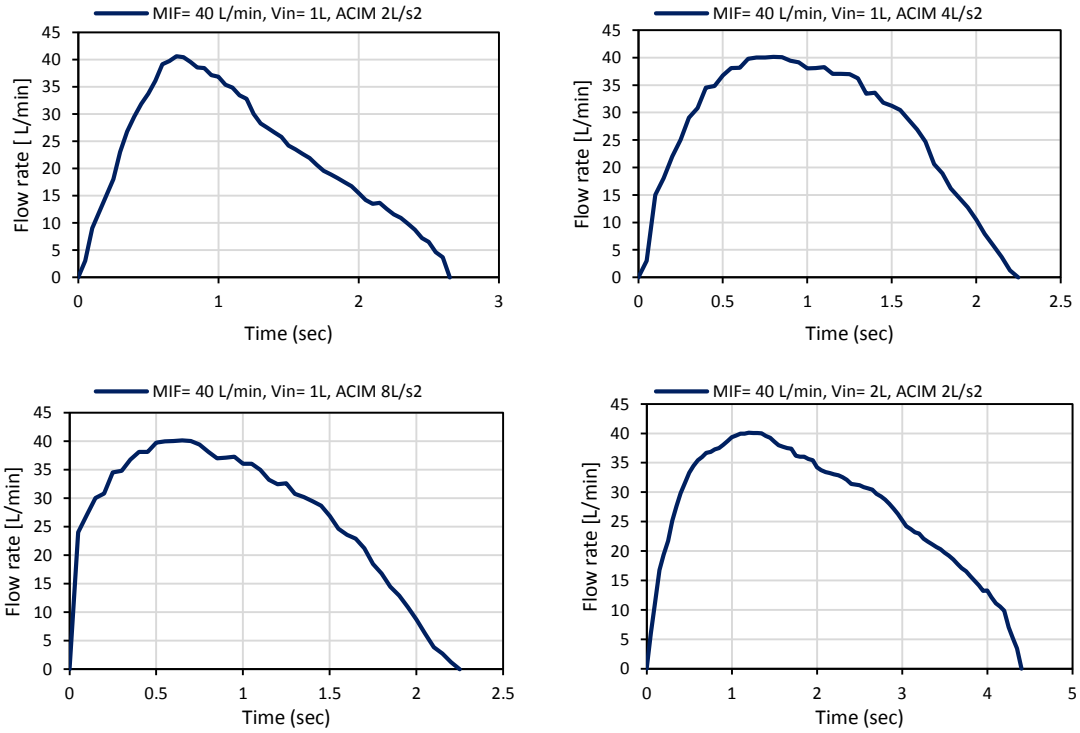
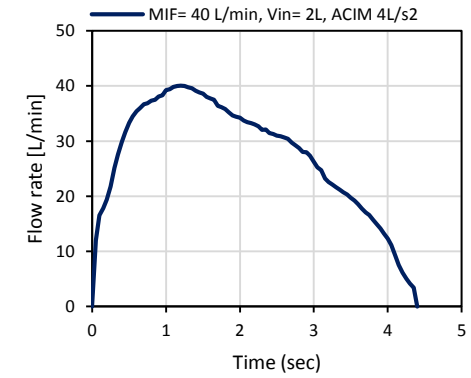
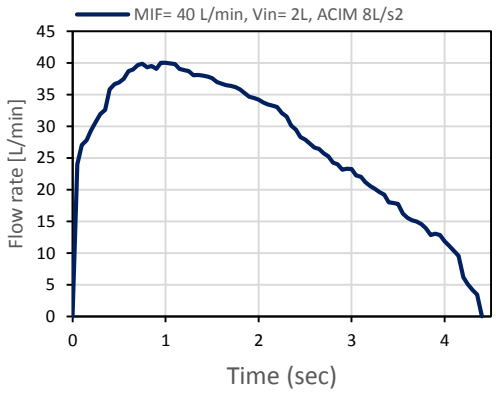
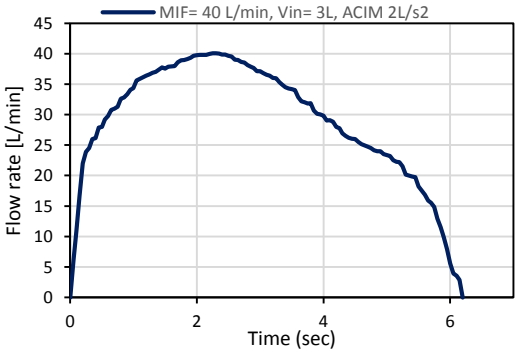
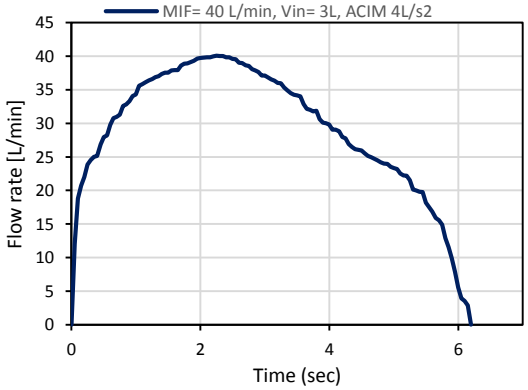
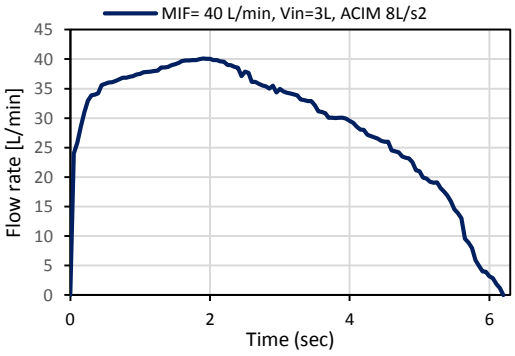


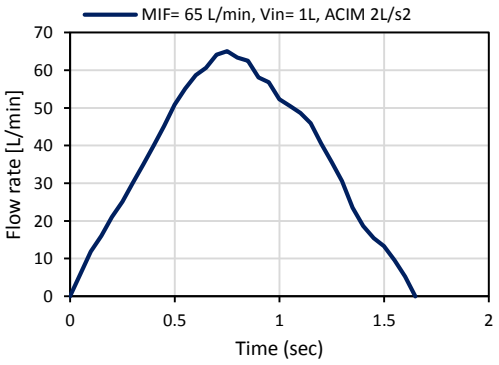

MIF= 40 L/min, Vin= 2L, ACIM 4L/s2
Flow rate [L/min]
Time (sec)

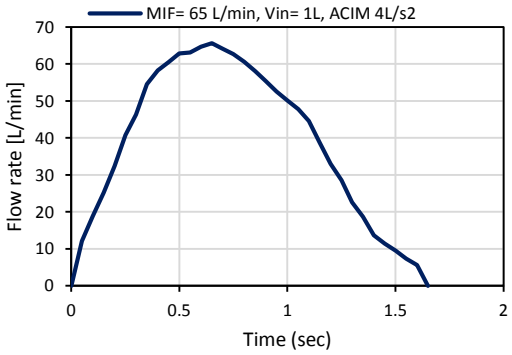

MIF= 40 L/min, Vin= 2L, ACIM 8L/s2
Flow rate [L/min]
Time (sec)

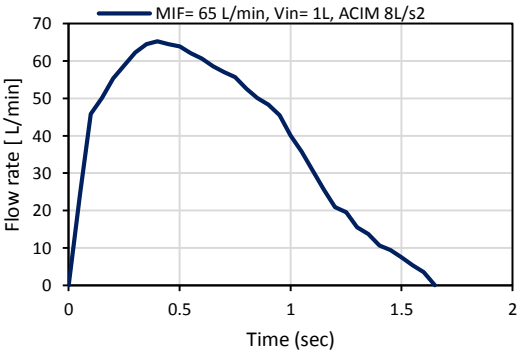

MIF= 40 L/min, Vin= 3L, ACIM 2L/s2
Flow rate [L/min]
Time (sec)

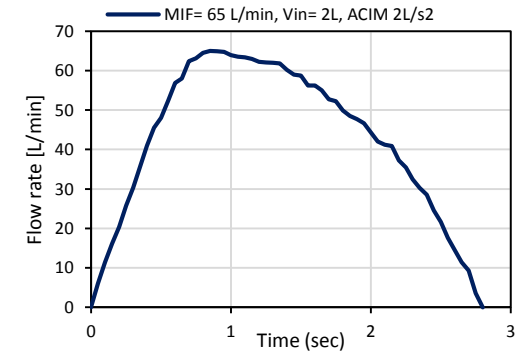

MIF= 40 L/min, Vin= 3L, ACIM 4L/s2
Flow rate [L/min]
Time (sec)

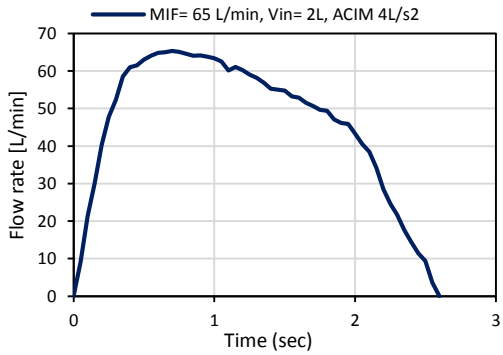

MIF= 40 L/min, Vin=3L, ACIM 8L/s2
Flow rate [L/min]
Time (sec)

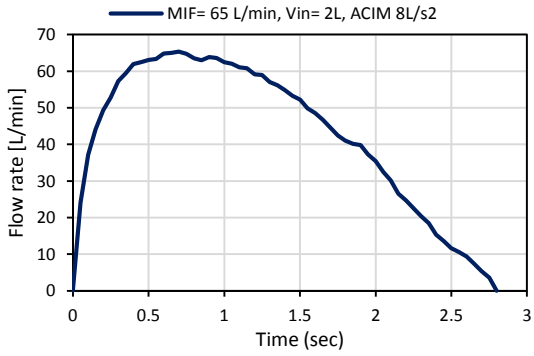

MIF= 65 L/min, Vin= 1L, ACIM 2L/s2
Flow rate [L/min]
Time (sec)

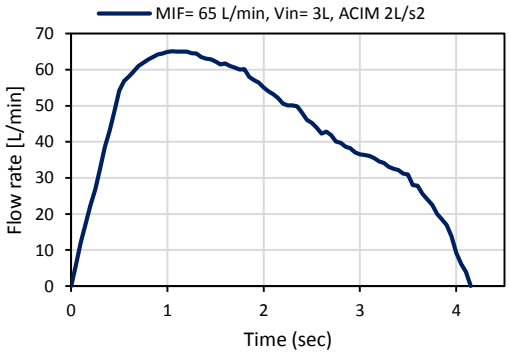

MIF= 65 L/min, Vin= 1L, ACIM 4L/s2
Flow rate [L/min]
Time (sec)

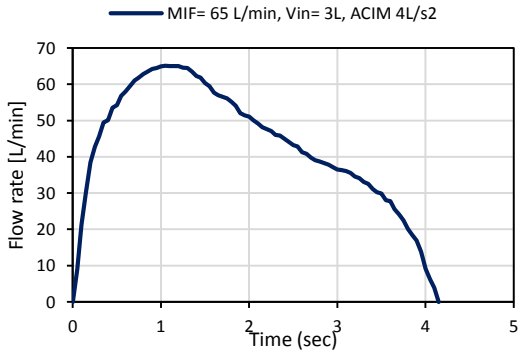

MIF= 65 L/min, Vin= 1L, ACIM 8L/s2
Flow rate [L/min]
Time (sec)

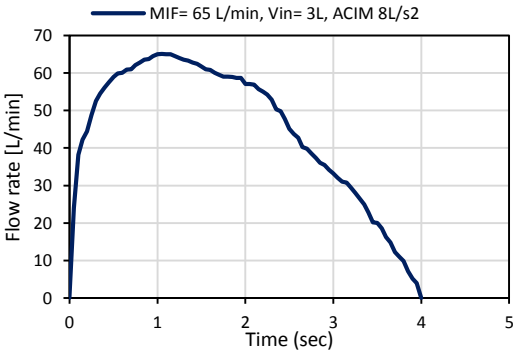

MIF= 65 L/min, Vin= 2L, ACIM 2L/s2
Flow rate [L/min]
Time (sec)

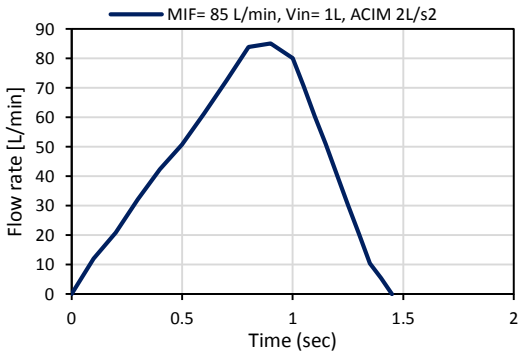

MIF= 65 L/min, Vin= 2L, ACIM 4L/s2
Flow rate [L/min]
Time (sec)

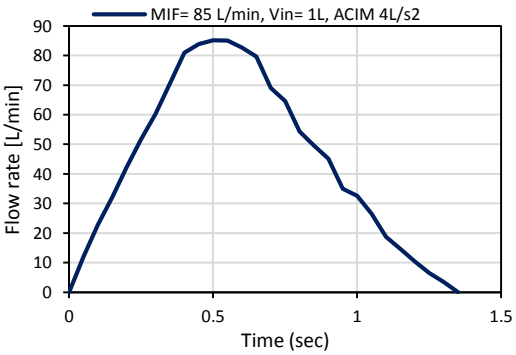

MIF= 65 L/min, Vin= 2L, ACIM 8L/s2
Flow rate [L/min]
Time (sec)

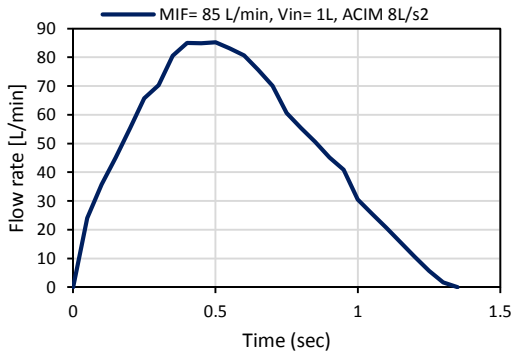

MIF= 65 L/min, Vin= 3L, ACIM 2L/s2
Flow rate [L/min]
Time (sec)

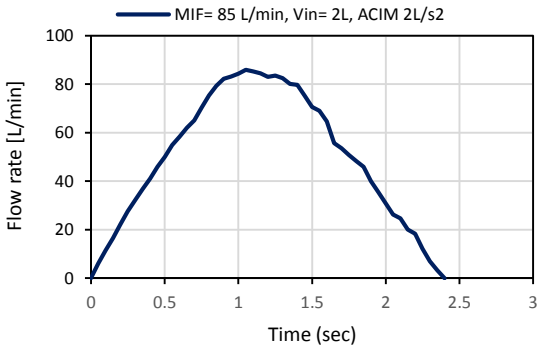

MIF= 65 L/min, Vin= 3L, ACIM 4L/s2
Flow rate [L/min]
Time (sec)

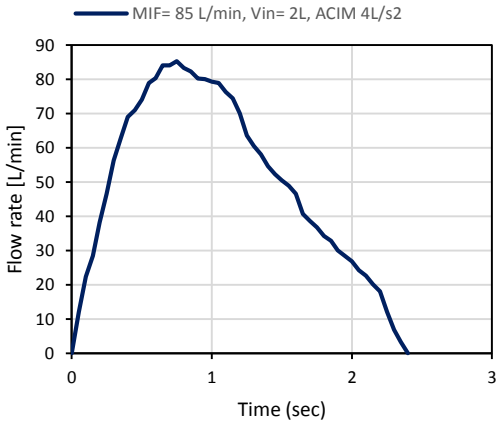

MIF= 65 L/min, Vin= 3L, ACIM 8L/s2
Flow rate [L/min]
Time (sec)

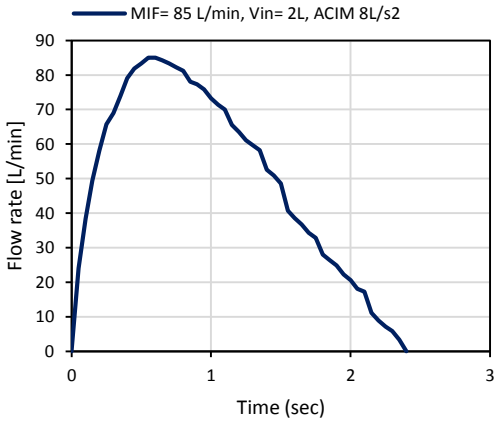

MIF= 85 L/min, Vin= 1L, ACIM 2L/s2
Flow rate [L/min]
Time (sec)

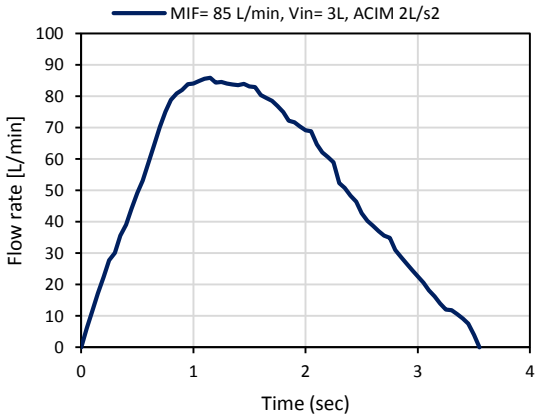

MIF= 85 L/min, Vin= 1L, ACIM 4L/s2
Flow rate [L/min]
Time (sec)

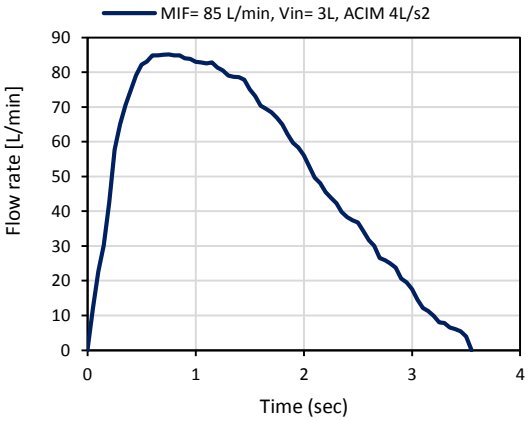

MIF= 85 L/min, Vin= 1L, ACIM 8L/s2
Flow rate [L/min]
Time (sec)

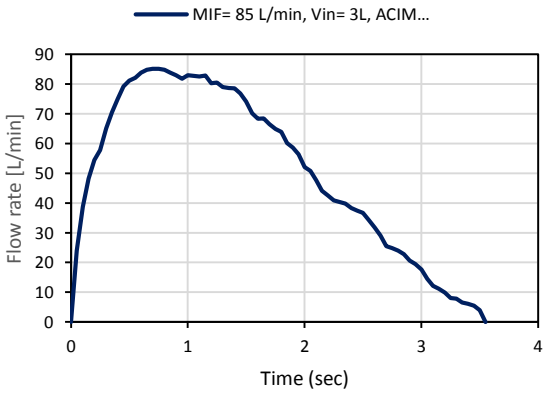

MIF= 85 L/min, Vin= 2L, ACIM 2L/s2
Flow rate [L/min]
Time (sec)


MIF= 85 L/min, Vin= 2L, ACIM 4L/s2
Flow rate [L/min]
Time (sec)


MIF= 85 L/min, Vin= 2L, ACIM 8L/s2
Flow rate [L/min]
Time (sec)


MIF= 85 L/min, Vin= 3L, ACIM 2L/s2
Flow rate [L/min]
Time (sec)


MIF= 85 L/min, Vin= 3L, ACIM 4L/s2
Flow rate [L/min]
Time (sec)


MIF= 85 L/min, Vin= 3L, ACIM...
Flow rate [L/min]
Time (sec)