RELATIVE BIOAVAILABILITY OF TERBUTALINE TO THE LUNGS FOLLOWING INHALATION USING DIFFERENT METHODS

M. E. A. Abdelrahim

PhD

UNIVERSITY OF BRADFORD

2009

RELATIVE BIOAVAILABILITY OF TERBUTALINE TO THE LUNGS FOLLOWING INHALATION USING DIFFERENT METHODS

Development and application of methodology to assay aqueous and urine terbutaline concentrations and to determine in-vitro aerodynamic characteristics and the relative lung and systemic bioavailability using different inhalation methods.

Mohamed Emam Abdelmobdy Abdelrahim, MPharm

Submitted for the degree of
Doctor of Philosophy

Postgraduate studies in clinical Pharmacy

Institute of Pharmaceutical Innovation, School of Pharmacy

UNIVERSITY OF BRADFORD

MAKING
KNOWLEDGE
WORK

2009

Relative bioavailability of terbutaline to the lungs following inhalation using different methods

Abstract

Keys words: terbutaline, urine, MMAD, FPD, relative lung bioavailability, Turbuhaler, spacers, nebulisers.

The primary aim was to validate and implement a urinary pharmacokinetic method for terbutaline to determine the relative lung and systemic bioavailability following inhalation and to measure the in-vitro characteristics of the emitted dose by these inhalation methods.

Two new robust, accurate and sensitive high performance liquid chromatography methods for the determination of terbutaline in aqueous and urine samples were validated in accordance with the FDA and ICH guidelines. Terbutaline was extracted using solid phase extraction with salbutamol and bamethane as internal standards. The accuracy, precision, lower limit of detection and recovery for both methods were within recognized limits.

The in-vitro characteristics of terbutaline sulphate inhalers were measured according to standard compendial methodology as well as adaptation of this methodology to simulate routine patient use. The dose emission of terbutaline sulphate from a Bricanyl Turbuhaler was determined using an inhalation volume of 4 L at inhalation flows of 10-60 L min^{-1}. The particle size distribution was measured using an Anderson Cascade Impactor (ACI) with a mixing inlet valve to allow measurement at different flows. A steady increase in total emitted dose (TED) and the fine particle dose (FPD) was observed as the inhalation flow increased thereby highlighting the flow dependent dose emission characteristics of the Turbuhaler.

The in-vitro dose emission characteristics of terbutaline sulphate from Bricanyl MDIs were measured according to the standard compendial methodology at a flow of 28.3 L min^{-1} using a 4 L inhalation volume. The TED and particle size distribution of terbutaline sulphate from the Bricanyl MDI were determined alone and with different spacers [AeroChamber Max (AMAX), AeroChamber Plus (APLUS), Fisonair and Nebuhaler]. The TED from the MDI alone was significantly higher than all MDI+spacers ($p<0.001$). The MDI with APLUS resulted in the smallest mass median aerodynamic diameter (MMAD) and the highest fine particle fraction (FPF). The MDI with AMAX resulted in the highest FPD.

The in-vitro characteristics of terbutaline sulphate from Bricanyl respules using the Aeroneb Pro (vibrating mesh) and Sidestream jet nebulisers were determined by the CEN methodology and the Next Generation Impactor (NGI) methodology. The Aeroneb Pro was found to have significantly better aerodynamic properties than the Sidestream. The results from the NGI method were significantly different from the CEN method suggesting further evaluation of both methods. Cooling the NGI decreased the evaporation effect.

Twelve healthy volunteers (6 females) completed in-vivo urinary terbutaline pharmacokinetic studies to determine the relative bioavailability following inhalation. The differences between the amounts excreted 0.5, 1, 2, 4, 6 and 24 hour post inhalation from a Bricanyl MDI (I) and oral (O) dosing of 500 µg terbutaline sulphate and with the co-administration of oral charcoal (IC and OC, respectively) were studied. No terbutaline was found in OC samples. The amount of terbutaline excreted 30 minutes post I and IC were significantly ($p<0.001$) higher than post O suggesting that the amount of terbutaline excreted 30 minutes post dosing can be used as an index of the lung deposition. The amount of terbutaline excreted 24 hour post I was significantly ($p<0.01$) higher than post O suggesting that the amount of terbutaline excreted 24 hour post dosing can be used as an index of the relative systemic bioavailability. The dose response relationships and the low inter and intra-subject variability studies confirm the feasibility of this method.

To demonstrate the application of the method the effect of inhalation technique on the lung and systemic bioavailability following inhalation from a dry powder inhaler was evaluated. The effect of different spacers on the dose emitted from the Bricanyl MDI and the effect of different nebulisers on the dose emitted were also studied using twelve healthy volunteers (6 females) for each study.

A fast inhalation flow using the Bricanyl Turbuhaler resulted in significantly higher amounts of terbutaline excreted 0.5 and 24 hour post dosing (2 doses of 500µg terbutaline sulphate from Bricanyl Turbuhaler) than slow inhalation flow ($p<0.001$). The Bricanyl MDI alone resulted in a significantly higher amount of terbutaline excreted 24 hour post dosing (2 doses of 250µg terbutaline sulphate from Bricanyl MDI) and significantly lower amounts excreted 30 minutes post dosing than the MDI+Spacers. The AMAX provided a greater amount of urinary terbutaline excreted 30 minutes post dosing than the APLUS and Nebuhaler. The Aeroneb Pro resulted in significantly higher amounts of terbutaline excreted 0.5 and 24 hour post dosing (1 dose of 5mg/2ml terbutaline sulphate from Bricanyl respule) than a Sidestream Jet nebuliser ($p<0.001$).

Further application of the method was demonstrated by 12 (6 female) COPD non-invasive mechanically ventilated patients. One dose of 2mg in 0.8ml terbutaline sulphate respiratory solution from Aeroneb Pro and one dose of 5mg in 2ml terbutaline sulphate respiratory solution from Sidestream jet nebuliser resulted in a similar amounts of urinary terbutaline excreted 0.5 and 24 hour post dosing. The results were consistent with the results of the ex-vivo study performed on the same patients.

The thesis highlights extension of the urinary pharmacokinetic method following inhalation to terbutaline and its application in volunteer and patient studies.

Dedicated to my father, mother and my beloved wife, they helped and supported me very much toward this achievement

List of Publication

Sections of this thesis have already been published in the following form:

1. **Abdelrahim, M.E.**, K. Assi, and H. Chrystyn, Relative bioavailability of terbutaline to the lung following inhalation, using urinary excretion: 1. Method Validation. J Aerosol Med, 2007. **20**(2): p. 168. Presented as poster in 15th Congress of the International Society of Aerosol in Medicine, Tour, France, June 2007.
2. **Abdelrahim, M.E.**, K. Assi, and H. Chrystyn, Relative bioavailability of terbutaline to the lung following inhalation, using urinary excretion: 2. Dose response relationship and application. J Aerosol Med, 2007. **20**(2): p. 169. Presented as poster and oral presentation in 15th Congress of the International Society of Aerosol in Medicine, Tour, France, June 2007.
3. **Abdelrahim, M.E.**, K. Assi, and H. Chrystyn, Dose emission and aerodynamic characterization of the terbutaline sulphate dose emitted from a Turbuhaler at low inhalation. J Pharm Pharmacol Supp 2007. **59(1)**: p. A39-40. Presented as poster in the British Pharmaceutical Conference (BPC) 2007 - Science on September 2007.
4. **Abdelrahim, M.E.**, and H. Chrystyn, Aerodynamic characteristics of nebulised terbutaline sulphate using the Next Generation Impactor (NGI) and CEN method (in press for publication in J Aerosol Med)

Acknowledgement

I am sincerely and deeply grateful for Prof. Henry Chrystyn Professor of Clinical pharmacy, and head of Pharmacy school, University of Huddersfield for his instructive supervision, valuable guidance, unlimited help, advice and encouragement during this work.

I would like to express my sincere gratitude and heartily gratefulness to Dr. Khaled Assi Doctor of Pharmaceutical Chemistry University of Bradford for his fruitful and continuous advice and encouragement and the supervision during this work.

I am very grateful for the patience, help and sponsorship I received from all the members of the Egyptian culture office in UK and the missions department in Egypt.

I would like to thank Dr. Paul Plant the consultant chest physician at St James's University hospital for letting me in the Respiratory care unit of the hospital and helping and directing me in the study part of this thesis which were done in St James's University hospital.

I would like to thank the doctors and nursing staff in St James's University hospital for their hospitality and help during my work in the hospital especially Sister Sarah and Sister Lindsay for their great assistant.

A special and deep gratitude to my father Prof. Emam A. Abdelrahim Professor of Biochemistry, Faculty of Agriculture, University of Cairo for his fatherhood and unlimited help and encouragement during the work.

A special and deep gratitude to my mother for her kindness and unlimited patient and encouragement during the work

I am deeply grateful for my wife for her unlimited and warm love, help and supports during the difficult times we faced in the work of this thesis. She made me a great environment that helped me to achieve this thesis. Without her help I could not have done this thesis.

I am also very grateful to Alan Lindley, Amir Amani, Anest Williams, Anka Heier, Angela Turcny, Asha Srinivasan, Dinesh Nadarassan, Hassan Ali, Hossam El-shawary, Karen Masey, Liz Dunphy, Mohamed El-Maizany, Maroan Mashat, Mohamed El-thanyan, Nausheen Sheik, Salman El-fadil and Samira Lobo. Without their help I could not have done this work.

I would like to thank all the members of the Institute of Pharmaceutical Innovation and University of Bradford for the friendly environment, which helped very much during the work.

I would like to thank all the members of Faculty of Pharmacy, University of Beni Suif for their patient on me and their friendship and help.

Finally I would like to offer a warm thanks to my mother in low, my Sister Yasmin E. Abdelrahim my brother Ahmed E. Abdelrahim and relatives for their constant encouragement, help and love.

List of content

List of Abbreviations

°C	Degree (s) Celsius
µg	Microgram
µm	Micrometer
ABG	Arterial Blood Gas
ACI	Anderson Cascade Impactor
AVR	Alveolar Ventilation Rate (ml/min)
BNF	British National Formulary
BP	British Pharmacopoeia
BTS	British-Thoracic-Society
cAMP	Cyclic Adenosine mono phosphate
CEN	Comité Européen Normalisation
CFC	Chlorofluorocarbons
CI	Confidence intervals
CITDAS	Copley Inhaler Testing Data Analysis Software
cm	Centimetre
cm H_2O	Centimetre of Water as a pressure unit
COPD	Chronic Obstructive Pulmonary Disease
CPAP	Continuous Positive Airway Pressure
CV	Coefficient of Variation
DAS	Dead Air Space (ml/respiration)
DPI	Dry Powder Inhaler
ED	Emitted Dose
EP	European Pharmacopoeia
ERV	Expiratory Reserve Volume

FDA	Food and Drug Administration
FEV_1	Forced Expiratory Volume in one second
FPD	Fine Particle Dose
FPF	Fine Particle Fraction
FRC	Functional Residual Capacity
FVC	Forced Vital Capacity
g	Gram
G	Generation
GOLD	Global Initiative for Chronic Obstructive Lung Disease
GSD	Geometric Standard Deviation
HFA	Hydrofluorocarbons
HLB	Hydrophilic Lyophilise Balance
HPLC	High Performance Liquid Chromatography
IC	Inspiratory Capacity
ICH	International Committee of Harmonisation
IFR	Inspiratory Flow Rate
IRV	Inspiratory Reserve Volume
kPa	Kilopascal (1 Pascal=force of 1 Newton par square meter)
L	Litre
L min^{-1}	Litre per minutes
LLOQ	Lower Limit of Quantification
LOD	Limit of Detection
LTOT	Long-Term Oxygen Therapy
MAOIs	monoamineoxidase Inhibitor
MDI	Metered Dose Inhaler
mg	Milligram

mg/L	Milligram per litre
min	Minute
ml	Millilitre
ml/min	Millilitre per Minute
mM	Millimolar
MMAD	Mass Median Aerodynamic Diameter
MMM	magnetic marker monitoring
MOC	Micro Orifice Collector
MRI	Magnetic Resonance Imaging
MSLI	Multistage Liquid Impinger
ng	Nanogram
NGI	New Generation Impactor
NICE	National Institute for Health and Clinical Excellence
NIV	Non-Invasive Mechanical Ventilation
nm	Nanometer
NPPV	Non-Invasive Positive Pressure Ventilation
P	Probability
PaO_2	Arterial Oxygen Tension
PEF	Peak Expiratory Flow
PEFR	Peak Expiratory Flow Rate
PET	Positron emission tomography
PIF	Peak Inspiratory Flow
PSV	Pressure Support Ventilation
r	Radius
R^2	Correlation Coefficient
RR	Respiratory Rate (Respiration/min)

RSD	Relative Standard Deviation
RV	Reserve Volume
SD	Standard Deviation
Sec	Second
SIGN	Scottish Intercollegiate Guidelines
SPE	Solid Phase Extraction
SPECT	Single Photon Emission Computed Tomography
SpO_2	Arterial Oxygen Saturation
TLC	Total Lung Capacity
TV	Tidal Volume
USP	United State Pharmacopoeia
UV	Ultraviolet
v/v	Volume per Volume
VC	Vital Capacity
w/v	Weight per Volume
w/w	Weight per Weight
α	Alpha
β	Beta

List of Tables

List of Figures

1.1 Introduction

Short acting β_2-agonists (Salbutamol and Terbutaline) are widely used in the management of asthma and COPD. In the British Thoracic Society (BTS) / Scottish Intercollegiate Guidelines Network (SIGN) guidelines short acting β_2 agonists are recommended as a first line treatment for the management of asthma. Short acting β_2 agonists are also recommended by the National Institute for Health and Clinical Excellence (NICE) guidelines for the management of COPD as a first line option. NICE recommend to treat breathlessness and exercise limitation initially with short-acting bronchodilators (β2-agonists or anticholinergics) when required. Salbutamol and terbutaline have been widely prescribed in the treatment of asthma and COPD and have been shown to be of benefit in both cases.

Terbutaline sulphate, introduced in 1970 after salbutamol by AstraZeneca (Brand name-Bricanyl), is available for inhalation as a metered dose inhaler (MDI), dry powder inhaler (DPI) and Respules for inhalation. The aerosol emitted from these inhalation methods has different characteristics. Part of the emitted dose contains particles that have an aerodynamic diameter of less than 5 µm. This portion of the dose has the greatest potential for deposition in the lungs and is referred to as the fine particle dose (FPD). Particles with an aerodynamic diameter more than 5 µm mostly deposit in the oropharyngeal area. Following an inhalation drug is deposited in the lungs, the mouth and the throat (oropharyngeal region) then it is absorbed into the systemic circulation where it faces metabolism and excretion. The swallowed portion can be metabolised in the stomach and the liver before it enters the systemic circulation. There is a lag time before the swallowed fraction enters the systemic circulation. Whereas that deposited into the lungs enters the body very quickly.

Several in-vitro and in-vivo methods have been described to predict lung deposition. In-vitro studies using compendial methods mostly use cascade impactors that have been

designed for a set of inhalation conditions. In-vitro methods are used as a quality assurance procedure to identify the quality of the inhaled product such as the total emitted dose, uniformity of dose and the aerodynamic characteristics. Further, they are often extrapolated to give an estimation of in-vivo deposition. The most commonly used techniques are inertial separation methods and laser diffraction.

In-vivo studies mostly use gamma scintigraphy, pharmacodynamic methods and pharmacokinetic methods to provide an insight into the lung deposition. The application of traditional pharmacokinetic methods to lung deposition studies is difficult, because the doses administered are small and the volume of distribution is large. Thus the resulting systemic drug concentrations are low and thus require sensitive assay methods (Newman et al., 1981).

Pharmacokinetic methods to evaluate the relative lung bioavailability of an inhaled drug have used plasma and urine concentrations of the drug. A plasma pharmacokinetic method has been reported for salbutamol by drawing blood samples in the absorption lag time of the orally swallowed portion of the drug. Hence the concentration of these samples would account mainly for the drug deposited in the lung after inhalation. The proposed times for plasma samples are 5, 10, 20 minutes post inhalation (Anhoj et al., 1999; Lipworth and Clark, 1997; Mobley and Hochhaus, 2001). However due to the low concentration many doses are required instead of those routinely inhaled.

The first urinary pharmacokinetic method used to evaluate the fate of an inhaled drug used urinary excretion with the co-administration of oral charcoal to block gastrointestinal absorption of the swallowed fraction of the inhaled dose. Later the methodology was extended to the lag time of the gastrointestinal absorption phase without the need to administer oral charcoal.

The charcoal block urinary pharmacokinetic method original reported by Borgstrom and Nilsson (1992) highlighted the value of this method to determine the relative amount of

drug delivered to the lung. In this study terbutaline sulphate was given to healthy volunteers by inhalation from a metered dose inhaler (MDI) with the oral administration of a charcoal slurry (5 gm before the inhalations, 5 gm after inhalation and 10 gm after 1, 2, 3 hours). Subjects provided urine samples at 0-12 hours, 12-24 hours, 24-36 hours and 36-48 hours interval post dosing. The drug excreted in urine was measured. Since the charcoal slurry adsorbed 97% of an oral dose then the oral contribution to the overall systemic bioavailability after inhalation, when charcoal was co-administered, could thus be neglected. The use of a charcoal dose taken before and after an inhalation prevents any oral absorption hence for patients with concomitant use of other drugs then the use of charcoal is not possible.

Using the lag time of the gastrointestinal absorption phase Hindle and Chrystyn (1992) developed a urinary pharmacokinetic method to determine the lung and systemic bioavailability of inhaled salbutamol. Their study demonstrated that following the oral administration of salbutamol, negligible amounts of salbutamol are excreted in the urine during the first 30 minutes post dose, and that significantly greater amounts ($p<0.001$) are excreted 30 minutes post inhalation. They showed that the salbutamol excreted in the urine 30 minutes post dosing was an index of the lung bioavailability and that the urinary salbutamol excreted 24 hours post dosing was an index of the systemic bioavailability. In this thesis application of this method has been investigated for terbutaline with extension to the in-vivo assessments together with in-vitro evaluations of the emitted dose.

1.2 Aim and objectives

1.2.1 Aim

To use in-vitro and in-vivo methods to determine the respective dose emission properties and relative lung deposition of terbutaline from inhaled products using different inhalation techniques.

1.2.2 Objectives

1. To modify and validate a previously reported salbutamol HPLC assays for terbutaline in aqueous and urine samples.
2. To determine the aerodynamic characteristics of the emitted dose of terbutaline sulphate from a dry powder inhaler (Turbuhaler), a MDI with different spacers and from nebulisers.
3. To optimise the in-vitro methodology for nebulised products to limit evaporation effects.
4. To validate the Hindle and Chrystyn urinary salbutamol pharmacokinetic method to measure the relative lung and systemic bioavailability of terbutaline to the lung following inhalation.
5. To consolidate the potential of the urinary terbutaline pharmacokinetic method by assessing its dose response relationship and intra- and inter-subject variability.
6. To demonstrate the application of the method by evaluating the effect of inhalation technique and inhalation methods on the lung and systemic bioavailability following inhalation from a dry powder inhaler (Bricanyl Turbuhaler), Bricanyl MDI alone and with spacers and from nebulisers (using healthy volunteers and non-invasive positive pressure ventilated patients).

1.3 Summary of the work

To achieve the aim and objectives the work in this research has been divided into three parts:

a. High performance liquid chromatography (HPLC) method validation for the determination of terbutaline in aqueous and urine samples.
b. In-vitro studies to determine the dose emission properties of terbutaline sulphate from different inhalation methods and when using different inhalation techniques.

c. In-vivo studies to determine the relative lung bioavailability of terbutaline following inhalation.

Terbutaline sulphate is a synthetic resorcinol derivative β_2-adrenergic agonist that is used as a bronchodilator in the treatment of bronchial asthma. Terbutaline like most other sympathomematics exists as a racemic mixture.

Aqueous and urine samples of terbutaline for in-vitro and in-vivo studies need an accurate and sensitive chromatographic method to analyse and separate them. Two sensitive and easy to perform assays to quantify salbutamol in aqueous and urine samples were developed and validated by Mazhar and Chrystyn (submitted for publication). The initial work was to adapt and validate similar assays for the quantification of terbutaline in aqueous and urine samples collected after in-vitro dose emission and in-vivo inhalation of the drug, respectively. Solid phase extraction (SPE) was used to isolate terbutaline from urine samples. The validated method for aqueous and urine samples used a C18 reversed phase column for HPLC separation and quantification using a fluorescence detector, set with an excitation/emission of 267/313 nm.

The in-vitro studies have been divided into three parts, the first part has focussed on the determination of the aerodynamic characteristics of the emitted dose, from a Bricanyl Turbuhaler at different inhalation flows (10-60 L min^{-1}). Previously dose emission below 30 L min^{-1} through a DPI has not been determined and is not a compendial method. However during routine use some patients do not achieve an inhalation flow of 30 L min^{-1} (Pedersen et al., 1990). Therefore, the Pharmacopoeia Methods were modified to determine dose emission characteristics to include low inhalation flows from a terbutaline sulphate Turbuhaler (Bricanyl, AstraZeneca, UK). To achieve this, a novel adaptation methodology using a mixing inlet valve has been incorporated into the compendial methods to enable the measurement of the particle size distribution at low inhalation flows.

The second part has focussed on the effect of different spacers on the dose emitted from a Bricanyl MDI at 28.3 L min^{-1} inhalation flow. The third part has focussed on the optimisation of methodology for the in-vitro assessment of the dose emitted from nebulised systems. The NGI and the CEN methods were used to determine the aerodynamic characteristics of the nebulised aerosol from two different nebulisers with optimisation of the NGI method to limit evaporation effects.

The in-vivo study of this research work commenced with the validation of a urinary terbutaline pharmacokinetic method to determine the relative lung and systemic bioavailability of terbutaline following inhalation in healthy volunteers. This method has then been used to study:

a. The effect of different inhalation flows on the relative lung and systemic bioavailability of terbutaline when using the Bricanyl Turbuhaler.

b. The effect of different spacers on the relative lung and systemic bioavailability following inhalation from a Bricanyl MDI.

c. The effect of different nebulisers on the lung and the systemic bioavailability of nebulised terbutaline on healthy volunteers and non-invasivly positive pressure ventilated patients.

1.4 Thesis structure

The work in this thesis is as follow: -

Chapter 1 is a general introduction with a brief summary of the research work.

Chapter 2 provides an overview of literature related to the areas of study.

Chapter 3 describes the validation of a HPLC assay to determine terbutaline in aqueous and urine samples. Terbutaline and its internal standard bamethane or salbutamol, as appropriate, were extracted from urine samples using a solid phase extraction method and then quantified using high-performance liquid chromatography (HPLC). The intra-day

and inter-day accuracy, precision, limit of detection and lower limit of quantification of terbutaline by the extraction method and the HPLC assay method have been determined.

Chapter 4 details the in-vitro studies to characterise the dose emission properties of the dose emitted from a terbutaline sulphate dry powder inhaler (Bricanyl Turbuhaler) at different inhalation flows (10-60 L min^{-1}).

Chapter 5 extends the in-vitro studies to characterise the dose emission properties of terbutaline sulphate emitted from a metered dose inhaler (Bricanyl MDI) alone and when it was attached to four different spacers.

Chapter 6 completes the in-vitro studies by detailing the characteristics of the dose emitted from terbutaline sulphate Respules (Bricanyl Respules) using two different nebulisers measured by two different in-vitro methods. The methodology includes optimisation of the NGI method to limit evaporation effects. A comparison has been made of the two different in-vitro (CEN and NGI) methods.

Chapter 7 describes an extension of the urinary salbutamol pharmacokinetic method to measure terbutaline. A validation of this method to the relative bioavailability of terbutaline to the lung following inhalation by healthy volunteers is provided. It is divided into three parts

a. Urinary excretion profiles following oral, oral with charcoal, inhalation (Bricanyl MDI) and inhalation (Bricanyl MDI) with charcoal administration.

b. Dose dependant kinetics of terbutaline excretion 30 minutes and 24 hours post inhalation.

c. Investigation of the intra- and inter-subject variability in the urinary terbutaline excretion 30 minutes post inhalation.

Chapter 8 describes applications of the urinary terbutaline pharmacokinetic method.

a. Relative lung and systemic bioavailability of terbutaline inhaled from a dry powder inhaler at different inhalation flows.

b. Relative lung and systemic bioavailability of terbutaline inhaled from a metered dose inhaler alone and when it was attached to different spacers.

c. Relative lung and systemic bioavailability of terbutaline inhaled from two different nebulisers (Aeroneb Pro and Sidestream Jet Nebuliser).

Chapter 9 details an application of the urinary terbutaline pharmacokinetic method and ex-vivo method to non-invasive positive pressure ventilated patients. Relative lung and systemic bioavailability of nebulised terbutaline using urinary drug excretion post inhalation has been determined.

Finally a general conclusion from these studies and suggestion for future work are described in chapter 10.

2.1. Respiratory tract

Studying, sleeping, and even exercising all have one common feature in that they all involve breathing. It is one of the first things that is checked to determine if the unconscious person is alive. Respiratory tract development in the embryo starts in the cephalcaudal (head-to-tail) direction with more turnovers till by the 28th week when the respiratory system develops sufficiently to sustain life (Nicpon-Marieb, 2004; Tortora, 2003).

Respiration includes the following processes:

(1) Ventilation, which is the movement of air into and out of the lung.

(2) Gas exchange between the air in the lungs and the blood, sometimes called external respiration.

(3) Transport of oxygen and carbon dioxide in blood and tissues, sometimes called internal respiration.

2.1.1. The respiratory system:

The respiratory system, described in Figure 2.1, consists of the upper respiratory tract which is the nasal cavity and the pharynx and the lower respiratory tract which is the larynx, the trachea, the bronchi and the lungs. The diaphragm and the muscles of the thoracic wall are required for respiratory movements. In the mouth, nose and throat the air is heated to 37 °C and moistened to 99% relative humidity. The pharynx is the common opening of both the digestive system and the respiratory system. It receives air from the nasal cavity and air, food and water from the mouth. Inferiorly the pharynx connects the respiratory system at the larynx and the digestive system at the oesophagus.

The larynx consists of an outer casing of nine cartilages that are connected to each other by muscles and two pairs of ligaments. The epiglottis prevents food and liquid from entering the larynx and air from leaving the lung.

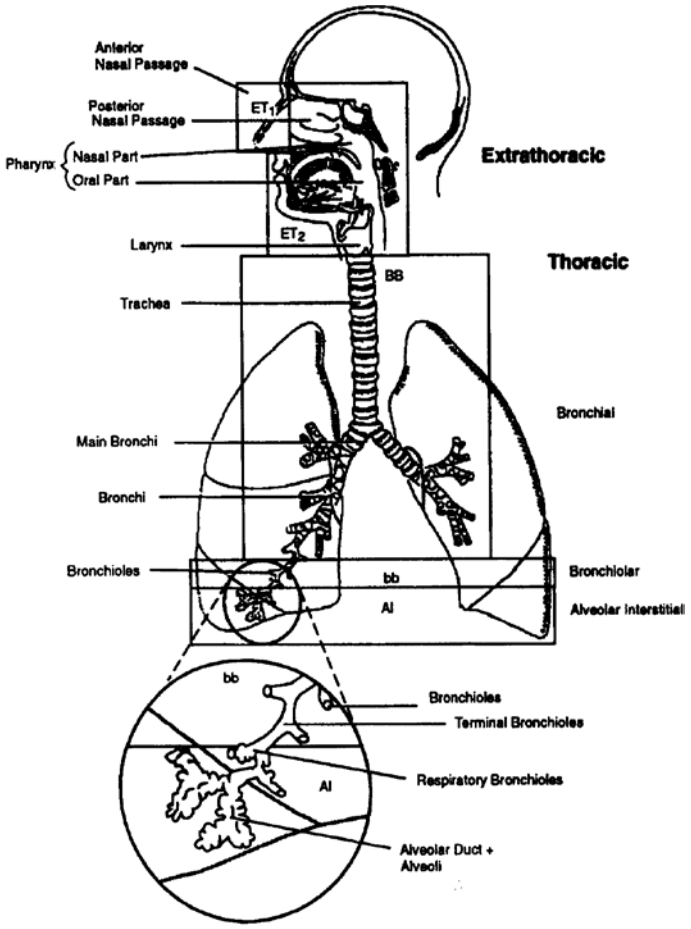


Figure 2.1 Schematic diagram of the respiratory system. [Reproduced from Berico et al., (1997)]

The trachea or windpipe is a membranous tube that consists of dense regular connective tissue that is reinforced with 15 to 20 C-shaped cartilages. The cartilages form the anterior and the lateral sides of the trachea. They protect the trachea and maintain an open passageway for the air. The posterior wall of the trachea is devoid of cartilage and consists of a ligamentous membrane and smooth muscle, because the oesophagus lies immediately posterior to the trachea (the cartilage free part).

The trachea divides into left and right bronchi. Like the trachea these two branches are supported by C-shaped cartilage rings. The rest of the bronchial tree is supported by numerous small cartilage plates embedded in the walls of the airways rather than the C-

shaped cartilage rings. Further down the respiratory tree the cartilages become smaller and smooth muscle becomes more abundant.

As shown in Figure 2.2 the point of entry of the bronchi to the lung is called the hilum or root. The lungs are the principle organs of respiration and on a volume base they are among the largest organs in the body. Each lung is conical in shape, with a base resting on the diaphragm and its apex extending superiorly. The right lung is larger (weight about 620 gm) than the left lung (weight about 560 gm). The right lung has three lobes and the left lung has two lobes. Between each two lobes a fissure is present. Each lobe is divided into lobules separated by connective tissue partitions and no blood or nerve supply passes it. Hence individual diseased lobules can be surgically removed. There are nine lobules in the left lung and ten lobules in the right lung.

The basic architecture of the tracheobronchial region is theoretically a series of dichotomous branches (Hillery et al., 2001). Every branching of the tracheobronchial tree leads to a new generation (G) of airways, for example, the trachea (G0) branches into two main bronchi (G1) and then following sequential branching into secondary bronchi (G2), tertiary bronchi, bronchioles, and ultimately the terminal bronchioles (G16), then the alveolar region (G17 to 23) as shown in Figure 2.2. Progression from the trachea to the extremities of the respiratory tree is characterized by a decrease in the diameter and length of the tubules with each branching and an increase in number of airways which results in an increase of the surface area.

The alveolar sac is composed of two or more alveoli that share a common opening. There are about 300 million alveoli in the lung. The average diameter of the alveolus is about 0.25 mm and its wall is extremely thin. They are surrounded by a network of capillaries as shown in Figure 2.3. In a normal adult the total surface area of the respiratory membrane is about 70 m^2 about half the size of a tennis court (Tortora, 2003).

In the alveolar region the lining is devoid of mucous and consists of simple squamous epithelium. There are two principal types, type I pneumocytes are thin cells for the diffusion of gas and drug molecules and type II pneumocytes are cuboidal cells that store, secret and re-use pulmonary surfactant. The surfactant decreases the surface tension and thereby maintains the morphology and function of the alveoli that is critical for respiration. In a newborn infant, a deficiency of surfactant is known as respiratory distress syndrome (RDS) and in adults as adult respiratory distress syndrome (ARDS). β_2-adrenergic agonists treat this type of respiratory distress by increasing the surfactant secretion from the type II pneumocytes (Isohama et al., 2001; Kresch et al., 1996; Singh et al., 2004). Macrophages are also present on the surface of the alveolar epithelium.

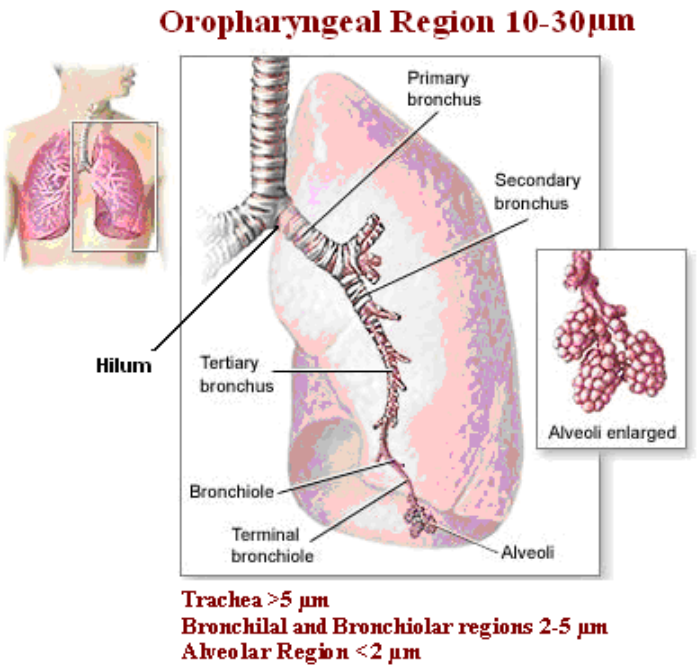


Figure 2.2 Diagram representing airway branching in human lung.

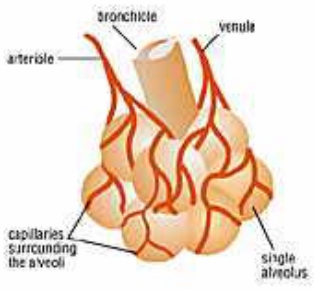


Figure 2.3 The alveolar duct, showing the blood supply to the alveoli.

2.1.2. Ventilation:

The lungs are very elastic and when inflated they are capable of expelling air and return to their original uninflated state, however, even when uninflated the lungs retain some air, which give them their spongy texture. The lungs are contained in the thoracic cavity. Each lung is surrounded by a separate pleural cavity formed by the pleural serous membranes.

The ventilatory apparatus consists of the lungs and surrounding chest wall. The chest wall includes not only the rib cages but also the diaphragm and abdominal wall (Figure 2.4).

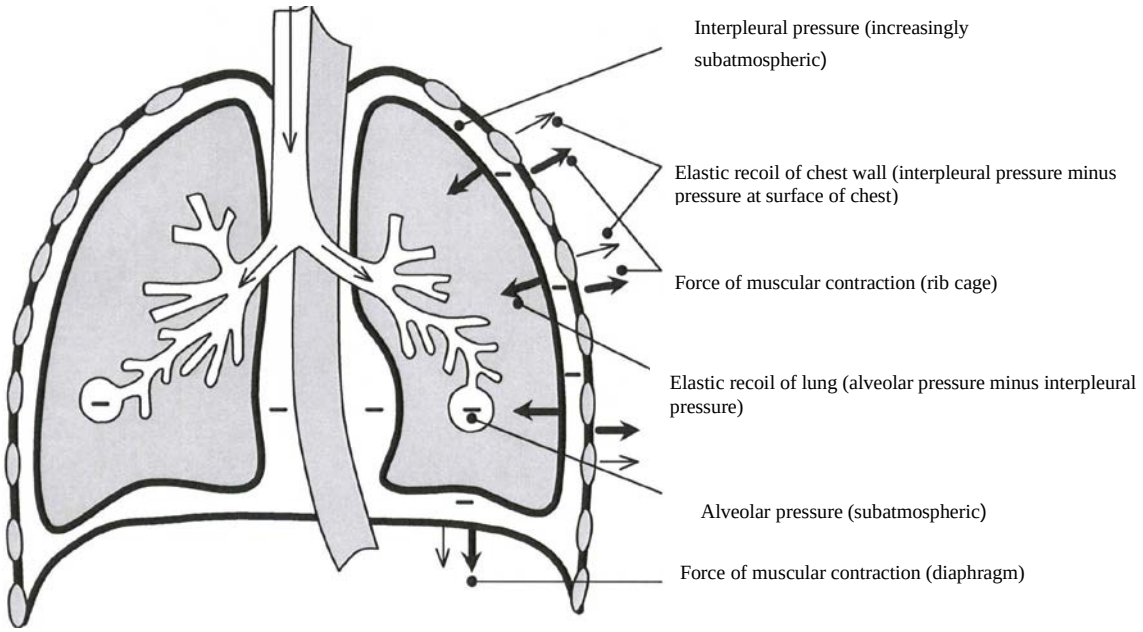


Figure 2.4 Forces and pressures during inspiration [Reproduced from Netter FH (1979)].

The muscles for inspiration include the diaphragm and those muscles that elevate the ribs, and the muscles of expiration consist of those that depress the ribs and sternum each contraction causing inspiration and expiration respectively.

Movement of air into and out of the lungs is driven by pressure differentials or gradients across the lungs. At the end of expiration the atmospheric pressure and the alveolar pressure are equal. Therefore, no movement of air into or out of the lungs takes place.

When inspiratory muscles (diaphragm and intercostal muscles) contract to expand the thoracic cavity, a force is applied to the lung surface, which causes expansion of the lungs. Lung expansion occurs because the lungs are compliant and distensible. By expanding, a negative pressure (approximately -1 cm H_2O) is created within the lungs, specifically in the airways and alveoli. This results in airflow movement in the direction from high to low pressure, which is in the direction of the alveoli. At the end of inspiration the thorax, the lung and the alveoli stop expanding. The atmospheric pressure and the alveolar pressure are now equal and no movements of air into or out of the lungs take place but the volume of the lung is larger than at the end of expiration. During expiration the volume of the thorax decreases as the diaphragm relaxes, and the thorax and the lung recoil. The decreased thoracic volume results in a decrease in the alveolar volume. This causes an increase in the alveolar pressure over the atmospheric pressure of approximately 1 cm H_2O. Hence air flows out of the lungs. As expiration ends, the decrease in the thoracic volume stops, the alveoli stop changing size and the inspiration process starts again.

The flow of air decreases when the resistance to airflow is increased by conditions that reduce the radius of the respiratory passageways. The resistance to airflow is proportional to the radius [r] of the tube raised to the fourth power [r^4] (Seeley, 2000). Hence a small change in the radius results in a large change in the resistance, which in turn decreases airflow. For example, asthma results in the release of inflammatory chemicals such as leukotrienes that cause severe constriction of the bronchioles. Emphysema produces increased airway resistance because the bronchioles are constricted.

2.1.3. Pulmonary volumes, capacity and indices*:*

Spirometry is the process of measuring volumes of air that move into and out of the respiratory system and it is measured by a spirometer. The different parameters describing pulmonary ventilation are shown in Figure 2.5.

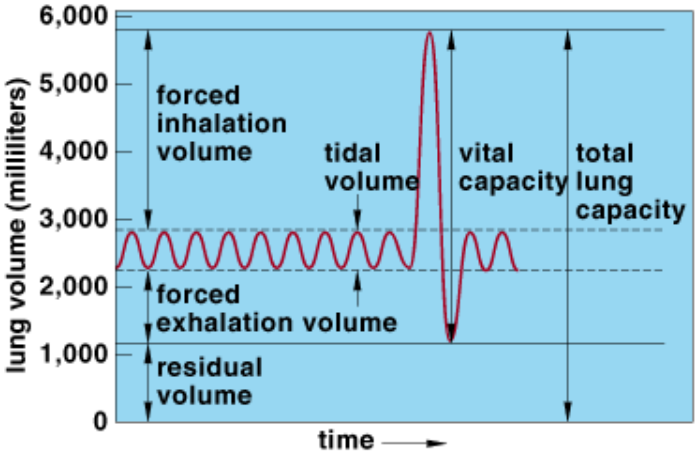


Figure 2.5 Spirometric tracing demonstrating different measures for lung volumes and capacities.

The pulmonary volumes are:

1. Tidal Volume (TV): The volume of air inspired or expired during normal breathing.
2. Inspiratory Reserve Volume (IRV): The amount of air that can be forcefully inspired after inspiration of a normal volume.
3. Expiratory Reserve Volume (ERV): The amount of air that can be forcefully expired after expiration of a normal volume.
4. Reserve Volume (RV): also known as the residual volume. The volume of air still remaining in the respiratory passages and lungs after the most forceful expiration.

Pulmonary capacities are the sum of two or more pulmonary volumes:

1. Inspiratory Capacity (IC): The sum of the TV and the IRV, which is the amount of air that the person can inspire maximally after a normal expiration.
2. Functional Residual Capacity (FRC): The sum of ERV and RV, which is the amount of air remaining in the lung at the end of normal expiration.
3. Vital Capacity (VC): The sum of IRV, TV, and ERV, which is the maximum volume of air that a person can expel from their respiratory tract after a maximum inspiration.

4. Total Lung Capacity (TLC): The sum of IRV, TV, ERV, and RV.

Furthermore performing a vital capacity manoeuvre with as much force as possible provides useful data. These spirometric measurements are the Forced Expiratory Volume in one second (FEV_1), the Peak Expiratory Flow (PEF) and the Forced Expiratory Vital Capacity (FVC).

1. The forced expiratory volume in one second (FEV_1) is the volume of air that is exhaled during the first second of a forced expiratory manoeuvre starting from the level of total lung capacity. FEV_1 is by far the most frequently used index for assessing airway obstruction, bronchoconstriction or bronchodilatation. FEV_1 can be expressed as a percentage of the Forced Vital Capacity (FVC) and as a percentage of predicted. It is the standard index for assessing and quantifying airflow limitation. In adults, but not in children and adolescents, FEV_1% declines with age.

2. Expiratory peak flow (PEF) which is the maximum flow generated during an expiration performed with maximal force and started after a full inspiration.

3. Forced expiratory vital capacity (FVC): The volume change of the lung between a full inspiration to total lung capacity and a maximal expiration to residual volume (RV). The measurement is performed during forceful exhalation. The manoeuvre is almost invariably performed in conjunction with the assessment of the FEV_1 and PEF.

2.1.4. Minute respiratory volume and alveolar ventilation rate:

The minute respiratory volume is the total amount of air moved into and out of the respiratory system each minute, and it is equal to the product of TV and the respiratory rate. Since resting TV is about 500 ml and the respiratory rate is about 12 breaths per minute, the minute respiratory volume average is about 6 L min^{-1}. The minute respiratory volume is not a measure of the amount of air available for gas exchange, which takes place in the alveoli, the alveolar duct, and the respiratory bronchioles. The volume of air

available for gas exchange is called the alveolar ventilation rate (AVR) and it is calculated as follow:

$$AVR=RR\ (TV-DAS)$$

AVR= Alveolar Ventilation Rate (ml/min)

RR = Respiratory Rate (Respiration/min)

TV = Tidal Volume (ml/ respiration)

DAS = Dead Air Space (ml/respiration)

The Dead Air Space is about 150 ml (Nasal cavity, pharynx, larynx, trachea, bronchi, bronchioles and terminal bronchioles) so the alveolar ventilation rate is 4.2 L min^{-1}.

2.2. Diseases of the Respiratory tract:

2.2.1 Asthma

Asthma (reversible airway obstruction) is a disease characterised by an increase in the constriction of the bronchi in response to various stimuli, resulting in a narrowing of the air passageways and decreased ventilation efficiency followed by inflammatory changes. Symptoms include dyspnea, wheezing, coughing, shortness of breath, a tight feeling in the chest and poor exercise tolerance. A cough producing sticky mucus can also be a symptom of asthma. The aetiology of asthma is poorly understood, but asthma is described as extrinsic when it is associated with exposure to a specific allergen, such as pollen, dust, or non-specific stimulus such as chemical irritant or exercise. It may be described as intrinsic when no external precipitating factor is identified. These triggers can cause the asthmatic's lungs to release chemical mediators such as histamine and leukotrienes released from the mast cells. These chemical mediators cause inflammation to the bronchial lining, constriction, and bronchial spasms. If the effect on the bronchi becomes severe enough to obstruct exhalation, carbon dioxide could build up in the lungs and might lead to unconsciousness or death.

In contrast to many other respiratory disorders, the symptoms of asthma typically reverse either spontaneously or with therapy. The exact cause of asthma is unknown. There are no definitive pathological features or diagnostic tests for asthma, but the three important features of the disease are chronic airway inflammation, airway hyperreactivity and airway obstruction.

Asthma is a form of hypersensitivity in which the bronchioles in the lungs are narrowed by inflammation and spasm of the lining of the airway wall. Inflammation occurs when irritated tissues swell and produce extra mucus, creating a condition known as bronchoconstriction as shown in Figure 2.6 (British Thoracic Society, 2007). Constriction or complete blockage of the airways can initiate symptoms of an asthma attack. During normal breathing, air is taken in through the nose and mouth. It goes down the windpipe, through the airways and into the air sacs. When breathing out, air is expelled from the lungs in the reverse order. During an asthma attack, the muscles around the airways tighten thereby making the opening in the airways smaller. The lining of the airways swells due to inflammation and oedema together with an increase in mucus that blocks the airways. Because it is more difficult to breathe out than to breathe in during acute bronchospasm then more air is retained in the air sacs in of lungs with each breath. It is this (the air- trapping phenomena) that requires emergency treatment because during an acute exacerbation this could lead to death.

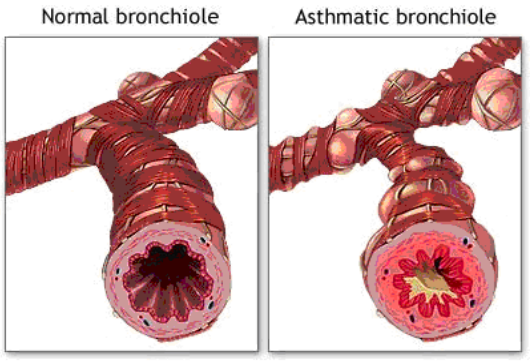


Figure 2.6 Normal bronchiole and asthmatic bronchioles (reproduced from WebMD medical reference 2005, www.mywebmd.com).

The changes that take place in the lungs of an asthmatic make the airways (bronchi and the smaller bronchioles) hyperreactive to many different types of stimuli that do not affect healthy lungs. In an asthma attack, the muscle tissues in the walls of the bronchi go into spasm, and the cells lining the airways swell and secrete mucus into the air spaces.

Many patients with asthma are prone to react to such foreign substances as pollen, house dust mites, or animal dander; these are called allergens. On the other hand, asthma affects many patients who are not allergic in this way. Asthmatic symptoms are usually quite variable; someone with asthma may go for periods of time without symptoms, and then suddenly have severe episodes for days. The most common symptom recognized by both physicians and patients is wheezing. Wheezing is a whistling or rumbling sound that comes from the chest expiration. It may be very loud or barely audible. Asthma can be classified as mild, moderate and severe according to the degree of obstruction and its severity. Asthma usually begins in childhood or adolescence, but it also may first appear during the adult years. While the symptoms may be similar, certain important aspects of asthma are different in children and adults. Children born to families with a history of allergies or asthma are more likely to have asthma. Asthma could also be occupational if the worker is exposed to a substance that causes narrowing or spasm of the windpipe. The term ‘Brittle Asthma’ is used when the asthma can change from being apparently well controlled to poorly controlled in a short space of time.

During an acute attack, the respiratory rate is rapid and tachycardia is common. The peak expiratory flow (PEF) and forced expiratory volume in one second (FEV_1) decrease to less than 50% of the subject’s predicted values. Life threatening features include exhaustion, cyanosis, bradycardia, hypotension, confusion, and coma.

2.2.2 Chronic Obstructive Pulmonary Disease (COPD)

Chronic obstructive pulmonary disease (COPD) is a major cause of ill health and mortality world wide, and is the only leading cause of death that is predicted to increase

over the coming years. Survey findings have shown that primary care physicians wrongly diagnose COPD and asthma (Pauwels et al., 2001). Chronic obstructive pulmonary disease (COPD) is characterized by airflow obstruction. The airflow obstruction is usually progressive, not fully reversible and does not change markedly over several months. The disease is predominantly caused by smoking (National Institute for Health and Clinical Excellence (NICE), 2004; Global Initiative for Chronic Obstructive Lung Disease (GOLD), 2007). The defining feature of chronic obstructive pulmonary disease (COPD) is irreversible airflow limitation measured during forced expiration (Global Initiative for Chronic Obstructive Lung Disease (GOLD), 2007) which is the result of a prolonged time constant for lung emptying caused by either an increase in the resistance of the small conducting airways (Yanai et al., 1992), an increase in lung compliance due to emphysematous lung destruction, (Mead et al., 1967) or both. Hence COPD includes a number of different disease processes, which result in airflow obstruction due to a combination of damage to the airways and lung tissue. Irritants like cigarette smoke, air pollution, or infection can produce a chronic inflammation of the bronchi (Jensen et al., 2000). The inflammation results in swelling of the mucous membrane lining the bronchi, an increase in the mucous production and a decrease in the movement of mucous by the cilia. If the irritant persist then the diameter of the bronchi decreases and ventilation is impaired causing bronchitis. Also emphysema results in the destruction of the alveolar walls. Loss of the alveolar walls decreases the respiratory membrane surface area, decreasing the gas exchange and loss of elastic fibers that decrease the ability of the lung to expel air out. Bronchitis and emphysema result in COPD.

Figure 2.7 reproduces classic data from a study by Fletcher et al (1977) showing the different rates of decline in FEV_1 with age for non-smokers and smokers who either do or do not develop COPD. The horizontal lines have been added to show the boundaries of COPD severity recommended by a global initiative on obstructive lung disease (GOLD)

(Global Initiative for Chronic Obstructive Lung Disease (GOLD), 2007). Fletcher et al (1977) showed that the rate of decline in the FEV_1 of many people who smoke is similar to that of non-smokers. These investigators also showed that in a susceptible minority of tobacco smokers (estimated at 15–20% of the total), lung function declines rapidly to levels consistent with moderate (GOLD 2), severe (GOLD 3), and very severe (GOLD 4) COPD. Their data also showed that stopping smoking had a beneficial effect on stopping the fast rate of decline at any age.

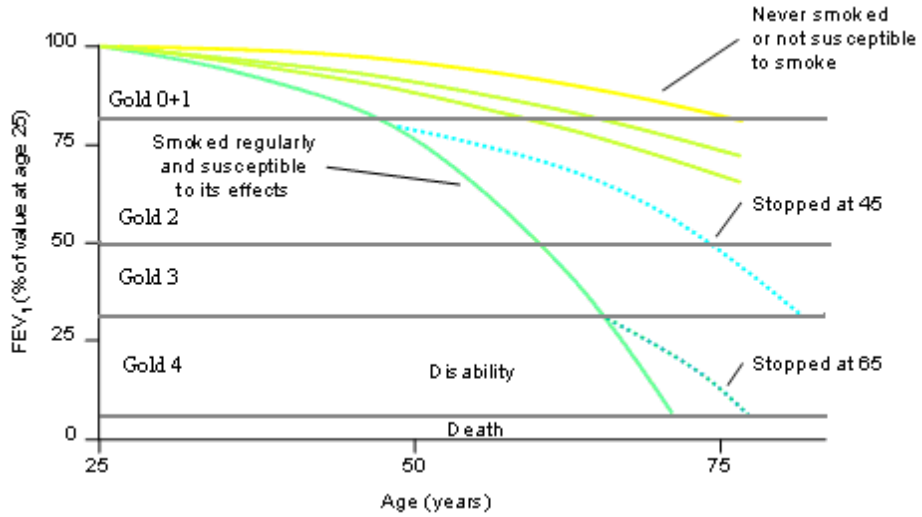


Figure 2.7 Natural history of chronic obstructive pulmonary disease at varying age population [Reproduced from Fletcher and Peto (1977)].

Although this shows rate of loss of FEV_1 for one particular susceptible smoker, other susceptible smokers will have different rates of loss, thus reaching disability at different ages (Fletcher and Peto, 1977) as the lung inflammation is present in everyone with a tobacco smoking habit (Hogg, 2004). The reason why only a minority of smokers experience an excessive decline in FEV_1 is unknown, but preliminary evidence suggests that the lung inflammatory response is amplified in the susceptible group (Retamales et al., 2001). Figure 2.7 highlights why smoking cessation is the first intervention for COPD patients as recommended by the NICE guidelines, whereas long-term oxygen therapy is able only to prolong survival in severe COPD patients (Gorecka et al., 1997).

Pharmacological therapies are aimed at relieving symptoms and reducing exacerbations of the disease.

COPD is a slow progressive disease usually following many years of smoking, although other risk factors, usually secondary, may also be responsible as shown in Table 2.1. COPD is not common in someone who has never smoked. Those non-smokers that develop COPD usually have a deficiency of alpha-antitrypsin.

Table 2.1 Associated risk factors for COPD.

- Tobacco exposure.
- Alpha-1 antitrypsin deficiency.
- Occupational exposure e.g. cadmium, silica or dusty environments.
- Low social class.
- Diet deficient in vitamin C.
- Pre-existing bronchial hyper-responsiveness.
- Low birth weight.
- Childhood respiratory infections.

However, there are some smokers that are not at risk, for reasons that are not fully understood, it may be relate to an individual's genetic profile that give rise to α_1 antitrypsin deficiency with resultant low levels of protease inhibitors in those smoker who develop COPD (Figure 2.8).

There has been an alarming increase in the hospital admissions in the past few years and this has created excess pressure on the hospitals as shown in Figure 2.9.

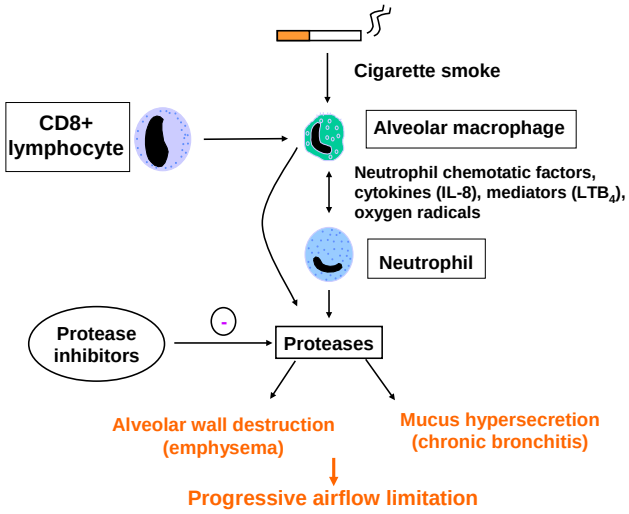


Figure 2.8 Disease processes in chronic obstructive pulmonary disease [Reproduced from Barnes (2000)].

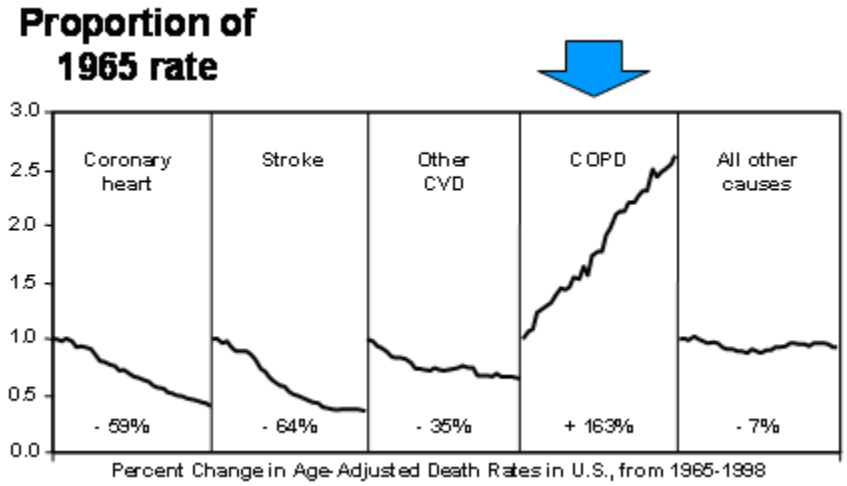


Figure 2.9 Increase in the death of patients suffering from chronic obstructive pulmonary disease (reproduced from Gold Guidelines http://www.goldcopd.com).

2.2.4 Difference between COPD and asthma

Although COPD and asthma have similar characteristics such as the signs of coughing and wheezing, they are two different conditions in terms of cause, disease onset, frequency of symptoms and reversibility of airway obstruction.

1. The onset of asthma typically occurs during childhood or adolescence (British Thoracic Society, 2007). COPD most often develops in smokers and former smokers who are in their mid-50s (Petty, 1995; Hogg, 2004).

2. Exacerbations of asthma - characterized by recurrent wheezing, shortness of breath, chest tightness and cough - often have identifiable triggers such as allergens, cold air, exercise, viral infection or bacterial infection (British Thoracic Society, 2007). However, exacerbations in COPD patients are commonly caused by respiratory tract infections (Pauwels et al., 2001).

3. With treatment the aim is for asthma patients to have near-normal lung function and be symptom-free between exacerbations (British Thoracic Society, 2007). COPD patients rarely experience a day without symptoms. Airflow obstruction in COPD sufferers is only partially reversible (National Institute for Health and Clinical Excellence (NICE), 2004).

4. In COPD patients there are more neutrophils compared to patients suffering from asthma. In asthmatic patients the percentage of eosinophils is more compared to patients suffering from COPD. Since glucocorticoids are effective against inflammation caused by eosinophils (Altman et al., 1981) then these agents are useful in asthma. Inflammation that is mediated by the neutrophils is more resistant to the effect of glucocorticoids agents.

Smoking cessation decreases the accelerated downward progression of lung function and breathlessness whilst bronchodilator use provides symptom relief (British Thoracic Society, 2007). Despite these differences, COPD is often misdiagnosed, and persons with COPD are treated instead for asthma (British Thoracic Society, 2007). In fact, a survey of 75 primary care physicians revealed that they prescribe similar medications for COPD and asthma even though the treatments differ (Kesten and Chapman, 1993).

The maintenance therapy for most patients with asthma is an inhaled corticosteroid to control inflammation, with the addition of a bronchodilator, when required to control symptoms (British Thoracic Society, 2007). However, the reverse is true for the treatment

of COPD. Bronchodilators are the first line maintenance treatment for COPD. Treatment with inhaled corticosteroids is reserved only for selected patients whose COPD is not adequately managed with bronchodilators (British Thoracic Society, 2007) that have moderate or severe COPD (FEV_1 50% predicted) and have frequent exacerbations (National Institute for Health and Clinical Excellence (NICE), 2004) A summary of the differences between COPD and asthma are shown in Table 2.2.

Table 2.2 Differential diagnosis for chronic obstructive pulmonary disease and asthma.

	COPD	**Asthma**
• **Smoker or ex-smoker**	Nearly all	Possibly
• **Symptoms under age 45**	Rare	Often
• **Chronic productive cough**	Common	Uncommon
• **Breathlessness**	Persistent and progressive	Variable
• **Night time waking with breathlessness and or wheeze**	Uncommon	Common
• **Significant diurnal or day to day variability of symptoms**	Uncommon	Common

2.2.5. Asthma Management

The treatment of asthma involves avoiding the causative stimulus if it is of the extrinsic type and administrating drug therapy (including prophylactic measures to reduce inflammation and airway resistance and to maintain flow with specific treatment for the acute attacks). Corticosteroids, leukotriene inhibitors and mast cell stabilizer, which prevent the release of chemical mediators from the mast cells, are used to reduce airway inflammation. Xanthines (e.g. theophylline), antimuscarinics (e.g. ipratropium and tiotropium) and β_2-adrenergic agents (e.g. salbutamol, Terbutaline) are commonly used to provide bronchodilatation.

The British Thoracic Society (BTS) has set step guidelines for the treatment of asthma. A summary of these guidelines are shown in Figure 2.10.

Step 1 of the BTS guidelines (British Thoracic Society, 2007) state that initially a short acting β_2-agonist (e.g. salbutamol, terbutaline) should be inhaled when required for symptomatic relief to open up the airways. There is no consistent evidence of any benefit from the regular (four times daily) use of short acting β_2-agonists compared with as required use (Dennis et al., 2000; Walters et al., 2003). Unless individual patients are shown to benefit from the regular use of inhaled short acting β_2-agonists then as required use is recommended. Using two or more canisters of β_2-agonists per month or >10-12 puffs per day is a marker of poorly controlled asthma (Scottish Intercollegiate Guidelines (SIGN), 2002b). If the patient is using a short acting β_2-agonist on a regular use, basis anti-inflammatory therapy is recommended.

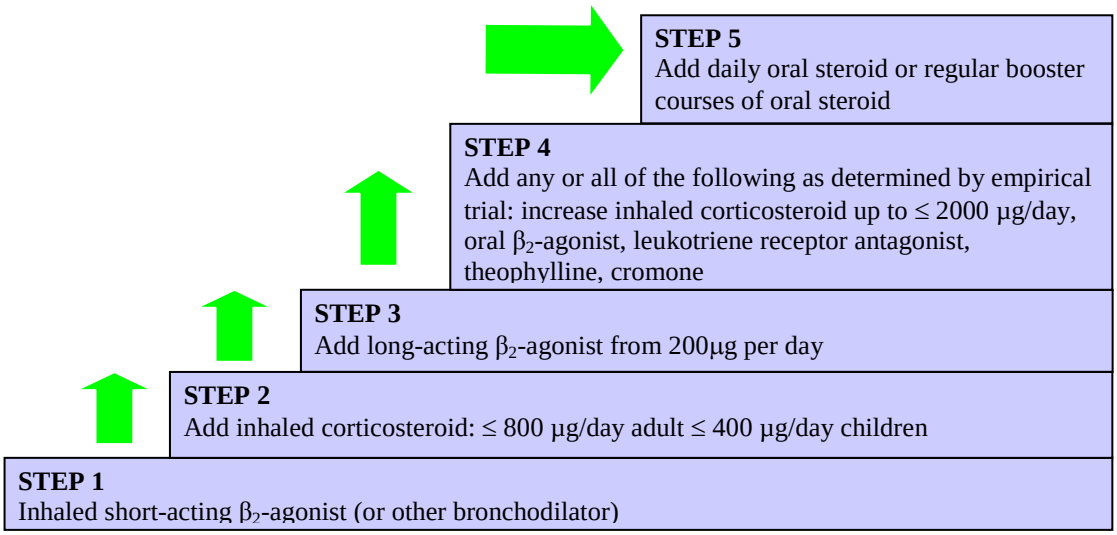


Figure 2.10 Proposed BTS / SIGN guidelines for the treatment of asthma (www.brit-thoracic.org.uk).

Step 2 has been judged on the ability to improve symptoms, improve lung function, and prevent exacerbations, with an acceptable safety profile. Inhaled steroids are the most effective therapy for asthmatic patients to achieve overall treatment goals (Scottish Intercollegiate Guidelines (SIGN), 2002d; Adams et al., 2005). The exact threshold for the

introduction of inhaled steroids has never been firmly established. Two recent studies have shown a benefit from the regular use of inhaled steroids in patients with mild asthma (O'Byrne et al., 2001; Pauwels, 2003). Benefit in these studies was seen even with an FEV_1 of 90% of predicted. Inhaled steroids should be considered for patients with any of the following (British Thoracic Society, 2007):

- Exacerbations of asthma in the last two years
- Using inhaled short acting β_2-agonist three times a week or more
- Symptomatic three times a week or more, or waking one night a week.

The lowest dose of inhaled steroids should be initiated at which effective control of asthma is maintained. Literatures also suggest the use of twice daily dosing is more effective than single dose (Scottish Intercollegiate Guidelines (SIGN), 2002c). However some inhaled steroids that have recently been introduced are once daily regiment (e.g. ciclesonide)

Step 3 focuses on add on therapy along with steroids. The use of high doses of steroids causes frequent side effects in patients. The BTS guidelines recommend a trial with add-on medications before stepping up the dose of steroids.

The first recommended choice is the use of a long acting β_2-agonist like formoterol or salmeterol to improve lung functions and symptoms and to decrease exacerbations (Scottish Intercollegiate Guidelines (SIGN), 2002a). Long acting β_2-agonist should always be used with an inhaled steroid and not alone in the management of asthma.

Step 4 recommends an increase in the dose of steroids if there is poor control when prescribed a moderate dose of inhaled steroid and add-on therapy. If there is a response to long acting β_2-agonist but control remains poor then the dose of the inhaled steroid should be increased and long acting β_2-agonist should be continued. If the add-on therapy still remains inadequate the use of leukotriene receptor antagonists, theophyllines, slow release β_2-agonist tablets are recommended in step 4 (Scottish Intercollegiate Guidelines (SIGN),

2002a). The maximum steroid dose that is recommended is equivalent to beclomethasone 2000 µg per day.

Step 5 recommends the use of oral steroids using the lowest dose that provides adequate control. A high dose of inhaled steroid at 2000 µg per day is advised. Consideration for other treatments to minimise the use of steroid tablets are also suggested.

It should be noted that the guidelines stated that before stepping up the patient treatments the patient's inhalation technique should be checked and compliance assessed.

2.2.6. Management of COPD

The diagnostic label COPD includes emphysema and chronic bronchitis with many patients presenting with both.

The goals of management of COPD are to:

- Enable early and accurate diagnosis
- Control symptoms
- Prevent deterioration
- Prevent complications
- Improve quality of life

2.2.6.1. Diagnosis and assessment

COPD produces a decrease in the peak expiratory flow (PEF) and forced expiratory volume in one second (FEV_1). The BTS/NICE guidelines have staged the disease severity as mild, moderate and severe (Table 2.3) on the basis of spirometry measurements, using the FEV_1.

Although by definition COPD is an irreversible disease, reversibility testing is important in assessing both diagnosis, prognosis of COPD and determining treatment choice. Hence the results of reversibility tests are important for future management, they should be clearly documented and be easily available for future reference. There are patients with an element of reversibility and those do respond to inhaled corticosteroids. Whether or not there is an association with asthma is difficult to identify.

Reversibility should be assessed in all COPD patients to detect those whose FEV_1 increases substantially after bronchodilator use. FEV_1 should be measured either before and 15 minutes post nebulised salbutamol (2.5-5 mg) or terbutaline (5-10 mg) or before and 30 minutes post nebulised ipratropium bromide (500 µg). These tests should be done when patients are clinically stable and free from infection. Patients should not take short acting bronchodilators within the previous six hours, long acting β_2-agonists in the previous twelve hours and sustained release theophylline within the previous 24 hours. Significant reversibility is present if the FEV_1 increases by more than 200 ml or by 15% over the pre-bronchodilator value.

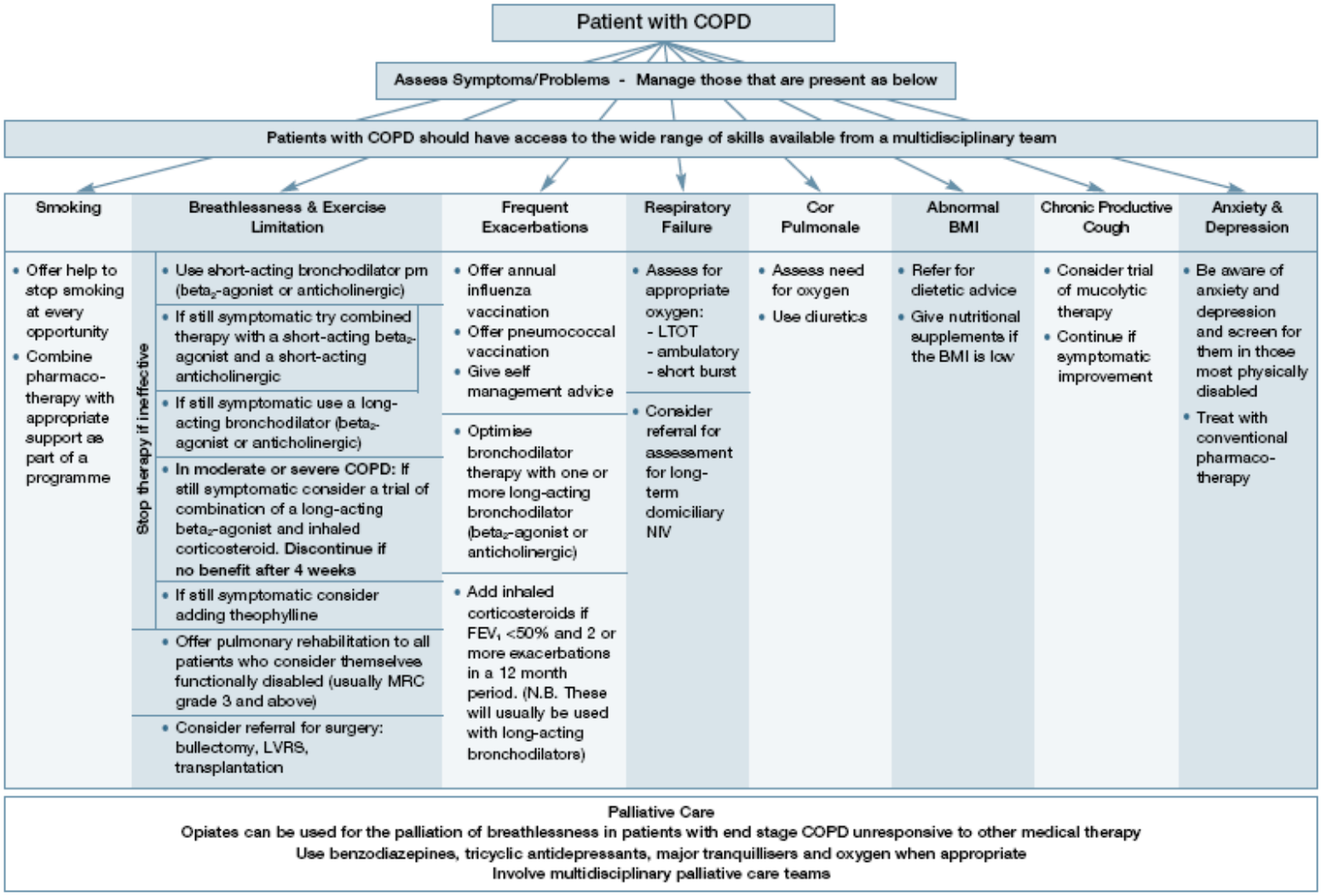
Table 2.3 Recommendations for staging disease severity in chronic obstructive pulmonary disease.

Disease Severity	FEV_1 % predicted	Symptoms and signs
Mild	≥80	No abnormal signs Smoker's cough Little or no breathlessness
Moderate	<50	Breathlessness with or without wheeze on moderate exertion. Cough with or without sputum. Variable abnormal signs (general reduction in breath sounds, presence of wheezes)
Severe	<30	Breathlessness on any exertion/at rest Wheeze and cough often prominent Lung overinflation usual with cyanosis, peripheral oedema and polycythaemia in advanced disease, especially during acute exacerbations

2.2.6.2. Treatment of stable COPD

A summary of National Institute for Clinical Excellence (NICE) therapeutic recommendation gives a broad outline to treat and stabilise COPD. A schematic design of the NICE guidelines recommendation for the therapeutic management of patients with stable COPD is described in Table 2.4.

Table 2.4 Summary of the recommended managements of stable COPD [reproduced from (National Institute for Health and Clinical Excellence (NICE), 2004)]



2.2.6.2.1 Smoking cessation

All COPD patients should be encouraged to stop smoking as this is the most effective way to improve outcomes and prevent further accelerated airway obstruction (Fletcher and Peto, 1977; National Institute for Health and Clinical Excellence (NICE), 2004). If patients continue to smoke, lung function will deteriorate at an accelerated rate which cannot be prevented by drug therapy. While stopping smoking may not improve lung function, it will stop the progress of the accelerated decline and, in 90% of cases, excess sputum production may cease (Fletcher and Peto, 1977; Hogg, 2004).

2.2.6.2.2 Bronchodilators

Even if the bronchodilator reversibility test does not show an objective improvement in lung function (i.e. the test is negative), inhaled bronchodilators may still reduce symptoms. Therefore all COPD patients should be given an inhaled bronchodilator to provide relief of symptoms (Celli et al., 2004). If they help the patient to perform normal daily activities or to improve exercise tolerance, it is worthwhile continuing this treatment (National Institute for Health and Clinical Excellence (NICE), 2004).

Inhaled short-acting β_2-agonists (e.g. salbutamol, terbutaline) have a relatively rapid onset of action and are often used as required to relieve symptoms (National Institute for Health and Clinical Excellence (NICE), 2004). Inhaled antimuscarinics (e.g. ipratropium bromide) are as efficacious as short-acting β_2-agonists in COPD and may provide a greater and longer bronchodilator response. However, due to their slower onset of action, antimuscarinics may be less suitable for symptom relief than β_2-agonists.

Antimuscarinics may be added or substituted for short-acting β_2-agonists where adequate control of symptoms is not seen or where regular maintenance therapy is required. The concurrent use of short-acting β_2-agonists with an antimuscarinic is not recommended unless the single drugs do not provide adequate symptom relief. Clinical trial evidence also recommends the use of long-acting β_2-agonists (e.g. salmeterol and formoterol) in

COPD. Long-acting β_2-agonists have a prolonged duration of action from 12-14 hours. In addition to their bronchodilator action, long-acting β_2-agonists also inhibit mast cell mediator release, plasma exudation and may reduce sensory nerve activation (Nials et al., 1994).

Long-acting antimuscarinic bronchodilators show muscarinic M_1 and M_3 receptor subtype selectivity. Tiotropium bromide, the first of a new class of selective and long-acting antimuscarinic agents was introduced for once daily maintenance treatment of COPD patients. The combination of long-acting β_2-agonists and tiotropium bromide exhibited additive effects in terms of daytime lung function improvements and sustained improvements during the night compared with the single components, despite the once daily dosing (Cazzola et al., 2004; Cazzola et al., 2005; van Noord et al., 2005). The NICE / BTS guidelines recommend that the combination of two long-acting bronchodilators with different pharmacological mechanisms of action should be considered in all patients with moderate to severe chronic obstructive pulmonary disease. The rational for using both of the anticholinergics and the β_2-agonist is that the anticholinergics will inhibit the vagal tone that exists in COPD. This will help the airways to relax so when the β_2-receptors are stimulated the bronchodilatation should be enhanced. A combination treatment of tiotropium and formetrol was more effective than the single agents with respect to bronchodilation in COPD patients (Cazzola et al., 2004; Cazzola et al., 2005). These two studies highlighted the value of using a long acting β_2-agonist together with a long acting anticholinergic agent. When a long-acting antimuscarinic agent like tiotropium bromide is used then the patient should not use a short-acting antimuscarinic agent like ipatropium bromide.

There is limited evidence showing the benefit of theophylline in COPD. In addition, theophylline may cause serious side effects which may occur within the normal dosage range. Such effects may be potentiated by concomitant drug therapy (e.g. erythromycin,

quinolone antibiotics). The common side effects of theophylline when the peak serum concentration reaches >20 mg L^{-1} are nausea, vomiting, headache and insomnia. As the peak serum concentration levels increase the side effects increase e.g. nausea, vomiting, stomach irritation, headache, cardiac arrhythmias and intractable seizures are produced. The use of theophyllines is therefore not strongly recommended and limited to those in whom other treatments have failed to control symptoms. Mucolytic therapies have recently had a lot of interest and should be considered in patients with a chronic productive cough. The aim of treatment is to reduce the frequency of cough and sputum production. A meta- analysis of such agents reveal that when used for more than two months there is a reduction in exacerbations by 29% correspond to placebo (Poole and Black, 2001).

2.2.6.2.3 Corticosteroids

The pathogenesis of airway obstruction in COPD is multifactorial, involving neutrophilic airway inflammation (Stanescu et al., 1996), protease-antiprotease imbalance (Tetley, 1993), oxidative stress (Repine et al., 1997), and recurrent infection. These mechanisms are interrelated such that reducing one factor may also reduce the stimulus to others.

An increased number of neutrophils are present in the lungs of cigarette smokers compared with that in nonsmokers. Cigarette smoke may attract neutrophils to the lung by stimulating alveolar macrophages to release a potent chemotactic factor for neutrophils (Hunninghake and Crystal, 1983). These increased neutrophils are associated with a rapid decline in the FEV_1 (Stanescu et al., 1996). Furthermore, neutrophil activation markers are elevated in the sputum supernatants of subjects with COPD (Keatings and Barnes, 1997), suggesting that neutrophils are active participants in airway inflammation. There is still an ongoing debate about the benefit of inhaled or oral corticosteroids in patients with stable COPD. While corticosteroids have no effect on neutrophilic inflammation they may also influence the cyctokine level (Keatings et al., 1996).

Inhaled budesonide was found to be of no clinical benefit in COPD patients recruited from the general population by screening (Vestbo et al., 1999). Although the ISOLDE study (Burge et al., 2000) showed benefits from inhaled fluticasone the TORCH study (Calverley et al., 2003) did not. Only those COPD patients with a positive response to a corticosteroid reversibility test should be considered for inhaled steroid therapy without long-acting β_2-agonists. It has also been noted that the combination of corticosteroid and long-acting β_2-agonists in a single inhaler improved lung function and severity of dyspnoea in patients with COPD (Cazzola and Dahl, 2004). The TORCH (Towards a revolution in COPD Health) has also been published and consolidated these findings (Calverley et al., 2003). A similar study by Szafranski et al (2003) has shown the benefit of budesonide in combination with formoterol (Szafranski et al., 2003). The NICE guidelines suggest that if a patient is prescribed a long-acting β_2-agonists and has a FEV_1 <50% with two or more exacerbations per year than a high dose of inhaled corticosteroid with a long-acting β_2-agonists should be considered. Recently, from the results of the TORCH study, the prescription licence for Seretide (Fluticasone and Salmeterol) has been changed to allow those with a FEV_1 <60% to be prescribed this inhaled combination.

Also the benefit of the combination of long acting β_2-agonist and an inhaled corticosteroid in a MDI was demonstrated in a study by Theophilus et al (2006). They demonstrated that there is a significant co-association of salmeterol and fluticasone propionate particles, leading to increased co-deposition when they are administered from the same inhaler. This provides a greater opportunity for a synergistic interaction between the two drugs to occur in the airways (Barnes et al., 2006) and may possibly be a significant factor contributing to the enhanced clinical effect seen in comparison with that observed when the drugs are administered separately from two inhalers (Theophilus et al., 2006).

Furthermore, COPD patients may develop Cor Pulmonale (a secondary heart disease) with pulmonary hypertension, right ventricular hypertrophy and right heart failure. Patients

with COPD frequently suffer acute exacerbations of their symptoms, and may require hospitalization (Vestbo et al., 1999). Treatment options include antibacterial agents and oxygen as necessary, together with appropriate managements of any associated cardiovascular disorder (Hogg, 2004; Martindale, 2002; Vestbo et al., 1999).

2.2.6.2.4 Supplemental long-term oxygen therapy (LTOT)

Supplemental long-term oxygen therapy (LTOT) improves survival, exercise, sleep and cognitive performance in hypoxaemic patients (Eaton et al., 2004; Global Initiative for Chronic Obstructive Lung Disease (GOLD), 2007). Arterial blood gas (ABG) assessment is the preferred method to determine oxygen need because it includes acid-base information. Arterial oxygen saturation as measured by pulse oximetry (SpO_2) is used as well in determining oxygen need. Physiological indications for oxygen include an arterial oxygen tension (PaO_2) <7.3 kPa (55 mmHg). The therapeutic goal is to maintain SpO_2>90% during rest, sleep and exertion. As a general principle, prevention of tissue hypoxia displaces CO_2 retention concerns. If CO_2 retention occurs, it is suggested to monitor for acidemia. If acidemia occurs, mechanical ventilation is essential for the survival of the patient.

Mechanical ventilation can be administered via non-invasive or invasive ventilation. Non-invasive is preferred whenever possible. Mechanical ventilation, either "invasive" or "non-invasive", is not a therapy but it is a form of life support until the cause underlying the acute respiratory failure is reversed with medical therapy (British Thoracic Society, 2007) Patients considered for mechanical ventilation should have a measurement of ABGs.

The institution of mechanical ventilation should be considered when, despite optimal medical therapy and oxygen administration, there is acidosis (pH<7.35), hypercapnia [$PaCO_2$>6–8 kPa (45–60 mmHg)] and respiratory frequency >24 breaths min^{-1}.

Non-invasive mechanical ventilation (NIV) can be instituted by two methods: non-invasive positive pressure ventilation (NPPV) by nasal or face masks; or negative pressure

ventilation (e.g. iron lung, not recommended). NPPV is by far the most popular mode of providing non-invasive ventilation. It is typically administered as a combination of continuous positive airway pressure [CPAP] plus pressure support ventilation [PSV] (Elliott et al., 1990; Ambrosino et al., 1995; Brochard et al., 1995; Lightowler et al., 2003; British Thoracic Society, 2007). The combination of some CPAP (e.g. 4–8 cmH_2O) and PSV (e.g. 10–15 cmH_2O) provides the most effective mode of NPPV. In the first hours, NPPV requires the same level of supervision as conventional mechanical ventilation. Contraindications for NPPV include the following: respiratory arrest; cardiovascular instability (hypotension, arrhythmias, myocardial infarction); impaired mental status, somnolence, inability to cooperate; copious and/or viscous secretions with high aspiration risk; recent facial or gastrooesophageal surgery; facial trauma and/or fixed nasopharyngeal abnormality; burns; and extreme obesity (Plant and Elliott, 1998). NPPV can be considered successful when ABGs and pH improve, dyspnoea is relieved, the acute episode resolves without the need of endotracheal intubation, mechanical ventilation can be discontinued and the patient is discharged from the hospital.

2.3 Pulmonary drug delivery:

Pulmonary drug delivery by inhalation is primarily used to treat conditions of the airways by delivering locally acting drugs to their site of action thereby reducing the dose needed to produce a pharmacological effect with minimal systemic effects (Dhand, 2000; Hillery et al., 2001; Kondili and Georgopoulos, 2002). Direct delivery of drug to the airways enables a rapid onset of action, avoids the gastrointestinal upset of oral therapy, and avoids the first pass effect in the intestine and the liver if this occurs (Barnes, 2004b; Dhand, 2000; Kondili and Georgopoulos, 2002; Witek, 2000). The lung may additionally be employed as a route for delivery of drugs into the systemic circulation. The type of drug and device used are very important factors in targeting drug to the respiratory tract (Barnes, 2004b; Bisgaard, 1998).

2.3.1. Mechanism of particle deposition in the airways:

There are three principal deposition mechanisms operating within the lower respiratory tract. The first mechanism is **inertial impaction** which is the dominant deposition mechanism for particles in the upper respiratory tract (mouth, pharynx, larynx, and tracheobronchial region). A particle with a large momentum (the product of velocity and mass) is unable to follow the changing direction of the inspired air as it passes the bending and branching of the upper respiratory tract. This large momentum leads to impaction on the airway walls. The probability of impaction is dependent upon the momentum, thus particles with a large diameter or high density travelling in the airstreams at higher velocity will show greater impaction. The airflow velocity in the main bronchi is estimated to be 100-fold higher than that in the terminal bronchioles, and 1,000 fold higher than in the alveolar region (Hillery et al., 2001).

The second mechanism of deposition is **sedimentation**. When the airstream velocity is low the particles will settle down under the effect of gravity. This occurs in the bronchioles and the alveolar region where the airflow is low. The fraction of particles deposited by this mechanism will be dependent upon the time the particles spend in these regions. Holding the breath after an inhalation increases the time the particles spend in these regions thus increases deposition by this mechanism (Hillery et al., 2001).

The third principle mechanism of deposition is **Brownian diffusion**. This usually occurs for particles with a particle size lower than 1μm, as particles below this size are displaced by random bombardment of gas molecules, which results in particle collision with the airway walls. The probability of particle deposition by diffusion increases as the particle size deceases and it is also more prevalent in regions where airflow is very low or absent, e.g. in the alveoli. Holding the breath after an inhalation increases deposition by this mechanism. Figure 2.11 shows a diagram of the above mentioned three mechanisms (Hillery et al., 2001).

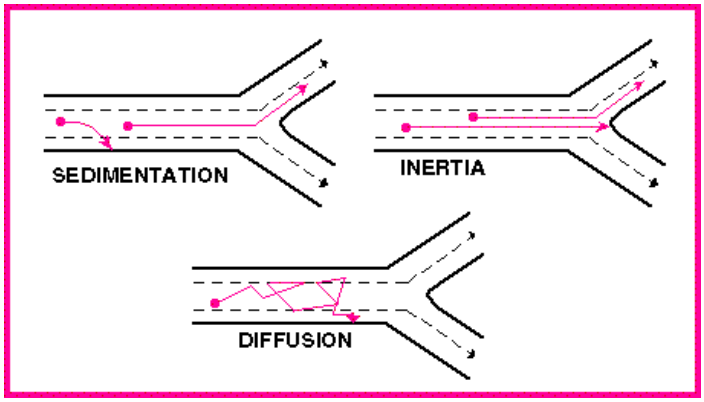


Figure 2.11 Particle deposition mechanisms at airway branching site.

From the above three mechanisms it is obvious that the lung morphology affects the particle deposition as the particles with large size are forced to deposit in the upper airways rather than stay airborne. To stay airborne particles must passes through a successive series of branching tubes of constantly decreasing size and must continually change directions. The increase in the inspiratory flow will also affect the particle deposition, as it will enhance deposition by impaction in the first few generations of the tracheobronchial region and also increase turbulence, particularly in the larynx and trachea, which itself will enhance impaction. The effect of the inspiratory flow on deposition from devices that use the energy of inspiration to generate the drug aerosol, e.g. dry powder inhalers (DPIs), are more complex, because the increase in the inspiratory flow will in most cases lead to the production of aerosol of smaller particle size.

An increase in inspiratory volume increases the penetration of the aerosol particles deeper into the lung thereby increasing the chance of deposition within the alveolar region. Breath holding also increases sedimentation and diffusion as it increases the time that particles stay in the lung and is used to optimize the pulmonary drug delivery (Dhand and Fink, 1999).

Pharmaceutical factors such as the aerosol velocity, particle size distribution generated from the aerosol, shape of the particle, density and its physical stability may also affect the

deposition. In general, for effective particle deposition in the lower airways, the aerodynamic diameter of particles should be <5 μm, however, particularly for patients with obstructive lung disease, all particles should ideally be within the 2–3 μm range (Terzano, 2001).

2.4. Inhalation devices:

Although there are several new treatments for asthma, which may be given orally (more specific drugs) or via injection (monoclonal antibodies), it is unlikely that these will be as effective as inhaled β_2-agonists or corticosteroids. Hence, inhaled therapy is likely to predominate for asthma treatment (Barnes, 2004a).

From a pharmaceutical perspective, an ideal inhaler would have the following characteristics; ease of manufacture, no propellant (i.e. environmentally friendly), uniform dosing throughout inhaler life, low potential of contamination and long shelf life (Hillery et al., 2001). However, these ideal characteristics are very much device-driven, rather than patient focused (Kohler, 2004). From a clinical perspective, to provide consistent clinical control, an appropriate inhaler should satisfy the criteria that are described in Figure 2.12. Perhaps, the most important characteristic from a patient and physician point of view is successful delivery of the therapeutic agent to the lungs and easy to use.

Most of the inhalation devices are more concerned with the local delivery of the drugs, such as the anti-asthmatics, directly to their site of action. An aerosol can be considered as colloidal, consisting of finely divided condensed matter in a gaseous container that on atomization produces a spray with small size drug loaded droplets suitable for inhalation. For maximum efficiency, these drug loaded droplets need to be less than 5μm in diameter. The principal categories of aerosol generator used for inhalation therapy are nebulisers, pressurized metered dose inhalers (MDIs) and dry powder inhalers (DPIs).

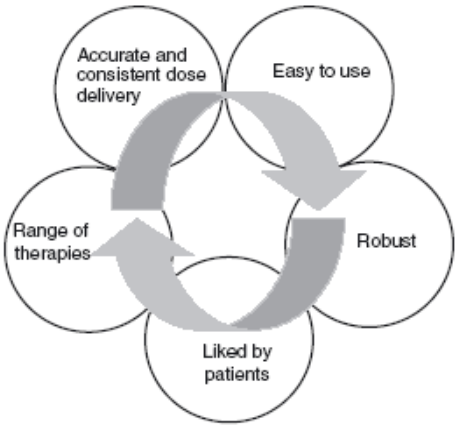


Figure 2.12 Criteria for an ideal inhaler. [reproduced from Chrystyn (2007)]

2.4.1. Nebulisers:

Nebulisers are devices that convert a solution or a micronized suspension of drug into an aerosol suitable for inhalation. Nebulisers have the potential to deliver relatively large amounts of drug to the patient, so they are frequently used for drugs that cannot be conveniently formulated into a MDI or a DPI or where the therapeutic dose is too large for delivery using an alternative system (McCallion et al., 1996; Taylor and McCallion, 1997). Energy is generated in the nebuliser by one of the following principal mechanisms; high velocity air stream dispersion (air-jet nebulisers), ultrasonic energy dispersion (ultrasonic nebulisers) and vibration energy dispersion (vibrating mesh nebulisers).

Nebuliser solutions are concentrated solutions from which aliquots are withdrawn and diluted before administration. Some concentrated solutions contain preservatives and antioxidants which sometime cause bronchospasm, and hence the standard practice is to use small unit dose isotonic solutions (Respules) free from preservatives and antioxidants. The physicochemical properties, e.g. viscosity and surface tension, of a nebulised solution significantly affect the nebuliser performance (McCallion and Patel, 1996; Taylor and McCallion, 1997).

2.4.1.1. Air-jet nebuliser:

Jet Nebulisers use compressed gas (air or oxygen) from a compressed gas cylinder, hospital air-line or electrical compressor to convert a liquid (respiratory solution) into a spray (Mercer, 1981). The jet of high velocity gas is passed either tangentially (concentric Nebulisers, e.g. Turret, Respirgard II) or co-axially (Inspiron Mini-neb) through a narrow Venturi nozzle, typically 0.3-0.7 mm in diameter. An area of negative pressure, where the air jet emerges, results in liquid being drawn from a fluid reservoir up a feed tube as shown in Figure 2.13. A proportion of the resultant (primary) aerosol leaves the nebuliser directly carried in the airstream. The remaining, large, non-respirable droplets impact on baffles or the walls of the nebuliser chamber and are recycled into the reservoir fluid. The aerosol leaving the nebuliser is diluted by atmospheric air and inhaled through a face-mask or mouth-piece. The droplet size of this aerosol is significantly modified within the nebuliser by the 'filtering' effect of the baffles in the jet nabuliser and due to droplet aggregation, solvent evaporation and condensation. The nebuliser has a tendency to concentrate the solution (Dhand and Tobin, 1997; McCallion et al., 1996). During nebulization from a jet nebuliser cooling of the reservoir solution occurs which together with the vapour loss, results in concentration of the drug solution. This can lead to crystallization with subsequent blockage within the device, or variation of the aerosol particle size (Taylor and McCallion, 1997; Hillery et al., 2001). The aerosol output from a jet nebuliser is a mixture of drug solution and solvent vapour which saturate the outgoing air (Ferron et al., 1976). This causes solute concentration to increase with time (Ferron et al., 1976) and results in a rapid decrease in the temperature of the liquid being nebulised by approximately 10-15°C (Taylor et al., 1992; Clay et al., 1983). The air-jet nebuliser is used more often in hospitals.

Nebulisers are operated continuously and since the inspiratory phase of breathing constitutes approximately 1/3 of the breathing cycle (McCallion et al., 1996), a large proportion of the emitted aerosol is not inhaled.

The rate of gas flow driving atomization is the major determinant of the aerosol droplet size produced by a jet nebuliser. Clay et al (1983) in a laser diffraction analysis of four jet nebulisers showed that a 50% reduction in the MMAD was produced when the flow was increased from 4 to 8 L min^{-1}. This was accompanied by a linear increase in the fine particle dose (containing droplet less than 5μm) and an increase in the polydispersity of the aerosol (Clay et al., 1983). Increasing the flow increases the shearing forces to which the fluid filaments and the surface of droplets are exposed, resulting in smaller droplets. Viscosity and surface tension might be expected to affect the output characteristics of nebulisers, since energy is required to overcome viscous forces and to create a new surface. Atomization theory suggests that the mean diameter of aerosol droplets will increase as viscosity increases (Mercer, 1973). McCallion et al. (1996) studied the viscosity effects on nebulization of aqueous solutions. They found that the low viscosity solutions offered less resistance to the essential aerosol disintegration process, thereby producing smaller droplets and higher outputs (McCallion and Patel, 1996).

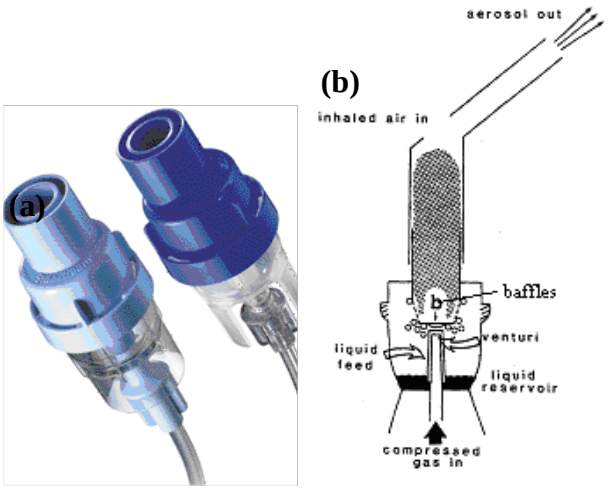


Figure 2.13 (a) Sidestream jet nebuliser as an example of air-jet nebuliser. (b) Schematic diagram of the jet nebuliser. [Reproduced from McCallion et al. (1996)].

This type of nebuliser is the one recommended for suspensions such as corticosteroid formulations, e.g. Pulmicort Respules, AstraZeneca (McCallion et al., 1996). Jet nebulisers have been used successfully to atomize recombinant human deoxyribonuclease (Cipolla et al., 1994). The activity and structural integrity of the enzyme was maintained, when jet nebulisers were employed, although ultrasonic nebulization caused denaturation of the protein, probably as a result of the elevated temperatures. However, studies with lactate dehydrogenase have suggested that during aerosolization in jet nebulisers there was an irreversible time-dependant loss of enzyme activity (Niven and Brain, 1994). Many different models of nebuliser and compressors are commercially available. Such devices cannot be considered equivalent. For instance, in a study of 18 different commercially available jet nebulisers, operated according to the manufacturer's guidelines, aerosols were produced with MMADs ranging from 0.9 to 7.2 μm (Waldrep et al., 1994). Clearly, the regional deposition within the lung of aerosols generated from such devices will vary enormously. Variability may not only exist between different nebulisers but also between individual nebulisers of the same type (Alvine et al., 1992) whilst repeated use of a single nebuliser may cause variability due to baffle wear and non-uniformity of assembly (Massey et al., 1982). In addition to factors relating to the design of nebulisers, gas flow rate, fill volume and the physicochemical properties of the fluid must be considered, alongside patient-related factors, when considering both the size of the aerosols produced and drug output.

2.4.1.2. Ultrasonic nebuliser:

The ultrasonic nebuliser uses a transducer made from a piezo-electric crystal to produce high frequency sound waves in the liquid in the nebulizing unit to produce vertical capillaries of liquid. When the amplitude of energy applied is sufficient the vertical capillaries break up to provide an aerosol, as shown in Figure 2.14. Ultrasonic nebuliser

causes a rise in the temperature of the nebulised solution so decrease its use with thermolabile drugs, e.g. proteins. They are less used than the jet nebuliser despite their compactness which makes them easy to be carried.

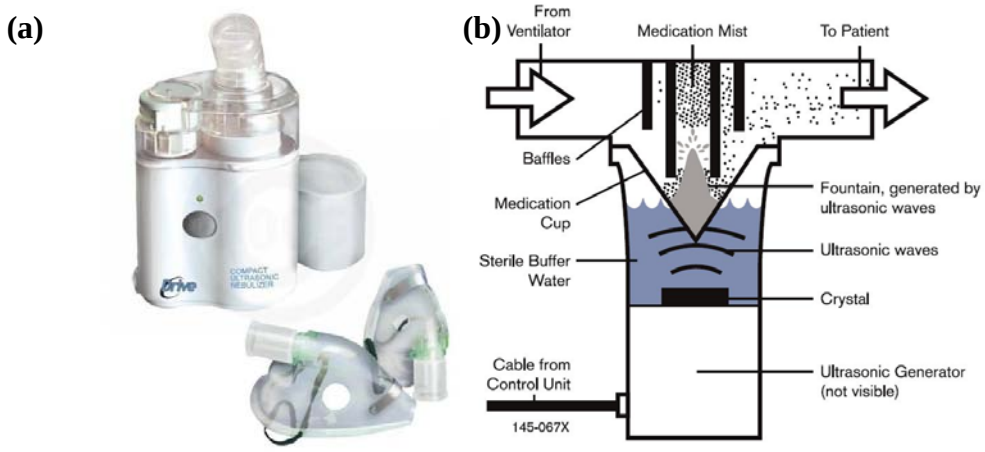


Figure 2.14 (a) An example of ultrasonic nebuliser. (b) Schematic diagram of an ultrasonic nebuliser [Rreproduced from Fink et al. (2001a)].

2.4.1.3. Vibrating mesh nebuliser:

Vibrating mesh nebulisers have recently been introduced. The drawbacks of conventional nebulisers are their lack of portability (an electrical or compressed gas source is needed for operation), poor efficiency and variability in performance between nebulisers of different brands (Smith et al., 1995). Recent technological advancements have led to the development of devices that can overcome many of the disadvantages of conventional nebulisers. The vibrating mesh nebulisers have much greater efficiency, precision and consistency of drug delivery to the lung than conventional jet or ultrasonic nebulisers. This new generation of nebulisers use a vibrating mesh or plate with multiple apertures to generate a liquid aerosol. Several manufacturers have developed aerosol devices that use a vibrating mesh or plate with multiple apertures to produce a liquid aerosol. These include Aerogen's Aerosol Generator (Aeroneb Portable Nebuliser System, Aeroneb Professional Nebuliser System, Aerodose Inhaler), Omron's Vibrating Mesh (NE-U03, NE-U22),

ODEM's TouchSpray Technology (TouchSpray inhaler devices) and Pari's device (the e-Flow). Some of them are shown in Figure 2.15.

Some vibrating mesh nebulisers have been designed for use during mechanical ventilation (Dhand, 2002). These generate aerosol continuously, though it can be adapted to generate aerosol only during inspiration. When it is connected to the inspiratory limb of the ventilator circuit, the aerosol that is generated is entrained in the airflow from the ventilator. The Aeroneb Pro has a three to five fold higher efficiency for delivering drug to the lungs than conventional jet or ultrasonic nebulisers (Fink et al., 2001; Fink and Schmidt, 2002; Kristin et al., 2003). Hence, similar clinical effects should be obtained with lower nominal doses of drugs with vibrating mesh nebuliser compared with conventional nebulisers.

Figure 2.15 Three examples of the vibrating mesh nebulisers a) e-flow (Pari's), b)Aeroneb Pro (Aerogen) and c) NE-U22 (Omron).

The aerosol generator, which is powered by alternating current or a rechargeable battery pack, consists of a vibrational element and a domed aperture plate as shown in Figure 2.16. The aperture plate has about 1,000 tapered holes that are electroformed in a sheet. The wider portion of the hole is toward the medication, and the narrower end is toward the

atmosphere. The medication is placed in a reservoir above (e.g. Aeroneb Pro) or against (e.g. e-flow) the domed aperture plate. When electric current is applied, the ceramic vibrational element expands and contracts, causing the domed aperture plate to move upward and downward by a few micrometers, which creates a micro-pump action that extrudes medication through the apertures to produce an aerosol (Dhand, 2002).

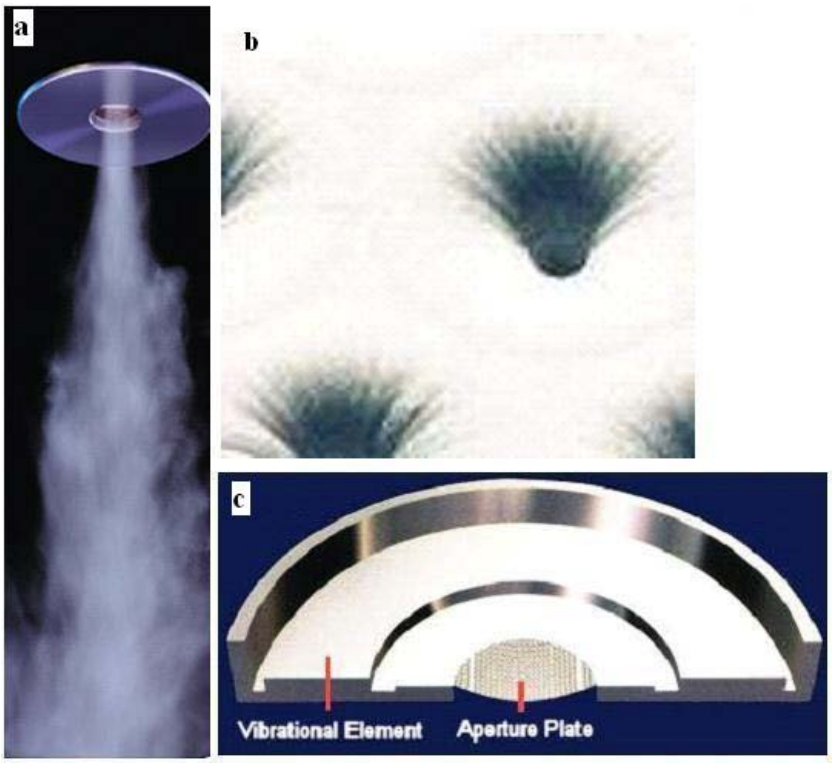


Figure 2.16 a) Picture of Aerogen's aerosol generator, b) A microscopic view of an aperture plate X 250 magnification and c) diagram of the aerosol generator components.

The aerosol particle size and the flow are determined by the exit diameter of the aperture hole. These can be modified for specific clinical applications. These nebulisers nebulise at a rate ranging from 0.3 to 0.6 ml min^{-1} and generally the nebulisation time is shorter compared with conventional nebulisers (Fink et al., 2001b). These devices are relatively quiet because they do not require any compressed gas flow or high vibration energy for aerosol generation. Moreover, the volume of solution remaining in these devices at the end of treatment (residual volume) is minimal. The energy required for nebulisation is applied to the vibrational element in the aerosol generator rather than to the nebulised

solution, hence the increase in temperature of the solution during operation is minimised (Fink et al., 2001a). There is, therefore a negligible risk of denaturing proteins or peptides and of reducing the activity of antibiotics during aerosolisation.

Blockage of the minute apertures with drug particles, especially when suspensions are aerosolised can occur. These devices, therefore, must be cleaned regularly to prevent build-up of deposit in the apertures.

A study by Fink et al (2001) compared the ability of the Aeroneb Pro and two small volume jet nebulisers, MistyNeb (Allegiance) and Vix One (Westmed), to deliver 2.5 mg of salbutamol sulphate to a simulated pediatric patient during mechanical ventilation with high frequency oscillation. The Aeroneb Pro delivered more salbutamol (582 ± 89 µg) to the end of the endotracheal tube than the MistyNeb (201 ± 87 µg) or the Vix One (197 ± 50 µg) ($p<0.02$). Operation of the Aeroneb Pro did not alter any of the monitored ventilator parameters. In contrast, both of the jet nebulisers increased mean airway pressure during operation by >5 cmH_2O and required adjustment of ventilator flow settings during operation to maintain ventilator parameters with initiation and discontinuation of nebulization. Opening the medication reservoir to refill the Aeroneb Pro did not result in any change of the ventilator parameters. In contrast, both of the jet nebulisers required interruption of ventilation in order to refill. This level of aerosol delivery without changes in airway pressure may make aerosol delivery during mechanical ventilation with high frequency oscillation more practical.

2.4.2. Pressurized metered dose inhalers (MDIs) and Add-no devices:

MDIs are effective and easily accessible when patients are away from home. They are the gold standard for the treatment of airflow obstruction since they provide reliable, reproducible, effort-independent dosing, protection of their contents from environmental humidity and bacterial infection and at the same time are the most readily pocketable, efficient and least expensive aerosol therapy devices available. When combined with add-

on devices they are also the most flexible delivery system because they are well suited to a variety of patients.

2.4.2.1 Pressurized metered dose inhalers (MDIs)

MDIs were introduced in the 1950s, and in the UK have remained the most popular method for achieving inhalation therapy (Crompton, 2004; Vaswani and Creticos, 1998). The pressurized metered dose inhaler (MDI) is the most widely used device for aerosol therapy. Over 70 million patients in the world use one, either alone or with a spacer fitted. The MDI has changed little since it was first developed (Terzano, 2001).

The MDI, as shown in Figure 2.17, is a remarkable small spray that repeatedly provides almost identical aerosol doses from the beginning to the end of the canister charge.

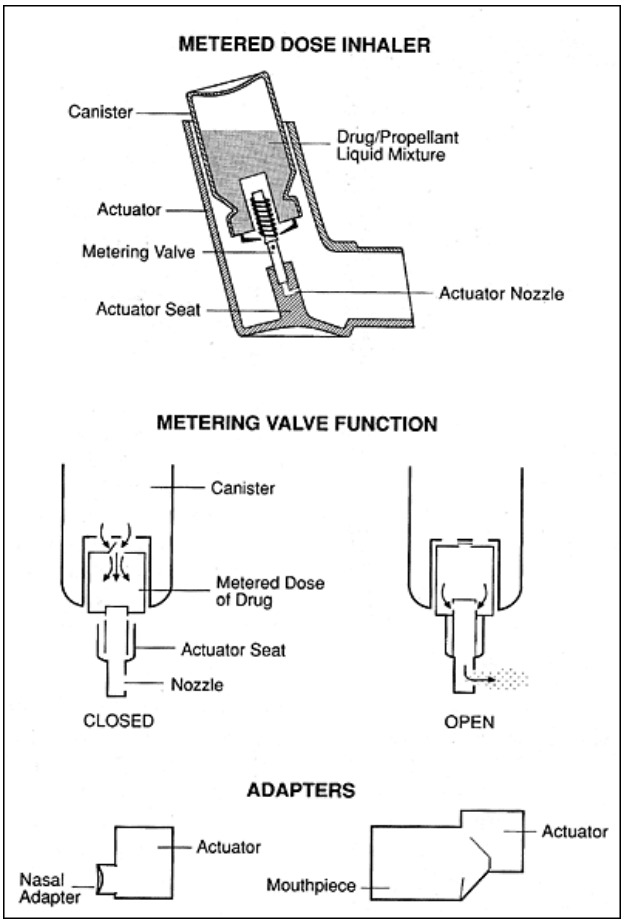


Figure 2.17 Pressurized metered dose inhalers (MDIs). [Reproduced from Daniel et al. (2003)]

The MDI consists of a canister with a metering valve containing a drug in suspension or in solution with surfactants, lubricants and a propellant at a pressure. The canister is lodged upside down in a plastic support called the actuator. By pressing the bottom of the canister, a pre-metered drug dose is released. When the canister is depressed, liquid propellant containing the drug substance is forced into the actuator where flashing occurs. The resulting gas under high pressure forces the propellant together with the excipient and the drug substance as particles or droplets of drug solution exit through the actuator orifice, thus creating the aerosol cloud. The aerosol cloud consists of a so-called heterodisperse aerosol consisting of a variety of particle sizes from approximately 1 μm to 35 μm. These emerge at a very high speed. The large particles have considerable inertia resulting in their deposition on the tongue and in the oropharynx. From 100 μg of a drug such as salbutamol in the Ventolin CFC formulation leaving the actuator orifice, approximately 10 μg remains in the actuator and mouthpiece, 75–80 μg is deposited in the mouth and throat and only about 10–15 μg reaches the lungs. Newman et al (1981) had reported a Controlled inhalations study by eight patients with obstructive airways disease showed that on average 8.8% of the dose was deposited in the lungs (3.0% in the alveoli and 5.8% on the conducting airways) and 80% in the mouth. The remainder of the dose was either expired [1.0%] or deposited in the aerosol actuator [9.8%] (Newman et al., 1981). While the MDI delivery system is quite inefficient overall, this dose is sufficient to provide maximum bronchodilatation in stable mild–moderate asthmatics (Ruffin et al., 1978).

The size of the particles delivered by MDIs is influenced by a variety of factors, including: (1) the pressure inside the canister; (2) the physical and chemical properties of the propellant and of the other additives; (3) the drug used, its concentration and the delivered volume; (4) the metering valve and delivery outlet design; (5) mouthpiece and delivery outlet cleanness (Terzano, 2001).

The particle size depends on the propellant evaporation time and on the distance to travel from the delivery outlet. For effective particle deposition in the lower airways, the aerodynamic diameter of particles should be <5 μm (Heyder et al., 1986). However, particularly for patients with obstructive lung disease, all particles should ideally be within the 2–3 μm range (Patel et al., 1990; Zanen et al., 1994; Zanen et al., 1996; Bisgaard, 1997b).

Until recently the MDIs were formulated using chlorofluorocarbons (CFC) propellants. In 1974 Molina and Rowland reported that CFCs were being added to the environment in steadily increasing amounts and were causing the destruction of the atmospheric ozone layer (Molina and Rowland, 1974). In 1987 the Montreal Protocol banned the use of all CFC propellant gases, this included MDIs, to protect the ozone layer from further depletion (Montreal, 2000). Pharmaceutical companies have reformulated MDIs with hydrofluorocarbons (HFAs) propellants which are 2000 times more potent greenhouse gases than CFCs but do not damage the ozone layer (Crompton, 2004).

The major limitation of a MDI is the patient's inability to use the correct inhalation technique, especially good co-ordination between aerosol discharge and inhalation. A number of studies have shown that between 25% of patients who have been taught how to use MDIs in a chest clinic (Crompton, 1982) and 75% of elderly patients (Allen and Prior, 1986) may not perform the inhalation manoeuvre very efficiently. A recent study of asthmatics revealed that only 7.5% used the correct technique (Al-Showair et al., 2007). Table 2.5 Shows the main problems connected with the use of MDIs.

Previously, MDI technique assessment has heavily focussed on co-ordinating the dose release with the start of an inhalation. However studies have shown that co-ordination of dose actuation and inhalation is not important as long as the patient is inhaling when the dose is released (Tomlinson et al., 2005) and a slow inhalation flow is used. Also a slow

inhalation flow through the MDI provides better lung deposition than a fast inhalation flow (Newman et al., 1982; Terzano and Mannino, 1999; Tomlinson et al., 2005).

Table 2.5 Main problems connected with the use of MDIs.

Canister not shaken energetically before delivery
Mouthpiece cap not removed
Patient breathes in before or after delivery or through the nose
Patient breathes out during delivery or before inhalation is complete
Patient delivers multiple doses during the same breath
Drug delivery and inhalation are not simultaneous
The inhalation flow is too fast
Inhalation is interrupted due to 'Freon effect' (early breath cut-off caused by a cold sensation provoked by CFCs in the mouth and the pharynx)

A new training aid, the 2Tone Trainer, which looks like a MDI but without a canister has been introduced to help training and maintaining a slow inhalation flow. In a clinical study using adult asthmatics the 2Tone Trainer helped patients to maintain the recommended MDI technique post verbal inhalation technique training with a resultant improvement in Asthma Quality of Life (Al-Showair et al., 2007).

In an effort to preclude the need for patients to co-ordinate device actuation with inhalation, breath-actuated MDIs were introduced in 1989. In the UK these are available as the Autohaler (Teva, UK) and the Easi-Breathe (Teva, UK). The Autohaler system is a metered aerosol equipped with a spring device which once loaded, is actuated by a moderate inspiratory flow (about 30 L min^{-1}), consequently delivering the drug dose in the form of an aerosol. The Easi-Breathe inhaler is primed when the mouthpiece cover is opened. When the patient breathes in, the mechanism is triggered and a dose is automatically released into the airstream. It has an integral mouthpiece cover and can be actuated at airflows of approximately 20 L min^{-1}.

Breath-actuated inhalers are likely to increase lung deposition in patients with a poor inhalation technique (Terzano and Mannino, 1996; Barry and O'Callaghan, 2003). They are much easier and more efficacious for patients with poor inhalation technique. It has

been shown that the Autohaler can be used easily by children over 7 years old (Pedersen and Mortensen, 1990). These devices can help to achieve a satisfactory level of lung deposition in poor co-ordinators, but may not deposit additional drug in patients who can use the MDI with the correct inhalation technique (Newman et al., 1991c). A small volume spacer, the Optimiser, has been evaluated for use with the Easi-Breathe inhaler. Oropharyngeal deposition in healthy subjects was reduced by 80% by the use of the spacer (Hardy et al., 1996). The Optimiser spacer removes most of the non-respirable drug, without compromising the fine particle dose delivered from the Easi-Breathe inhaler (Hardy et al., 1996). At present there is no evidence that a breath actuated MDI helps to overcome the 'cold Freon' effect, as the spray is fired direct into the oropharynx just like a standard MDI.

One marketed MDI actuator, the Neohaler, and another in development, Tempo, use novel technologies to slow the aerosol cloud before it leaves the actuator (Newman and Clarke, 1993; Shrewsbury et al., 2006). The Neohaler device was formerly known as the Gentlehaler and Spacehaler. This device has been shown to reduce oropharyngeal deposition because it removes most of the non-respirable drug, while lung deposition is either increased (Newnham et al., 1993) or unchanged (Newman and Clarke, 1993). The Neohaler device is similar to the MDI but has a slightly elongated mouthpiece as show in Figure 2.18. The Tempo device also includes a breath-actuation mechanism, and markedly increases lung deposition compared with a conventional actuator (Shrewsbury et al., 2006).

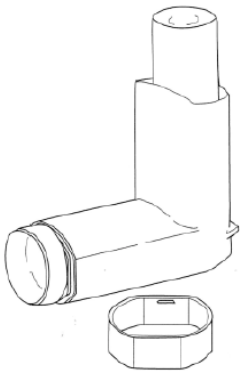


Figure 2.18 Diagram of Spacehaler MDI

2.4.2.2. Spacer:

Spacers were first introduced in the 1970s as an extension tube attached to the MDI mouthpiece to eliminate patient MDI co-ordination problem, A spacer device, as shown in Figure 2.19, is a tube extension to a MDI or a holding chamber with a port at one end to which the MDI is attached, a mask or mouthpiece being fitted at the other end. Patients dispense a dose (one puff at a time) into the spacer and inhale by breathing normally through the mask or mouthpiece. As mentioned before, approximately 25% of adult patients and, in practice, all children have difficulty synchronizing actuation of the MDI with inhalation of the aerosol (Crompton, 1982). Spacer devices attached to the MDI can help to overcome this difficulty and improve inhalation technique. The use of a spacer reduces both the velocity and the size of the aerosol particles and decreases the need for patient co-ordination between actuation of the MDI and inhalation of the aerosol.

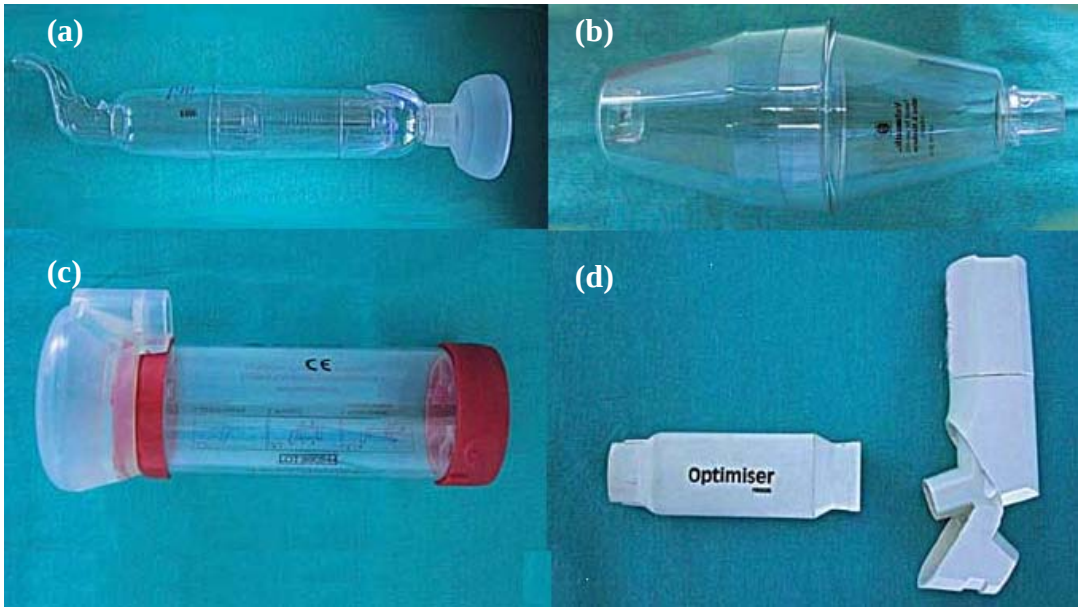


Figure 2.19 Four examples of spacer, (a) Babyhaler (b) Volumatic, (c) Aerochamber with mask and (d) Optimiser used with a breath-actusted MDI.

An in-vitro study by Terzano and Mannino (1999) compared the MDI alone to the MDI plus a large-volume holding chamber (Volumatic). They estimated the particle characteristics of fluticasone propionate, flunisolide and beclomethasone dipropionate, at

inhalation flows of 30 L min^{-1} from the MDI alone and the MDI attached to the Volumatic spacer (GlaxoSmithKline, Ware, UK). A significant reduction of the MMAD and increase of the fine particles dose was obtained for all three drugs with the MDI plus Volumatic spacer (Terzano and Mannino, 1999).

Moreover spacers have a size selective function, retaining the non-breathable particles, reducing the 'cold-Freon effect' and drug deposition in the oropharynx. Furthermore valved holding chambers reduce drug loss associated with poor hand-breath co-ordination (Aswania and Chrystyn, 2001; Fink, 2000; Terzano, 2001; Vaswani and Creticos, 1998).

Spacers may not improve the clinical effect in patients able to use a MDI properly, but may reduce the systemic bioavailability of the inhaled drug (Newman, 2004).

Recent guidelines (British Thoracic Society, 2007) indicate that a MDI plus spacer is the preferred method for the delivery of β_2-agonists and inhaled corticosteroids in children below 5 years of age.

Almost all spacer devices are constructed of plastic and so unless specially treated the surfaces can accumulate static charge, especially when handled by the patient. Drug delivery from a spacer may be increased by placing an antistatic lining on the inner walls of the device (O'Callaghan et al., 1993). It has been found that washing a plastic spacer in detergent without subsequent rinsing in tap water and then allowing it to air dry (Kenyon et al., 1998; Pierart et al., 1999; Wildhaber et al., 2000), priming by using the spacer several times (Bisgaard et al., 1995) or using a spacer made of metal [Figure 2.20] (Bisgaard, 1995) can decrease the electrostatic charges on the spacer surface which decrease drug output from plastic spacers. Rubbing the spacer with a cloth increases the electrostatic charge. In a metal spacer the aerosol half life is about 30 seconds compared with about 10 seconds in a new plastic spacer (Bisgaard, 1997a); a short half-life increases the need for co-ordination between actuation and inhalation. Therefore non-electrostatic spacers deliver

a significantly higher dose than plastic spacers. Similarly, using a large volume spacer increases the lung dose in adults by approximately 50% (Bisgaard, 1997a).

Several spacers and holding chambers are available. Dose delivery varies considerably depending on design. Some devices can be used to deliver aerosols from MDIs to intubated or tracheotomised patients (Cates, 2003) and the mechanically ventilated patient (Dhand and Tobin, 1996; Manthous et al., 1995).

Figure 2.20 Nebuchamber a metal spacer (AstraZeneca, Sweden) 250 ml

Inhalation from the spacer must be slow and multiple actuations should be avoided because this will reduce drug output from the spacer (Barry and O'Callaghan, 1994).

Certain spacers (Volumatic, Nebuhaler) are designed to fit only a single type of MDI, whereas others can be used with all types (AeroChamber). The right kind of spacer must be used, choosing the most suitable kind, ideally after testing a number of different devices for the individual patient. The disadvantages of spacers are that they are bulky, and difficult to carry around; in addition, the valves sometimes stick or become otherwise faulty.

During an acute attack the use of a high dose (10–15 puffs) of short-acting β_2-agonist via a MDI and a large volume spacer was found to be an effective alternative to its use via a nebuliser (Cates et al., 2003; Cates, 2003; Duarte et al., 2000; Marik et al., 1999). In addition, cost analysis studies indicate that for hospitalized adult patients with asthma exacerbations, treatment with either MDIs plus spacers or nebulisers produce equivalent

responses, and MDI plus spacer use is not associated with longer periods of hospitalization (Chou et al., 1995).

There are many types of spacer, ranging from large volume spacer e.g. Nebuhaler, Volumatic and Fisonair to small volume e.g. Aerochamber and Inhalet.

The spacers are classified into three categories, simple tube spacer, holding chambers devices and reverse-flow devices. The simple tube spacer is an extension to the inhaler mouthpiece. Holding chamber devices usually contain a one-way valve in the mouthpiece. A reverse-flow device is a device in which the spray is fired away from the patient into a bag or chamber, from which the patient then inhales. The three categories are shown in Table 2.6 (Newman and Newhouse, 1996; Newman, 2004).

Table 2.6 Types of spacer device for pressurised metered dose inhalers [reproduced from Newman and Newhouse (1996)].

Device (manufacturer)	Volume in ml
Simple tube spacer	
MicroSpacer (Respiratory Delivery System)	20
Optimiser (Norton Healthcare)	50
Inhalet (AstraZeneca)	80
Azmacort spacer (Aventis)	110
Syncroner (Aventis)	150
Holding Chambers	
Jet spacer (Chiesi)	100
AeroChamber (Trudell Medical)	149
Space Chamber (Orthopaedic Appliances)	200
Inhacort spacer (Boehringer Ingelheim)	250
Nebuchamber (AstraZeneca)	250
Babyspacer (AstraZeneca)	260
Rondo (Leiras)	270
Integra (GlaxoWellcome)	312
Babyhaler (GlaxoWellcome)	350
Nebuhaler (AstraZeneca)	750
Volumatic (GlaxoWellcome)	750
Fisonair (Fisons)	750
Reverse-flow devices	
Optihaler (Respironics)	70
Aerosol Cloud Enhancer (DHD Corporation)	170
InspirEase (Schering-Plough)	600

An example of a large volume spacer, the Nebuhaler is based on a design from 1976 (Barry and O'Callaghan, 2003). It is 750 ml in size. Its shape was intended to replicate the shape of the aerosol cloud emitted from the MDI, and early versions of the Nebuhaler were described as the pear-shaped extension tube.

Another large volume spacer, the Volumatic, is a 750 ml diamond-shaped valved spacer that increases fine particle delivery of drug in-vitro (Barry and O'Callaghan, 1996), increases lung deposition of drug compared to the MDI alone (Newman, 2004) and reduces oropharyngeal deposition.

The Fisonair is another large volume spacer that has been shown to be as effective as the properly used sodium cromoglycate MDI in the prevention of exercise induced asthma in children (Comis et al., 1993).

It had been found that, taking multiple tidal breaths from a large volume spacer may be more practical than taking single deep breaths, especially in children, and fortunately this appears to result in a satisfactory bronchodilator response (Castro-Rodriguez and Rodrigo, 2004; Gleeson and Price, 1988).

The Aerochamber was first described as a ‘portable, breath actuated particle size selective medical aerosol spacer (Barry and O'Callaghan, 2003). The Aerochamber has undergone a number of modifications in design and construction from the original design. Aerochamber is available in 3 sizes; adult (with and without a mask), child (with mask) and infant (with mask). The size of the Aerochamber is 149ml with the difference for the patient group being in the mouthpiece and the size of the mask.

A device developed specifically for the treatment of babies and young children (Kraemer, 1995), the Babyhaler, is a polycarbonate tube of only 350 ml volume which could be easier for a child to empty than larger devices (Newman, 2004). Inspiratory and expiratory valves are fitted in an exhalation area of 36 ml. Also the addition of a facemask to the spacers helps with the management of infants and young children (O'Callaghan and Barry, 2000).

The facemask is a useful alternative to the nebulisers in infants and small children (Fok et al., 1998; British Thoracic Society, 2007).

2.4.4. Dry powder inhalation devices (DPIs):

Dry powder inhalers basically contain four functional elements; the powder container, the metering system, the disintegration principle and a mouthpiece. Based on these functional elements, dry powder inhalers can be divided into two major groups, single dose and multi-dose inhalers (Table 2.7).

Table 2.7 Dry powder inhalers available in the market.

Inhaler device (manufacturer)
Single dose inhalers
Spinhaler (Aventis)
Cyclohaler (Pharmachemie)
Rotahaler (GlaxoWellcome)
Aerolizer (Novartis Pharma)
Inhalator (Boehringer Ingelheim)
Handihaler (Boehringer Ingelheim)
Multi-dose inhalers
Multiple unit-dose inhalers
Diskhaler (GlaxoWellcome)
Aerohaler (Boehringer Ingelheim)
Diskus / Accuhaler (GlaxoWellcome)
Reservoir systems
Turbuhaler (AstraZeneca)
Clickhaler (Innovata Biomed/ML labs celltech)
Easyhaler (Orion Pharma)
Pulvinal (Chiesi)
Novolizer (Viatris)

The multi-dose inhalers are divided in two different types of design: the reservoir systems and the multiple unit-dose inhalers. An example for the reservoir system is Turbuhaler, Clickhaler, Pulvinal, Novolizer and Easyhaler. In this type of inhaler, the powder formulation is stored in a reservoir from which single doses are measured volumetrically and dispensed with a special dose metering unit. Accurate dose metering for this type of inhaler requires careful manipulation of the device by the patient. In the multiple unit-dose inhalers, single doses are filled by the manufacturer into suitable dose compartments, such

as blisters. Examples are Diskhaler, having the blisters on a disk (Rotadisk), and Diskus (know as Accuhaler in UK) with the blisters on a long strip.

The early DPIs were all unit dose systems such as the Spinhaler which was introduced in 1969 (Figure 2.21), and the Rotahaler in 1977. As single dose inhalers, both utilize premetered doses dispensed into hard gelatine capsules with a different mechanism of powder delivery. The capsule cap and body must be separated before inhalation (Rotacaps for Rotahaler) or the capsule has to be pierced at both ends; as for the capsules for the Aerolizer, the Spincaps for the Spinhaler, and the Spiriva Handihaler.

The Diskhaler, introduced in 1980, (Figure 2.21) has individual doses contained within a blister on a disk. The dose is released by piercing the upper and the lower surfaces of one of the blisters before an inhalation.

The Diskus/Accuhaler (GlaxoSmithKline) was launched in 1994. It contains 60 discrete dispensed doses packed on a strip which is coiled within the inhaler.

The multiple dosing reservoir devices contain 100-200 doses with each dose metered immediately before an inhalation. The Turbuhaler was introduced in 1988, (Figure 2.21) and was designed to deliver 500μg per dose of terbutaline sulphate devoid of carrier, and then it was used for other drugs. It is the most frequently prescribed DPI that produces good deposition of drug in the lungs provided that a sufficient inspiratory flow has been achieved by the patients [i.e. 60 L min^{-1}] (Dhand and Fink, 1999). Patients with reduced inspiratory capacity may not get the full benefit from the Turbuhaler (Barry and O'Callaghan, 2003).

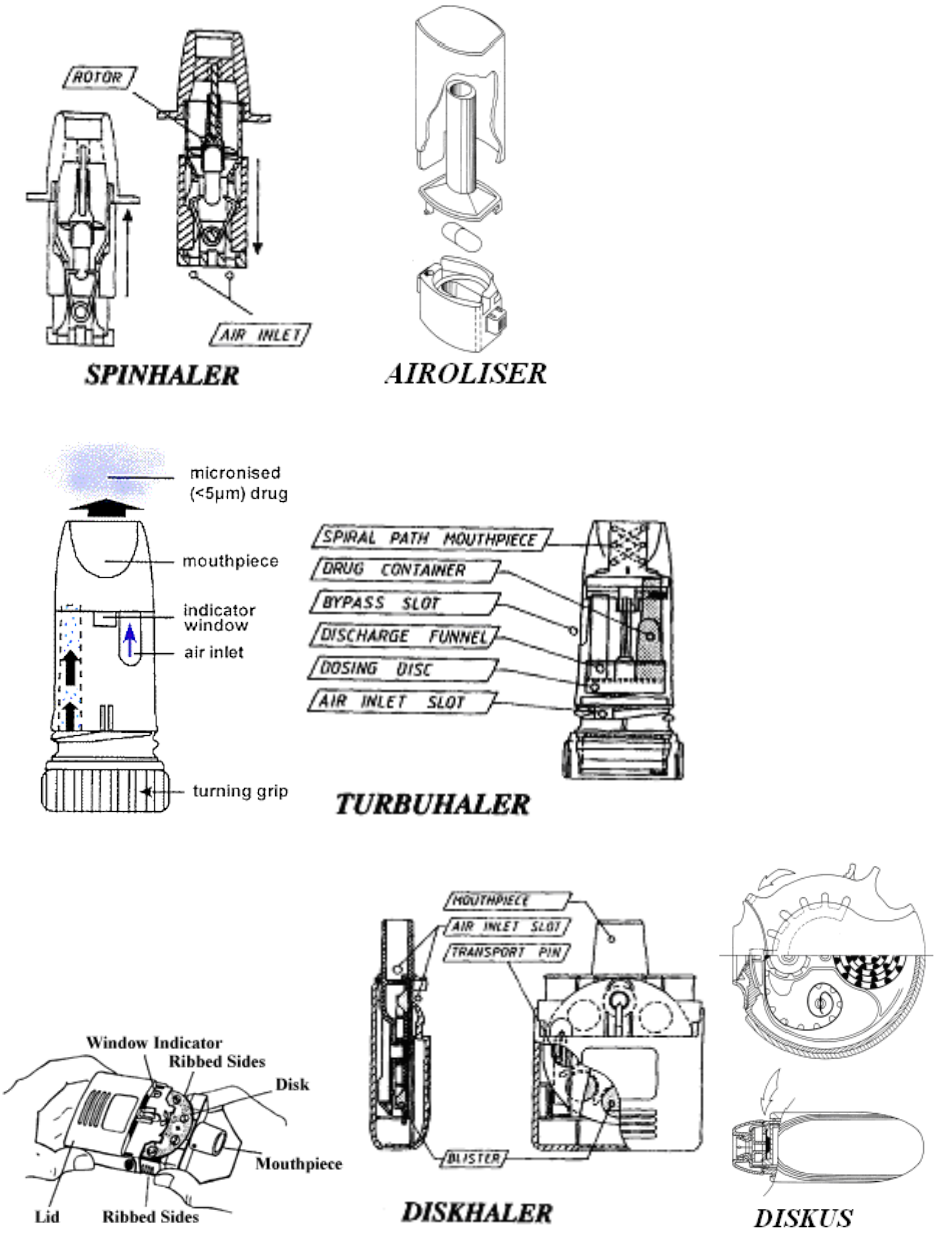


Figure 2.21 A schematic diagram of different types of inhalers available in the market. [Reproduced from de Boer et al. (1996)].

Recently many multiple dosing reservoir DPI devices have been introduced e.g. Clickhaler, Pulvinal and Easyhaler which give a fairly consistent emitted dose irrespective of the inhalation technique used by patients of all age groups (Chrystyn, 2006). In 2001 the Novolizer (Viatris, Germany) entered the market (Crompton, 2004). It is a multiple dosing reservoir device with a refillable cartridge system. The dose has to be inhaled at a flow more than 35 L min^{-1} to release the dose (Kohler, 2004; Richter, 2004). No dose is released

when the patient inhalation flow is less than 35 L min^{-1}. A study to assess whether asthmatic children may generate sufficient peak inspiratory flow through the Novolizer was done by Vogelberg et al (2004). They found out that the medium to low intrinsic resistance of the Novolizers permits a relatively high PIF [mena (SD) of 69.0 (18.0) L min^{-1}] through this device. Together with the feedback mechanisms, this makes the Novolizer particularly valuable for inhalation therapy in asthmatic children with drugs such as salbutamol, formoterol or budesonide (Vogelberg et al., 2004).

For the marketed dry powder inhalers, only two different types of powder formulations are currently used; spherical pellets and adhesive mixture. Spherical pellets are used in the Turbuhaler. In this type of formulation, the micronized drug particles are agglomerated into much larger spherical units without a binding agent, behaving as a free flowing powder. Some micronized diluents such as lactose or glucose may be added to the active component when the dose is low (e.g. Formoterol 6 and 12µg), but the formulation does not contain coarser carrier crystals. Spherical pellets have to disintegrate nearly completely during inhalation into much smaller agglomerates or even primary particles that have the required size-range for deep penetration into the respiratory tract.

All other DPIs are filled with adhesive mixtures. This type of formulation consists of relatively large carrier crystals, mostly α-lactose monohydrate, carrying the micronized drug particles distributed over their surface. During inhalation, the drug particles have to be released from the carrier crystals to generate the aerosol with particles of the desired particle size, which are able to enter the lower respiratory tract. The fraction of drug not detached may cause local side effects, such as candidiasis with inhaled corticosteroids, in the upper respiratory tract (mouth and throat) where the carrier crystals, and other larger particles, are deposited.

All currently available DPIs are breath actuated, thus a dose capable of delivery to the lungs is produced in response to the patient's inspiratory effort. The principle of operation

for a DPI is to use the patient generated inspiratory flow as an energy source for the release of the dose and the delivery of fine drug particles into the respiratory tract. The particle size of the adhesive mixtures or the spherical pellets is far too large for lung deposition. Therefore, the pellet or mixture has to be disintegrated to make an aerosol cloud, which contains a high fraction of non-agglomerated drug particles with the desired particle size (<5 µm). Many different disintegration principles exist. They may vary from a simple screen (Rotahaler, Diskhaler), to twisted powder channels (Turbuhaler). The applied disintegration concept in the design of a dry powder inhaler largely determines the resistance to airflow of the inhaler device.

Inhalers without a recognisable disintegration principle Figure 2.22, for example Diskhaler and Accuhaler, often have a low resistance to airflow. Due to the low resistance to airflow, larger variations in peak inspiratory flow are found. However, the fine particle output is more or less constant over a broad range of inspiratory flows at a low level (de Boer et al., 1996), although there is a tendency for this to increase slightly as the PIF increases.

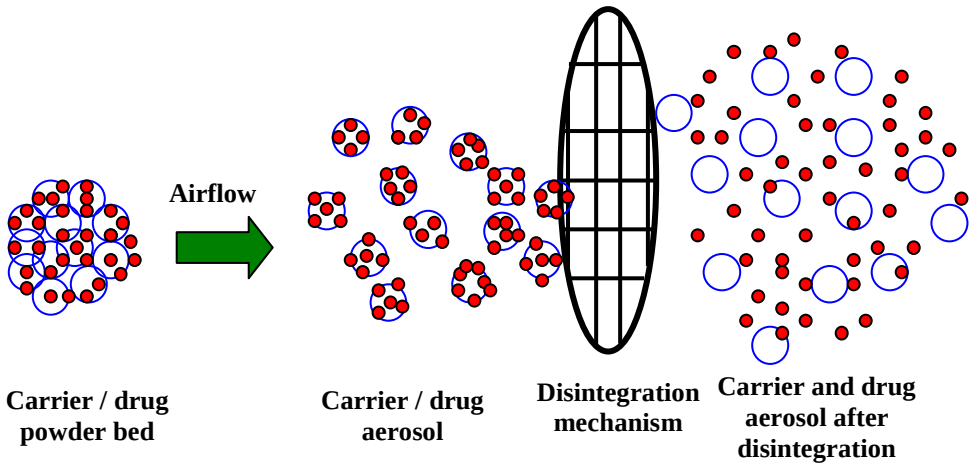


Figure 2.22 Schematic diagram of the disintegration of micronized drug particles from carrier crystals through a non-specific disintegration system.

More specific disintegration systems like the Turbuhaler use inspiratory flow more optimally as the energy source for disintegration and delivery of fine particles into the

airflow (Figure 2.23). This usually results in an increased resistance to airflow through the inhaler. Due to the inhaler design, the fine particle output depends more strongly on the patient's inspiratory performance. As a result, the fine particle output is more or less flow dependent. The optimum aerosol dispersion threshold for the high internal resistance Turbuhaler occurs at a peak inhalation flow (PIF) of at least 60 L min^{-1}. Patients who cannot achieve the recommended optimal PIF through the Turbuhaler may not obtain the maximum benefit from their medication (Cegla, 2004; Chrystyn, 1999). The peak inspiratory flow achieved by a patient through a powder inhaler depends upon the specific resistance to flow of the device and the patient inspiratory capability for a set of inspiratory effort. The lower the specific resistance in the DPI the higher will be the peak inspiratory flow (Clark and Hollingworth, 1993). Therefore, resistance to airflow is one of the design parameters for DPIs that could be used to control the inspiratory flow profile and optimize particle deposition in the airways (de Koning et al., 2002).

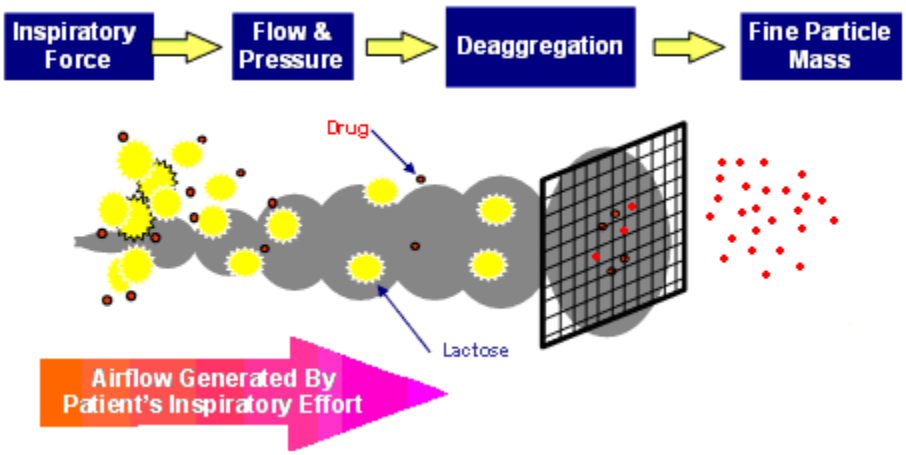


Figure 2.23 Schematic diagram of the disintegration of spherical pellets through a specific disintegration mechanism [Reproduced from Chrystyn (2003)].

The performance of DPIs will depend upon the peak inspiratory flow (PIF) generated through the device. The relationship between inhalation rate and the resistance in an inhaler is described by the relationship

F= $\sqrt{\Delta P}$ / R (Clark and Hollingworth, 1993)

F= flow through the inhaler (L min^{-1})

ΔP= pressure difference developed across the device (cm H_2O)

R= Resistance in the inhaler device resistance in (cm $H_2O)^{0.5}$ / L min^{-1}

Thus the more resistance there is inside the inhaler then the lower will be the inhalation flow for a set inspiratory effort.

The high resistance to airflow limits the range of possible inhalation flows. However, due to the higher disintegration efficiency, the fine particle output is higher compared to the non-specific disintegration systems (de Koning et al., 2002; Srichana et al., 1998; Hawksworth et al., 2000). The mouthpiece may be used to control the resistance to airflow of the inhaler and the direction of the aerosol cloud in the mouth and throat, in order to reduce drug deposition in the oropharyngeal cavities (de Boer et al., 1997). The most important factors affecting the dose delivered are the peak inspiratory flow of the patient, inspiration time, resistant to flow of the device, acceleration rate and type of drug in the inhaler (de Boer et al., 1996).

The total emitted dose and fine particle fraction released by some DPIs have been shown to vary considerably and be affected by differences in inspiratory flow (Chrystyn, 2007). The inhalation profile employed may affect both drug delivery and particle size distribution (Miller et al., 2000). A study was conducted to compare the emitted dose and the fine particle fraction of salbutamol from Easyhaler, Turbuhaler and Diskus (Palander et al., 2000). They concluded that the emitted dose and the fine particle fraction showed less flow dependency with Easyhaler and Diskus than with Turbuhaler. Hence patients cannot use all DPIs equally well (Gustafsson et al., 2005). A study by Tarsin et al (2006) have measured the aerodynamic characteristics of the emitted dose for both active constituents from Seretide Diskus (salmeterol xinafoate 50 µg; fluticasone propionate 500 µg) and Symbicort Turbuhaler (formoterol 6 µg; budesonide 200 µg). They found that the Turbuhaler dose characteristics were more dependent on the patient's inhalation flow (Tarsin et al., 2006). Sometime patients are not able to achieve the required energy. This energy requirement differs from one DPI to another. Patients using the Turbuhaler have to use a significantly

higher inspiratory effort compared to other DPIs in order to achieve the same inspiratory flow inside the device (Cegla, 2004; Chrystyn, 1999). Hence, patients when using DPIs for the relief of symptoms during acute exacerbations of asthma may have problems (Dhand and Fink, 1999). Also changing a delivery device can have adverse effects on both the safety and efficacy of an inhaled drug. Hence DPIs should not be regarded as interchangeable (Chrystyn, 2005; Williams and Chrystyn, 2007). Some of the recent DPIs incorporate an additional energy source to supplement airflow to the inspiratory force of the patient, in order to aerosolize the drug particles into the inhaled air stream, but not quite used yet and will have the patient co-ordination problems.

Comparative studies assess different DPIs at the constant pressure drop across the device (Clark and Hollingworth, 1993). This concept is used by all the compendial methods (European Pharmacopeia, 2001; British Pharmacopoeia, 2005a; United States Pharmacopeia, 2005). However these conditions are quality control indications and do not reflect patient use. Patients with COPD have been reported to have a lower inhalation flow than adult asthmatics when they use a dry powder inhaler (Tarsin et al., 2001). This study also showed that asthmatic children (aged 5-6 years) achieved the highest inhalation flows and that the more severe the airway obstruction the lower was the inhalation flow through a variety of dry powder inhalers (Tarsin et al., 2001). Another study has also recommended that the Turbuhaler device was not suitable for pre-school children because of the low flow achieved by this group (Pedersen et al., 1990)

Some advantages and disadvantages of dry powder inhalers are summarised in Table 2.8.

2.5. Methods of determination of the bioequivalence of inhaled products:

Two products may be pharmaceutically equivalent if they have the same composition and in-vitro performance. They may be bioequivalent if they have the same pharmacokinetic and lung deposition profiles. They may be clinically equivalent if they have the same therapeutic effects and side effects (Barry and O'Callaghan, 2003).

Table 2.8 Advantages and disadvantages for dry powder inhalers versus metered dose inhalers (Ashurst et al., 2000).

Advantages of dry powder inhalers	Disadvantages of dry powder inhalers
• Propellant free	• Performance depends on the patients inspiratory flow profile
• Less need for patient co-ordination	• Resistance to airflow of the device
• Less potential for formulation problems	• Potential difficulties to obtain dose uniformity
• Less potential problems with drug stability	• Less protection from environmental effects and patient abuse
• Less potential for extractables from device components	• More expensive

The assessment of pulmonary drug absorption and deposition is becoming increasingly important in drug development. Several methods are available to investigate pulmonary drug absorption and deposition. The methods include pharmacokinetic and pharmacodynamic studies, gamma scintigraphy method and in-vitro studies (Witek, 2000; Pauwels et al., 1997). In combination, these methods can indicate the fate of an inhaled drug, as the pulmonary fate of the aerosolized drug is influenced by where the aerosol particle is deposited in the lung (Chrystyn, 2000; Chrystyn, 2001; Mobley and Hochhaus, 2001).

2.5.1. Pharmacokinetic methods (using plasma or urine samples):

As shown in Figure 2.24, the inhaled drug will be deposited in the lung and ingested into the gastrointestinal tract, then into the systemic circulation where it faces metabolism and excretion.

The ingested portion may undergo the first pass effect (e.g. fluticasone propionate) and is absorbed after a lag time. The pharmacokinetic methods to evaluate the inhaled drug use either urinary excretion or blood concentrations of the drug.

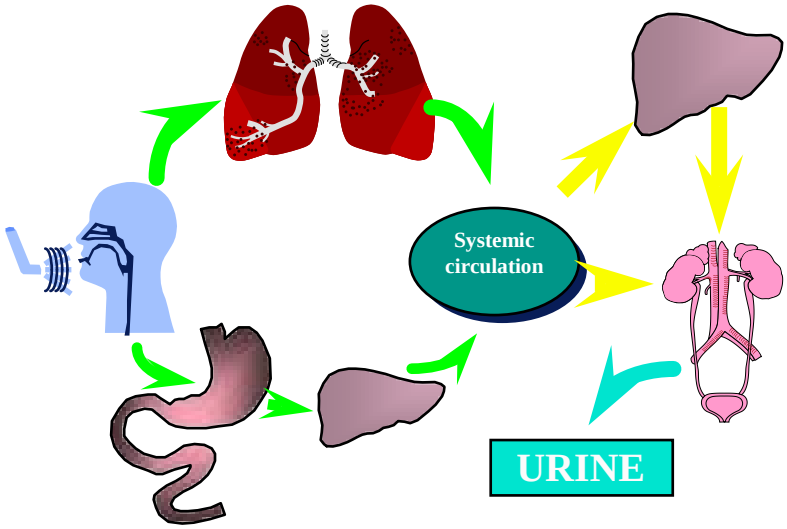


Figure 2.24 Pharmacokinetics of inhaled drug [Reproduced from Chrystyn (2001).]

Pharmacokinetic methods (using plasma or urine samples) can be used to identify the relative lung deposition of the drug (the effective lung dose) and total systemic delivery. The plasma or the urine concentration of the drug accounts for the total absorption from lung and gastrointestinal tract of the inhaled drug. The identification of the relative lung deposition using pharmacokinetic method, for a drug with an extensive first pass effect in the gastrointestinal tract (e.g. fluticasone) is easy as the oral absorption of it would be negligible. Hence sampling of urine or plasma after inhalation would be accurate to measure the relative lung deposition. The use of oral charcoal doses taken before and after the inhalation to block the gastrointestinal absorption of the oral ingested portion has been demonstrated (Borgstrom and Nilsson, 1990). The original study by Borgstrom and Nilsson (1990), as shown in Figure 2.25 highlighted the value of charcoal blockage of the gastrointestinal absorption to determine the relative amount of drug delivered to the lung. However, because this method uses oral charcoal it would be unethical to extend this method to patient studies due to their concomitant oral therapy (Chrystyn, 2000).

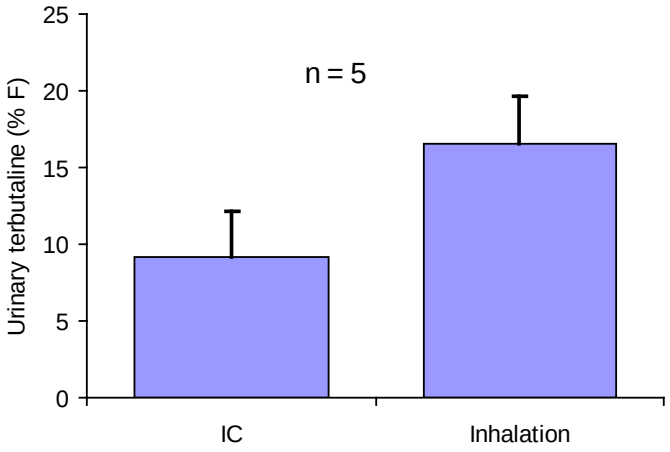


Figure 2.25 Mean amount of urinary terbutaline excreted 36 hours post dose with and without the administration of charcoal (Borgstrom and Nilsson, 1990).

The measurement of either plasma concentration or amount excreted in urine post inhalation during the absorption lag time of the orally swallowed portion represents the drug deposited in the lungs. The proposed time for the plasma sampling method is 5, 10 and 20 minutes post inhalation (Anhoj et al., 1999; Lipworth and Clark, 1997; Mobley and Hochhaus, 2001) and for urine it is 30 minutes after the start of an inhalation (Hindle and Chrystyn, 1992).

The drug concentrations in plasma or serum are very low because the inhaled dose is small and the volume of distribution of the drug is very large, specially for polar or basic drugs which are cleared from the blood very fast (Chrystyn, 1994).

Using the lag time of the gastrointestinal absorption Hindle and Chrystyn (1992) developed a urinary pharmacokinetic method to determine the relative lung and systemic bioavailability of inhaled salbutamol. They showed that the urinary salbutamol excreted 30 minutes post dosing was an index of the lung bioavailability and that the urinary salbutamol excreted 24 hours post dosing was an index of the systemic bioavailability. The amount excreted 30 minutes post inhalation was significantly different from that after oral dosing ($p<0.001$) as shown in Figure 2.26. The method is very simple and non-invasive.

The method has been extended to assess the relative lung bioavailability of inhaled sodium cromoglycate (Aswania and Chrystyn, 2001; Aswania and Chrystyn, 2002; Aswania et al., 1997; Aswania et al., 1999), nedocromil (Aswania et al., 1998), gentamicin (Al-Amoud et al., 2002), tobramycin (Barber, 2002) and formoterol (Nadarassan et al., 2007).

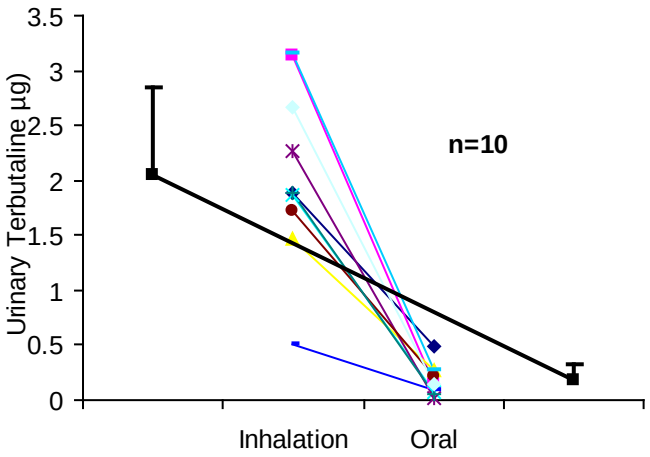


Figure 2.26 Mean and individual amounts of urinary salbutamol excreted 30 minutes post inhalation and oral dosing (Hindle and Chrystyn, 1992).

The use of this pharmacokinetic method provides useful information about the relative lung deposition and the relative systemic delivery to compare different methods, devices or technique and allows the use of the original products. The disadvantages of the pharmacokinetic methods are the need to differentiate between the swallowed and inhaled fraction of the inhaled dose. They do not identify dose deposition into different zones of the lungs and some assays do not have the sensitivity to measure the low concentrations (Chrystyn, 2001).

2.5.2. Gamma scintigraphy:

There are two types of gamma scintigraphy, two dimensional and three-dimensional imaging methods (Chrystyn, 2000; Newman and Wilding, 1999).

The two dimensional gamma scintigraphy (planer imaging) method usually uses 99m Technetium adhered to either the formulation or the drug molecules in the dosage form

(physical attachment). The subjects inhale the combination and then use rapid imaging of a radionuclide to identify the drug deposition in the lung following inhalation (Bondesson et al., 2003; Borgstrom and Newman, 1993; Newman and Wilding, 1999). The planar images obtained with this method may be insensitive to the relative deposition in the different zones of the lungs.

Three-dimensional imaging methods (SPECT and PET) have recently been introduced to overcome the disadvantage of planner imaging. SPECT (single photon emission computed tomography) is similar to two-dimensional gamma scintigraphy (physical attachment of the radiolabelling of the drug) except that the gamma camera rotates through 360^{o}C. This increases the data collection time. Hence, a very large dose is required. The dose may be 40 times larger than that required for the planar imaging and thus introduces formulation and preparation problems (Newman and Wilding, 1999).

PET (Positron emission tomography) is a direct incorporation of a radiolabel into the drug molecule (chemical attachment). The ones recently used are positron emitters such as ^{11}C (short half life) or ^{18}F (long half life). The positron emitters used so far have short half-lives and the method is very expensive. ^{11}C has been introduced into triamcinolone acetonide and studies have highlighted the greater peripheral deposition when a spacer is attached to an MDI (Heald et al., 1997). This was mainly due to a substantial increase in the total amount of drug deposited in the lungs (13.6% with and 4.9% without the spacer). This technique has also been used for fluticasone (Berridge et al., 1998).

Gamma scintigraphy produces data of the total lung dose that is absorbed through the airways and cleared by mucociliary clearance. Since the former is the part of the dose that is responsible for the therapeutic action in the airways then gamma scintigraphy will overestimate the effective dose reaching the lung. The charcoal block method using urinary excretion of terbutaline (Borgstrom and Nilsson, 1990) has been compared with total lung deposition measured by gamma scintigraphy (Borgstrom et al., 1992). The mean (SD)

terbutaline excreted in the urine post inhalation with concurrent charcoal administration was 21.1 (3.2) % of the nominal dose whilst gamma scintigraphy showed the total lung deposition to be 26.9 (3.8) %. The difference obtained is because part of the inhaled dose is cleared by mucociliary clearance. This fraction of the dose delivered to the lungs is identified by gamma scintigraphy but not by pharmacokinetic methods.

The long term safety and the expensive study costs are considered disadvantages of gamma scintigraphy (Chrystyn, 2001). In addition, the labelling procedure involves manipulations of the formulations. Consequently, the radiolabelled formulation is different from the formulation in the original product although in-vitro tests are carried out to confirm similarity (Snell and Ganderton, 1999). However, a previous report has shown that when using the Andersen Cascade Impactor the mass median aerodynamic diameter (MMAD) of a labelled drug was larger than the original product and that there was a difference in the homogeneity of the size distribution (Newman et al., 1982). Furthermore, particle size ranges should be quoted as amounts emitted rather than a percentage, and in-vitro determinations should use the same number of doses that were used in the scintigraphic study. Agencies such as the FDA are very cautious in using results from imaging studies for assessing bioequivalence (Mobley and Hochhaus, 2001).

There are also non-radioactive assessment methods such as nuclear magnetic resonance imaging (MRI) and magnetic marker monitoring (MMM) but their use is not well established yet (Newman and Wilding, 1999).

2.5.2. Clinical studies:

Clinical studies using spirometry or bronchoprovocation challenge have been used to identify the bioequivalence between two inhaled products (Eiser et al., 2001; Rodriguez-Carballeira et al., 2001).

A method often used in the evaluation of the efficacy of inhaled drugs is the protective effect on methacholine or histamine induced bronchoconstriction (Tattersfield, 1987;

Britton et al., 1988). The inhalation of a short-acting β_2-agonist increases the provocative dose of inhaled methacholine or histamine from 1.1 to 3.9 times (Casterline et al., 1976; Cockcroft et al., 1977). More recent studies have demonstrated that salbutamol increases the provocative dose of methacholine by 2.8 to 3.1 times (Wong et al., 1997; Seppälä et al., 1998).

Most clinical studies are carried out using measurements at the flat (plateau) portion of the dose-response relationship. For instance, a doubling of the therapeutic fluticasone inhaled dose has been shown to increase the peak expiratory flow rate (PEFR) by only 4.3 L min^{-1} from a baseline of almost 200 L min^{-1} (Dahl et al., 1993). Also for beclomethasone the FEV_1 (forced expiratory volume in the first second) increased by 0.18 L above baseline after 200 mg inhaled twice daily and by 0.21 L after 400 mg twice daily (Raphael et al., 1999). For the β_2-agonists the maximum response from therapeutic inhaled doses has been studied (Barnes and Pride, 1983). It has been found that in normal subjects a maximum airway response to inhaled salbutamol was achieved with a cumulative dose of 110 µg. By contrast the dose required to produce a maximal bronchodilator response in asthmatic subjects was significantly higher and increased as the severity of bronchoconstriction increased (Barnes and Pride, 1983). Newman et al. (1991) also demonstrated this in asthmatic subjects except that they also measured lung deposition using gamma scintigraphy. When these subjects inhaled radiolabelled salbutamol from a MDI and a MDI attached to a large volume spacer the total lung deposition was 12.3 and 23.1% (of the dose), respectively, but there was no difference in spirometry measurements (Newman et al., 1991b). Also bronchoprovocation challenge cannot differential between different inhalation techniques due to the large variability of the method (Tomlinson et al., 2005). The inter-patient variability in clinical studies is high. Thus sensitivity to detect a difference is low and so a large number of subjects need to be studied (Barry and

O'Callaghan, 2003). Furthermore the bronchoprovocation agents may stimulate different receptors to those of the drug studied causing deterioration of lung function.

2.5.3. In-vitro methods:

In-vitro methods are used as a quality assurance procedure to identify the quality of the inhaled product such as the total emitted dose, uniformity of dose and the aerodynamic particle size distribution. Further, they are often extrapolated to give an estimation of in-vivo deposition. The most used techniques are inertial separation methods and laser diffraction. Microscopic methods have also been used.

Aerosol particles are not perfectly spherical, instead they are of an uneven shape, weight and surface area that would be impossible to be described accurately by any one variable. Instead, such particles are generally described by their aerodynamic diameter. This is the diameter of a unit density sphere that has the same settling velocity in air as the particle. This aerodynamic diameter takes into account particle density, shape and size (Hickey, 1992). The mass median aerodynamic diameter (MMAD) of an aerosol is the diameter that separates the mass of the particles equally by 50% and is the term along with the geometric standard deviation (GSD) that is often used to describe an aerosol. The GSD is a measure of the polydispersity, or spread, of an aerosol. A monodisperse aerosol has a GSD of 1 and heterodisperse aerosol has a GSD greater than 1.2. The amount of an aerosol contained in particles with an aerodynamic diameter less than 5μm, is generally referred to as the fine particle dose [FPD] (Newman, 1991). This is the quantity of drug in the prescribed dose of an inhaled product that is generally considered to be of a size capable of penetrating the lung during inhalation i.e. respirable amount. The FPD expressed as a percentage of the emitted dose is the fine particle fraction (FPF). Although the respirable fraction does not equal the amount of aerosol deposition in the lung, it provides an estimate of the fraction of the dose that has the potential to be deposited into the lungs (Barry and O'Callaghan, 2003; Dhand and Fink, 1999).

2.5.3.1 Total emitted dose:

(a) MDI dose emission unit:

The delivered dose is the total amount of drug emitted from the drug device and hence available to the user. Its uniformity is critical to the safety and efficacy of all orally inhaled drug products. Based on an original design by Charles Thiel (a chemist who is credited with producing the first MDI, 1956) in the laboratories of 3M Healthcare, the MDI dose emission unit, as shown in Figure 2.27, has been designed specifically for the sampling and testing of MDIs. It is used to perform those tests specified by the relevant compendial standards (European Pharmacopeia, 2001; United States Pharmacopeia, 2005; British Pharmacopoeia, 2005a), namely 'Delivered Dose Uniformity' and in the case of multidose inhalers, 'Delivered Dose Uniformity over the Entire Contents'.

The sample collection tube is fitted with a 25 mm glass fibre filter, with 99.98% aerosol retention and a pore size of 1 micron. It has a volume of approximately 50 ml, which approximates to that of the human oropharynx.

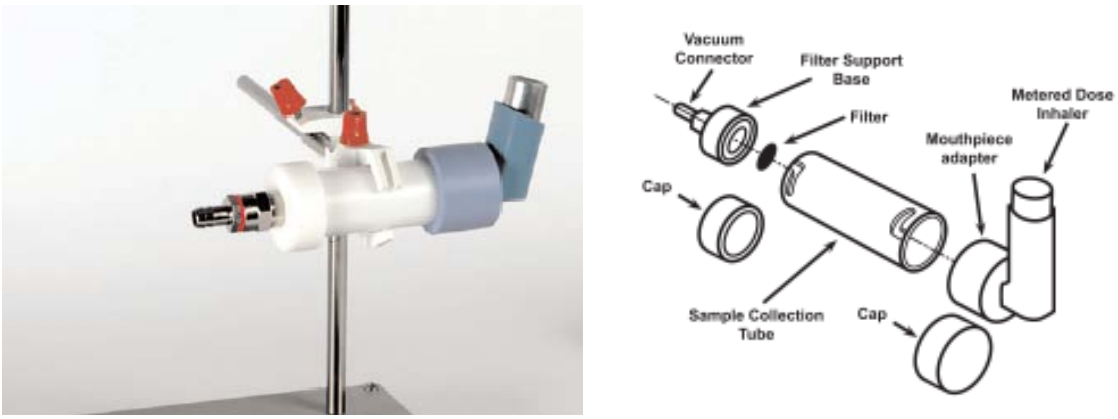


Figure 2.27 Parts and fitting of the MDI dose emission unit (Reproduced from Copley 2008).

(b) DPI dose emission unit:

A second and larger version of the MDI dose emission unit capable of sampling at a variety of flows up to 100 L min^{-1} is available for use with Dry Powder Inhalers (DPIs).

Like the MDI dose emission unit, the DPI dose emission unit, as shown in Figure 2.28, is used to perform those tests specified by the Pharmacopoeia (European Pharmacopeia, 2001; United States Pharmacopeia, 2005; British Pharmacopoeia, 2005a) that relate to content uniformity namely 'Delivered Dose Uniformity' and in the case of a multi-dose DPI, 'Dose Uniformity over the Entire Contents'. In the case of DPIs, both the emitted and fine particle dose is affected by the strength and duration of the patient's inspiration (Newman et al., 1991a; Ross and Schultz, 1996). Furthermore, different inhalers provide varying degrees of resistance to flow (Clark and Hollingworth, 1993). For these reasons, according to the compendial methods, it is essential, particularly when testing DPIs of intermediate to high flow resistance, to determine the appropriate test flow and duration based on the pressure drop developed over the specific inhaler under test (European Pharmacopeia, 2001; United States Pharmacopeia, 2005; British Pharmacopoeia, 2005a). Assuming this is done, it is then important to ensure that critical (sonic) flow occurs in the flow regulating valve employed in the system. This ensures that the flow through the dose emission unit is set as required and that it is unaffected by minor fluctuations in the pump. The resulting airflow that produces a drop of 4.0 kPa over the inhaler to be tested, should then be used for the determination of the delivered dose and particle size distributions as recommended by the compendial methods (European Pharmacopeia, 2001; United States Pharmacopeia, 2005; British Pharmacopoeia, 2005a).

The only exception to this criterion is for those low resistances DPI that produce a flow in excess of 100 L min^{-1}. In this case, a flow of 100 L min^{-1} should be used.

When using the DPI dose emission unit, it is necessary to use a critical flow controller to determine the pressure drop over the inhaler, to ensure critical (sonic) flow conditions and to set the duration, and hence volume of inspiration.

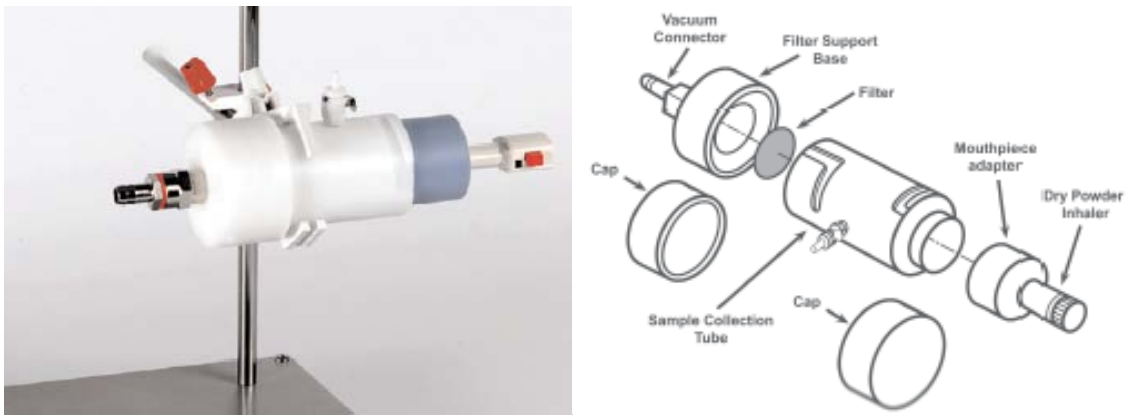


Figure 2.28 Parts and fitting of the DPI dose emission unit (Reproduced from Copley 2008)

2.5.3.2 Characterisation of the emitted dose:

Inertial impaction as a size separation factor by cascade impaction methods has been widely used as the 'gold standard' to determine the aerodynamic characteristics of the emitted dose from aerosols (European Pharmacopeia, 2001; United States Pharmacopeia, 2005; British Pharmacopoeia, 2005a). The application of this class of particle size analysis to the assessment of medical aerosols has recently been extensively reviewed, focusing on the types of impactor that are in current use together with their strengths and limitations for measurements with the different classes of inhalers (Mitchell and Nagel, 2003).

(a) Twin Stage Impinger:

The Twin Stage Impinger shown in Figure 2.29 (United States Pharmacopeia, 2005) can be operated using inhalation flows of 30 and 90 L min^{-1}. It has been retained in the Pharmacopeias because of its value as a simple and inexpensive quality control tool. However, it is generally accepted that an impactor or impinger should have a minimum of five stages, and preferably more, if it is to provide detailed particle size distribution data.

The Twin Impinger is likely to be withdrawn from compendial methods. Although they are useful for rapid quantification of the FPF, it provides insufficient rapid resolution in the critical aerodynamical diameter range of 0.5 to 5.0 µm (Newman and Kenyon, 1994).

Figure 2.29 The Twin Stage Impinger (Reproduced from Copley, 2008).

(b) Multistage Liquid Impinger:

The Multistage Liquid Impinger [MSLI] (United States Pharmacopeia, 2005) consists of a metal throat, impaction stages and a final filter. The MSLI operates at 60 L min^{-1} and has cut-off diameter of 25, 13, 6.8, 3.1 and 1.7 µm, thus giving a much more detailed particle size distribution then the Twin Stage Impactor (Figure 2.30). However the number of cut-off diameters is limited.

Figure 2.30 Multistage Liquid Impinger (Reproduced from Copley, 2008).

(c) Anderson Cascade Impactor:

As shown in Figure 2.31 the Anderson Cascade Impactor (ACI) consists of a stack of eight plates, each containing a series of precision drilled holes, and a final filter stage (European

Pharmacopeia, 2001; United States Pharmacopeia, 2005; British Pharmacopoeia, 2005a). The diameter of the holes decreases progressively in each succeeding stage. Therefore, the jet velocity increases as a particle travels through the impactor. The ACI operates at a flow of 28.3 L min^{-1} with cut-off diameters of 9, 5.8, 4.7, 3.3, 2.1, 1.1, 0.65 and 0.43 µm, respectively. This method allows a more detailed description of the particle size distribution than either the MSLI or the Twin Impinger. Although the ACI is designed to be used at inhalation flows of 28.3 L min^{-1}, modifications are available for the use at high flows 60 and 90 L min^{-1}. For an inhalation flow of 60 L min^{-1} stages 0 and 7 are removed and replaced by stages -1 and -0 on the top of the ACI. For an inhalation flow of 90 L min^{-1} stages 0, 6 and 7 are removed and replaced by stages -2, -1, and -0 on the top of the ACI. Alternatively the standard ACI can be operated at different flows with the cut-off stages altered according to the following equation ($ECD_{F2} = ECD_{28.3}\,(28.3/F2)^{0.5}$). Where ECD_{F2} = the Effective Cut-Off Diameters at the other flow; $ECD_{28.3}$ is the ECD at the manufacturers flow (28.3 L min^{-1}) and F2 is the other flow rate in L min^{-1} (Van Oort, 1995).

When sampling DPI aerosols, a pre-separator with a small amount of solvent is added to the ACI to prevent those particles greater than 10µm from bouncing into the ACI stages (Figure 2.31).

The high flows these devices operate at may result in the extensive evaporation of aqueous particles such as nebulised aerosols, thus underestimating the particles size (O'Callaghan and Barry, 1997). The respiratory tract has a highly complex anatomy along with the effects of temperature, humidity and pathological changes. Hence such in-vitro methods cannot accurately predict lung deposition. Differences in relative humidity and temperature within the respiratory tract may also lead to a change in the size of particles.

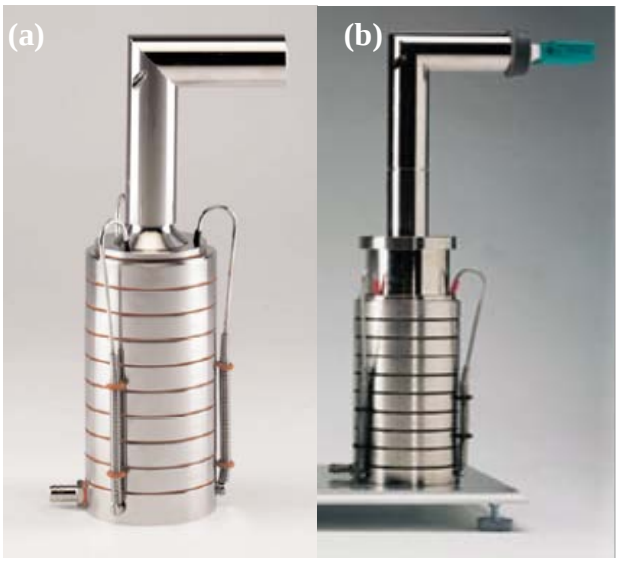


Figure 2.31 (a) Anderson Cascade Impactor set for MDI. (b) Anderson Cascade Impactor set for DPI (Reproduced from Copley, 2008).

(d) Next Generation Impactors:

The Next Generation Impactor has been designed specifically for pharmaceutical inhaler testing (Figure 2.32). This impactor has seven stages and is intended to operate at any inhalation flow between 30 and 100 L min^{-1}.

The cut-off size ranges from 0.54 µm to 11.7 µm aerodynamic diameter at 30 L min^{-1} and 0.24 µm to 6.12 µm at 100 L min^{-1}. The NGI has several features to enhance its utility for inhaler testing:

1. Particles deposited on collection cups are held in a tray from the impactor as a single unit, facilitating quick sample turn-around times if multiple trays are used.

2. The user can add up to approximately 40 ml of an appropriate solvent directly to the cups for more efficient drug recovery.

3. The Micro-orifice Collector (MOC) captures, in its collection cup, extremely small particles normally collected on the final filter of other impactors. The particles captured in the MOC cup can be analyzed in the same manner as the particles collected in the other impactor stage cups.

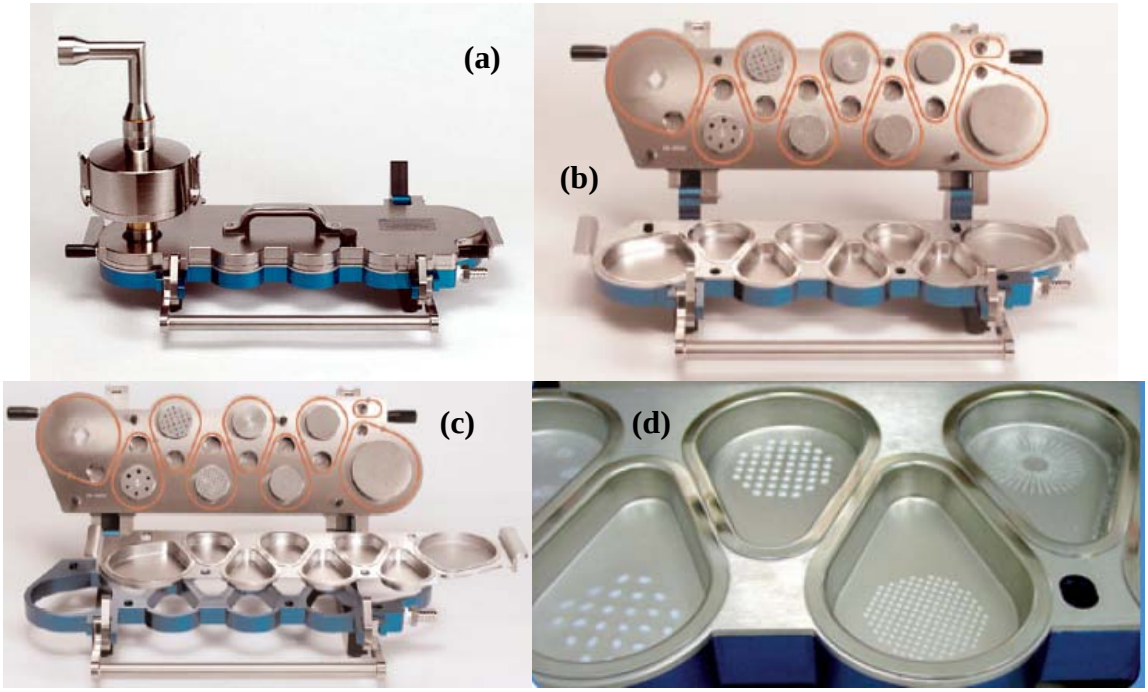


Figure 2.32 Next Generation Impactor (Reproduced from Copley, 2008). (a) NGI including preseparator and induction port. (b) NGI (open view) showing nozzles & collection cups. (c) NGI (open view) showing cup tray removed. (d) Collection cups showing typical deposition pattern.

2.5.3.3 Principles of operation of the cascade impactors:

Cascade impactors operate on the principle of inertial impaction. Each stage of the impactor comprises a single or series of nozzles or jets, as shown in Figure 2.33, through which the sample laden air stream is drawn directing any airborne particles towards the surface of the collection plate for that particular stage. Whether a particular particle impacts on that stage is dependent on its aerodynamic particle size. Particles having sufficient inertia will impact on that particular stage collection plate, whilst smaller particles with insufficient inertia will remain entrained in the air stream and pass to the next stage where the process is repeated.

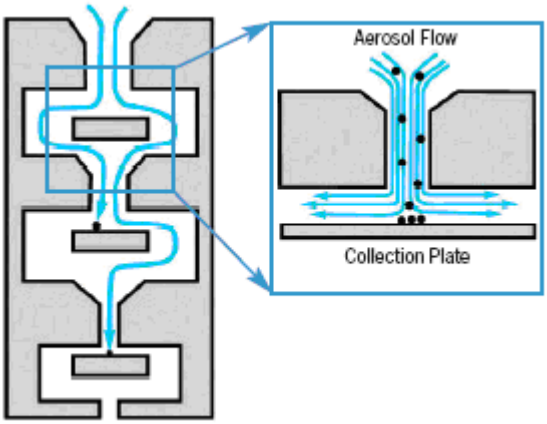


Figure 2.33 Principal of Cascade impactors operation (Reproduced from Copley, 2008).

The stages are assembled in a stack, in order of decreasing particle size. As the jets get smaller, the air velocity increases and finer particles are collected. Any remaining particles are collected on a final filter. At the end of the test, the particle mass relating to each stage collection plate is recovered using a suitable solvent and then analysed usually using HPLC to determine the amount of active drug actually present.

By analysing the amount of drug deposited on the various stages in this manner, it is then possible to calculate the Fine Particle Dose (FPD) and Fine Particle Fraction (FPF) and following further manipulation, the Mass Median Aerodynamic Distribution (MMAD) and Geometric Standard Deviation (GSD) of the active drug particles collected.

The term ‘impactor’ is generally used for an instrument where the particles ‘impact’ on a dry impaction plate or cup. If the collection surface is liquid, as in the case of the multi stage liquid impinger, then the term ‘impinger’ is used. The general principles of inertial impaction apply to both ‘impactors’ and ‘impingers’

In some instances, particles may bounce in response to impact when they contact the collection plate, in which case they are normally re-entrained into the air stream and carried to a lower stage. This can be a particular problem with a DPI and certain MDIs (where measurements are based on a limited number of actuations from the inhaler). This tendency may be avoided by coating the collection plate with a suitable surface coating

(Allen, 1990). Also particle deposition on impactor parts other than the collection plates is called ‘Inter-stage Losses’ (Kamiya et al., 2004).

2.5.3.4 In-vitro characterisation of the dose emitted from a Nebuliser:

For the in-vitro characterisation of the dose emitted from a nebuliser, the European Respiratory Society Nebuliser Guidelines were published to standardise nebuliser performance and therapy (Boe et al., 2001). These guidelines recommend the Comité Européen Normalisation (CEN) method to measure the aerodynamic particle size of the dose emitted from a nebuliser (Boe et al., 2001; Comité-Européen-Normalisation., 2001). This in-vitro method uses the Marple 298X Cascade Impactor, which is shown in Figure 2.34, because it is operated at low flows that correspond close to that of a normal sinus breathing inhalation flow. However the sampling of a limited fraction of the emitted dose due to the limited loading capacity of the cascade stages, filter fitting (and availability), desorption problems and its suitability for operation with drug formulations may each affect assessments (Jauernig et al., 2002; Jauernig et al., 2004). Also it has been shown that when using the Marple Impactor only 13% of the aerosol emitted is sampled (Jauernig et al., 2004). This impactor has 8 stages and a final filter. The stages have effective cut-off diameters of 21.3, 14.8, 9.8, 6.0, 3.5, 1.55, 0.93 and 0.52 µm respectively, at the recommended flow of 2 L min^{-1}.

Figure 2.34 The Marple 298X Cascade.

As previously described; the NGI has been introduced for the determination of the aerodynamic particle size distribution of the dose emitted from metered dose and dry powder inhalers using compendial procedures (United States Pharmacopeia, 2005; British Pharmacopoeia, 2005a). The methodology was calibrated to work with a flow range of 30-100 L min^{-1} and later at 15 L min^{-1} for nebulisers (Marple et al., 2004b). The cut-off diameters of the stages at 15 L min^{-1} flow were 14.1, 8.61, 5.39, 3.3, 2.08, 1.36 and 0.98. Studies have assessed the use of the NGI without the preseparator, with a filter in or after the Micro-Orifice Collector (MOC) [Figure 2.35] to collect any extra-fine particles that would bypass this component (Marple et al., 2004a; Berg et al., 2007) and with cooling the NGI either in a water bath (Jauernig et al., 2003) or refrigerator at 5^{o}C for 90 minutes before use (Berg and Asking, 2004; Berg et al., 2007). A consortium of pharmaceutical companies was formed in 2002 (the EPAG group–European Pharmaceutical Aerosol Group) to investigate the NGI at a flow of 15 L min^{-1} in compliance with the CEN recommendations for nebulisers, and sampling the whole air stream leaving the Nebuliser.

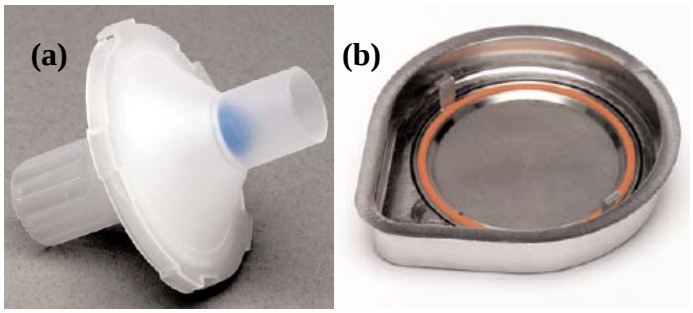


Figure 2.35 (a) Electrostatic filter holder placed after the MOC. (b) MOC with the internal filter holder. (Reproduced from Copley, 2008)

2.5 β_2-adrenergic drugs:

The β-receptor is a glycoprotein embedded in the plasma membranes of a number of cell types. Three distinct subtypes of β-receptors are now known, β_1, β_2, and β_3, found predominately in cardiac muscle, airway smooth muscle and adipose tissue, respectively

(Sears and Lotvall, 2005). Following β_2-adrenoceptor activation, the β_2-receptor induces cyclic AMP to produce an intracellular signaling which mainly produces airway relaxation through phosphorylation of muscle regulatory proteins and modification of cellular Ca^{2+} concentrations (Johnson, 2001; Sears and Lotvall, 2005; Thirstrup et al., 1997). As shown in Figure 2.36; β_2-agonists have been categorized into those which directly activate the receptor (salbutamol and terbutaline), those, which are taken up into a membrane depot (formoterol) from which it is thought they progressively leach out to interact with the β_2-receptors and those, which interact with a receptor-specific, auxiliary binding site and remains in the outermost monolayer, and slowly diffuses from the membrane (salmeterol) (Sears and Lotvall, 2005; Waldeck, 2002). These differences in mechanism of action are reflected in the kinetics of airway smooth muscle relaxation and bronchodilatation in asthmatic patients. A number of polymorphisms of the β_2-receptor have been described which appear to alter the behavior of the receptor, including a degree of downregulation (which produce tolerance to the β_2-agonists) and response to β_2-agonists (Brodde et al., 2002; Johnson, 2001; Sears and Lotvall, 2005).

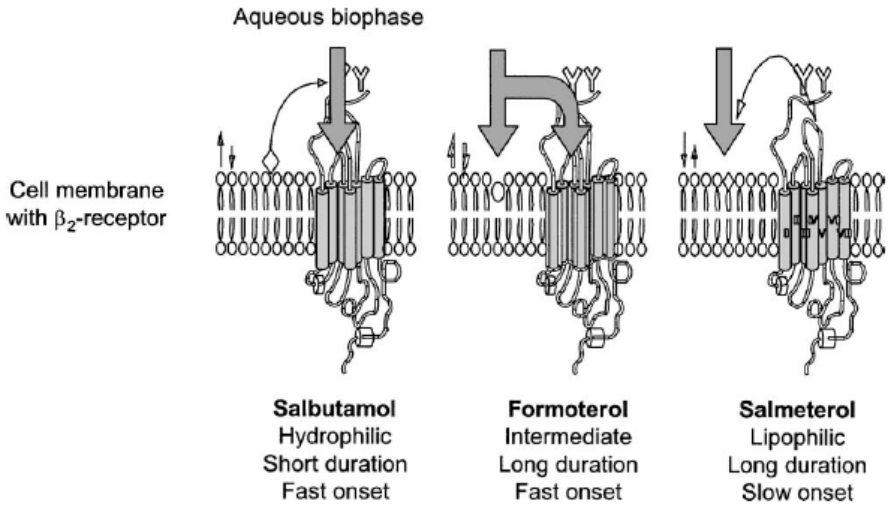


Figure 2.36 Diagrammatic representation of the diffusion microkinetic hypothesis. [reproduced from Anderson (1993)]

β_2-agonists and corticosteroids are the standard drugs routinely used in the management of asthma. β_2-agonists have been shown to be effective for the protection against exercise-induced bronchoconstriction (Vilsvik et al., 2001) and preferably administered by inhalation to deliver the drug directly to the desired site of action. Short acting inhaled β_2-agonists such as salbutamol and terbutaline are the initial drug of choice for acute bronchospasm because of their immediate bronchodilatation effect.

The first β-agonist introduced to treat the symptoms of asthma was isoprenaline (isoproternol), which was a non selective β-agonist. Its use was subsequently discontinued in favour of more selective β_2-agonists because of the epidemic morbidity and mortality in asthmatic patients associated with the use of non selective β-agonists such as isoprenaline in the 1960s (Pearce et al., 1991). But even the selective β_2-agonists had a second epidemic morbidity (Crane et al., 1989; Pearce et al., 1990; Grainger et al., 1991; Pearce et al., 1991; Pearce et al., 1995). It occurred in New Zealand and Canada in the late 1970s to 1980s associated with the use of the long acting β_2-agonist fenoterol (Pearce et al., 1995) and the same results in Canada and Japan for salbutamol (Spitzer et al., 1992; Beasley et al., 1998). However, an analysis of the deaths in the New Zealand study could not identify such a risk of using β_2-agonists other than fenoterol (Pearce et al., 1995). The meta-analysis of the accumulated data to 1992 concluded that the increase in morbidity due to β_2-agonists was slight and doubtful (Mullen et al., 1993). Since the last epidemic, the doses of β_2-agonists have fallen, selective short acting agonists have became more preferred, and even the frequency of administration has changed especially for the long acting β_2-agonists whilst regular administration of the majority has changed to administration as required.

2.5.1. Terbutaline Sulphate:

Terbutaline sulphate is a synthetic resorcinol derivative β_2-adrenergic agonist that is used as a bronchodilator in the treatment of asthma. It was first introduced in 1970s (Sears and Lotvall, 2005; Waldeck, 2002).

Its Molecular Weight is 548.658 and its empirical formula is $(C_{12}H_{19}NO_3)_2.H_2SO_4$. Its chemical name is [(1RS)-1-(3,5-Dihydroxyphynyl)-2-(tert-butylamino)-ethanol] sulphate (2:1 salt) (British Pharmacopoeia, 2005b). Figure 2.37 shows the molecular structure of terbutaline sulphate.

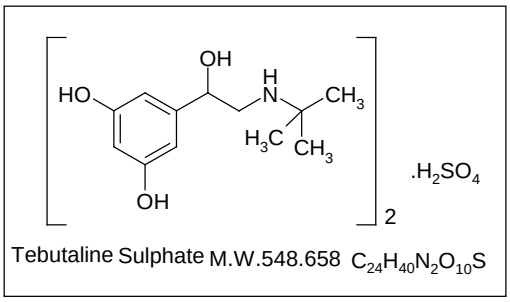


Figure 2.37 Molecular structure of terbutaline sulphate.

It is a hydrophilic white to grey-white crystalline powder, odourless or with a faint odour of acetic acid, soluble in water and 0.1N-hydrochloric acid, insoluble in chloroform, slightly soluble in methanol (United States Pharmacopeia, 2002).

The commercially available terbutaline sulphate is a racemic mixture. Its melting point range differs according to the crystal type, as there are two types of the crystal form. Crystal form A has a melting point range from 268 ^{o}C to 271 ^{o}C, and the crystal form B has a melting point range from 258 ^{o}C to 260 ^{o}C (Analytical Profiles, 1990).

Terbutaline sulphate is present in many dosage forms (inhalation, oral solution, injection and tablet) for prophylactic and acute bronchodilator treatment. Many methods have been established to determine terbutaline in these dosage forms (Daraghmeh et al., 2002; Henze et al., 2001; Lv et al., 2003).

Terbutaline Sulphate is a direct selective β_2-agonist, administered as the sulphate salt for its bronchodilating properties in reversible airways obstruction diseases and in patients with COPD. It also decreases uterine contractility by relaxing the uterus smooth muscles hence may be used to arrest premature labour in several dosage form, even as a vaginally applied gel (Bulletti et al., 1997; de Moustier et al., 1997; Elliott et al., 1999; Elliott et al., 2002;

Smigaj et al., 1996; Sophie Thayer, 1996). However in most cases it causes pulmonary oedema and other cardiovascular effects to the mother. The British National Formulary (BNF) does not recommend its prolonged use as a maintenance treatment in premature labour (Martindale, 2002) because of the risk to the mother increases after 48 hours and there is a lack of evidence of benefit from further treatment.

After inhalation, the bronchodilating effect of terbutaline usually begins within 5 minutes and last for about 3 to 4 hours. In regular use it should be inhaled as one or two doses of a 250μg terbutaline sulphate inhalation, every 4 to 6 hours upto a maximum of 8 inhalations in 24 hours (Martindale, 2002). Spacers may be used with terbutaline MDIs for better lung deposition (Comis et al., 1993). A dry powder inhaler (DPI) delivering 500μg terbutaline sulphate per dose is also available, with a maximum of 4 inhalations in 24 hours.

For oral administration the dose starts with 2.5 to 3 mg three times daily upto a maximum of to 5 to 6 mg three times daily, and in children the dose should be calculated with respect to body weight with a suggested dose of 75μg/kg. The onset of action of orally administrated terbutaline is about 30 minutes and its duration of action is up to 8 hours (Martindale, 2002). Modified release tablets are also available with a daily dose of 7.5mg twice daily. Severe unresponsive bronchospasm may require the administration of nebulised terbutaline sulphate which is available as 5 mg/2 ml terbutaline sulphate.

2.5.2. Pharmacokinetics:

Terbutaline sulphate is variably absorbed from the gastrointestinal tract. The resorcinol structure of terbutaline sulphate prevents it from being metabolised by the Catechol-O-Methyltransferase or monoamine oxidase (Analytical Profiles, 1990; Sears and Lotvall, 2005; Waldeck, 2002). About 60% of the absorbed dose undergoes first pass effect metabolism by conjugation with sulphuric acid, and some conjugation with glucuronic acid (in rate) in the liver and the gut wall (Martindale, 2002). Terbutaline like most other sympathomematics exists as a ± racemic mixture. The (-) enantiomer is the

pharmacologically active one. The oral bioavailability of the racemic mixture is 14.8%. Several studies have tried to separate the enantiomers from each other or determinate the concentration of each one of them in dosage forms and in biological samples (Desiderio and Fanali, 1995; Huynh et al., 1995; Boulton and Fawcett, 1996; Szeman et al., 1997; de Boer and Ensing, 1998; Lu and Cole, 1998; Kim et al., 2001a; Kim et al., 2001b; Roig et al., 2002; Lee and Jung, 2003).

The Terbutaline sulphate volume of distribution is 1.6 L kg^{-1} (Nyberg, 1984) and it is excreted in the urine as an inactive conjugate and unchanged terbutaline. Its half-life is 3 to 4 hours. There is some placental transfer and traces are delivered into the breast milk. Terbutaline plasma protein binding is low, 14 to 25% in contrast, binding to erythrocytes is more pronounced producing a erythrocyte: plasma concentration ratio of 2 to 2.5 (Analytical Profiles, 1990). Several methods have been described for the extraction and the determination of terbutaline in biological samples (Borgstrom and Nilsson, 1990; Boyd D, 1996; Croes et al., 1995; Herring and Johnson, 2000; Polettini et al., 1995; Van der Vlis et al., 1995; Van Vyncht et al., 1996).

Terbutaline and other β-agonists, especially large doses, may cause fine tremors of skeletal muscles (particularly the hand), palpitations, tachycardia, nervous tension, headaches, peripheral vasodilatation and rarely muscle cramps (Waldeck, 2002). Potentially serious hypokalaemia has been reported after large doses especially after parental or nebulised administration and is potentiated by concomitant administration of corticosteroids, diuretics, or xanthines [e.g. theophylline] (Smith and Kendall, 1986). Potassium blood concentrations should therefore be monitored in severe asthma when administrating large doses, as hypokalaemia may lead to arrhythmias. The concomitant administration of terbutaline with an monoamineoxidase inhibitor (MAOIs), e.g. toloxatone, may cause a symptom resembling pheochromocytoma (A rare catecholamine secreting tumour of adrenal medulla, accompanied with hypertension, headache, palpitation, and excessive

sweating), and this interaction is more typical of the older, irreversible, less selective MAOIs (Martindale, 2002). Some inhaled powder formulation of bronchodilators (including terbutaline) have been found to cause tooth erosion due to their pH, which is below 5.5, and it was suggested that might contribute to the dissolution of the enamel surface of the teeth (O'Sullivan and Curzon, 1998).

Some studies suggest that regular inhalation of a short acting β_2-agonist, although it continues to produce bronchodilatation, increases the airway hyperresponsiveness and may reduce its protective effect against bronchoconstriction provoked by stimuli such as allergen due to tolerance and tachyphylaxis (Lipworth et al., 1998; Hancox et al., 2000). Also, it have been shown that enhancement of Interleukin-8 production is one of the pathways via which β_2-adrenergic agonists can influence inflammatory responses (Kavelaars et al., 1997). Hence anti-inflammatory therapy such as corticosteroids is also required (Korn et al., 1998).

There is an inactive prodrug of terbutaline called bambuterol (Terbutaline Bisdimethyl Carbamate). It is a long acting bronchodilator (at least 24 hours) because when administrated orally at bedtime it is slowly hydrolysed to terbutaline and carbamic acid in the systemic circulation. The Preliminary clinical studies have indicated that the mean terbutaline half-life after bambuterol ingestion is about 21 hours (Zeng et al., 1995). Bambuterol inhibits the plasma cholinesterase activity like salbutamol, which can be correlated with the prolonged action of bambuterol and its prolongation of activity of sympathomematics (e.g. Suxuamethonium) when co-administered with bambuterol (Staun et al., 1990).

There is also another prodrug of terbutaline, which is ibuterol the diisobutyryl ester of the resorcinol function of terbutaline. After inhalation, ibuterol is 3-times as effective as terbutaline. In-vivo studies have shown that ibuterol is absorbed more rapidly than terbutaline, but both lung and serum terbutaline concentrations were lower after inhaled

ibuterol administration than those obtained after the administration of the free terbutaline, indicating that the prodrug acts as a reservoir releasing parent drug over a prolonged period (Andersson, 1976).

3.1 Introduction

The use of pharmacokinetic methods to identify the relative lung and systemic bioavailability of inhaled drugs require a sensitive, reliable and robust assay for the drug in samples of any body fluid. Traditional high performance liquid chromatography is used with solid phase extraction to isolate the drug from a body fluid matrix.

Recently, Mazhar and Chrystyn (submitted for publication) have modified the reversed-phase ion-pair HPLC assay for salbutamol in the urine, previously published by Hindle and Chrystyn (1992). The aim of the work in this section was to adapt this recent method of Mazhar and Chrystyn to develop a sensitive, robust and reliable HPLC assay for the determination of terbutaline concentrations in aqueous samples for in-vitro testing of inhaled products and urine samples collected from subjects following terbutaline inhalation and oral administration.

3.2 Analysis of terbutaline sulphate in aqueous samples

The reversed-phase HPLC method, for the terbutaline sulphate, was based on the assay developed by Mazhar and Chrystyn (submitted for publication) for the determination of salbutamol in aqueous samples. The mobile phase used was buffer : acetonitrile (75:25), the buffer was 5mM potassium dihydrogen orthophosphate buffer adjusted to pH 2.5, with orthophosphoric acid. A constant flow-rate of 1 ml min^{-1} was used with florescence detector was set at an excitation/emission of 267/313 nm. An operating temperature of 30^{o}C was used throughout the analysis using a column chiller model 7950 (Jones Chromatography, UK).

3.2.1 Materials

3.2.1.1 Standards

Terbutaline sulphate: HPLC grade; Sigma (UK).

Bamethane sulphate: HPLC grade; Sigma (UK).

3.2.1.2 Mobile phase

Acetonitrile: HPLC grade; Fisher Scientific (UK).

Water: Highly purified double distilled water

Potassium dihydrogen orthophosphate: Analytical grade; BDH (Poole, UK).

3.2.1.3 HPLC conditions

Stationary phase: Spherisorb, ODS1, column 5µm 4.6 x 250mm, C-18 (Water Chromatography, UK).

Mobile phase: Buffer : acetonitrile (75:25), the buffer was 5mM potassium dihydrogen orthophosphate buffer adjusted to pH 2.5, with orthophosphoric acid. The mobile phase was filtered through a 45mm membrane filter (Millipore, Whatman Ltd, UK) and degassed under vacuum in an ultrasonic bath for 10 minutes prior to use.

Internal standard: Bamethane sulphate 300µg L^{-1}

Flow rate: 1 ml min^{-1}

Pump: Gilson model 307

Injector: Shimadzu Corporation SIL-9A Liquid Chromatography Automatic Sample Injector fitted with a 200µl loop

Detector: Shimadzu RF-551 fluorescence detector, set at an excitation/emission of 267/313 nm.

Integrator: Shimadzu C-R6A Chromoatopac

Temperature: 30^{o}C using a column chiller model 7950 (Jones Chromatography, UK).

3.2.1.4 Standards

Aqueous stock solutions of terbutaline sulphate 1000 mg L^{-1} (w/v) were prepared and stored below –20^{o}C. From the terbutaline sulphate stock solution, working standards were prepared by serial dilution to yield nominal terbutaline sulphate concentrations of 10, 25,

50, 100, 200, 300, 400, 500, 600, 700 and 800µg L^{-1} (w/v). Working solutions were stored below –20°C in well closed, light resistant containers prior to analysis. Stability studies over 21 days of storage at –20°C, 15 days at room temperature and through three thawing cycles showed no significant change in the analyte concentration.

3.2.2 Calibration

Calibration curves were performed using eleven terbutaline sulphate standards between 10µg L^{-1} and 800µg L^{-1} with 300µg L^{-1} bamethane as an internal standard. Three injections were performed for each terbutaline sulphate standard. The peak height ratio of terbutaline sulphate and bamethane was plotted against the concentration of the terbutaline sulphate standard. A straight line was fitted to the data using linear regression. A representative plot, described by the equation y = 0.012x + 0.0086 (r^2=0.9989) is shown in Figure 3.1. Representative chromatograms are shown in Figure 3.2. The detector response was shown to be linear over the range of 10 to 800µg L^{-1} of terbutaline sulphate in aqueous samples containing 300µg L^{-1} of bamethane with correlation coefficients of 0.9995.

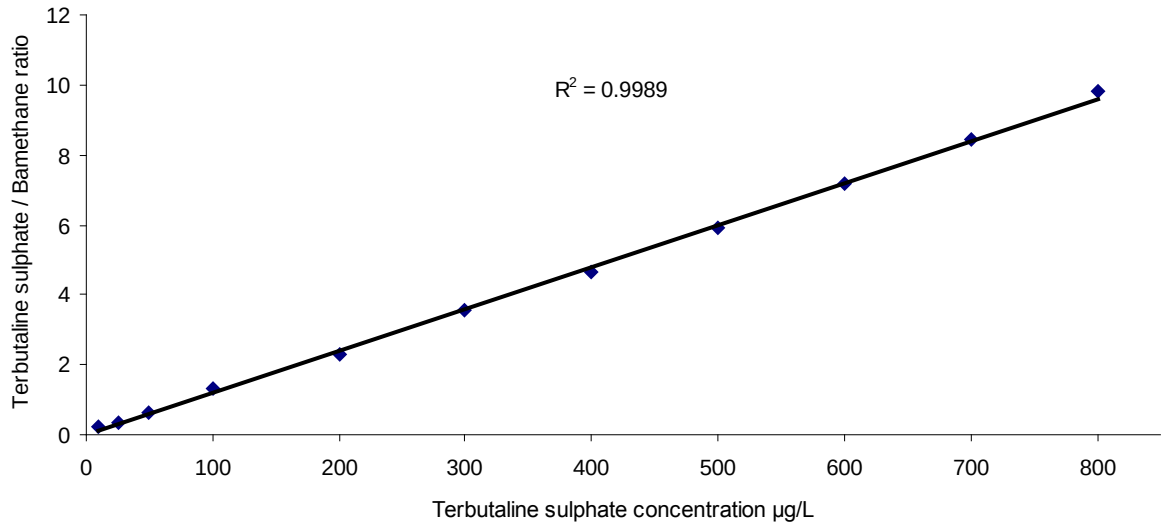


Figure 3.1 A representative calibration curve of the peak height ratio of terbutaline sulphate and bamethane against the concentration of terbutaline sulphate.

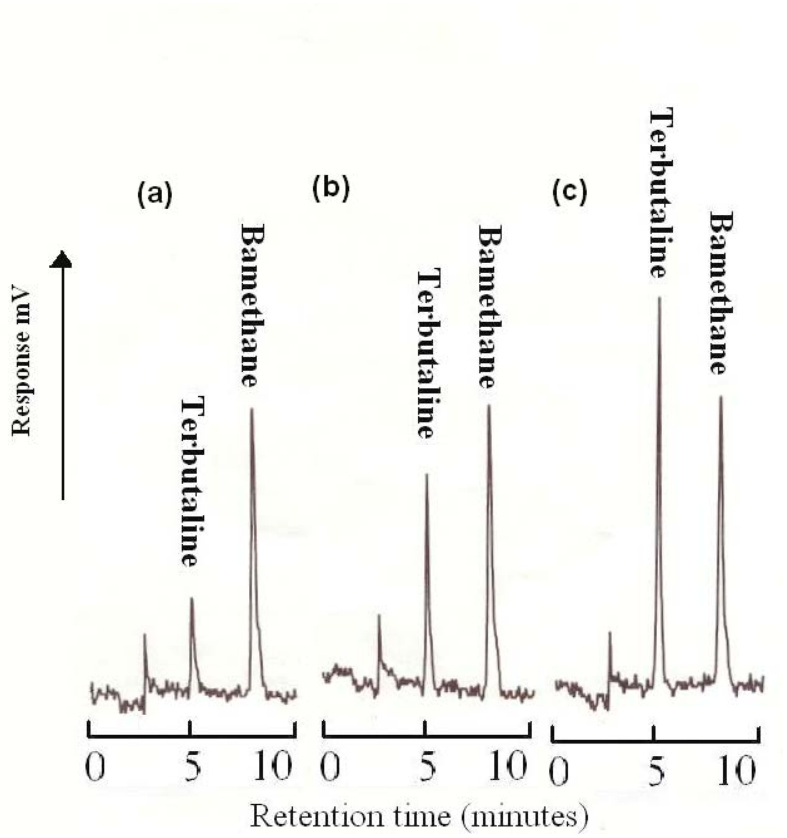


Figure 3.2 Chromatograms obtained from the analysis of the aqueous standard samples containing (a) 50, (b) 100 and (c) 200µg L^{-1} terbutaline sulphate, and 300µg L^{-1} bamethane represented as a, b and c, respectively.

Representative chromatographic parameters are shown in Table 3.1. The results are expressed as the mean (SD) from 10 samples.

Table 3.1 Chromatographic parameters for aqueous standards, (n=10)

Parameter	Mean (SD)
R_t ter (min)	6.0 (0.03)
R_t bam (min)	9.0 (0.06)
k' ter	2.8 (0.05)
k' bam	4.6 (0.07)
α (ter/bam)	1.5 (0.03)
R (ter/bam)	4.2(0.3)
N	14270.1(235.5)

Where R_t is retention time, *k*' is capacity factor, α is the separation factor, R is resolution and N is number of theoretical plates.

3.2.3 Precision

This is a measure of the distribution of individual measurements around the mean. This parameter is generally assessed by repeated analysis of the same solution and expressed as the Relative Standard Deviation (RSD) otherwise known as the Coefficient of Variation (CV). The lower the value the better is the assay performance. Three concentrations (low 50, medium 300 and high 700μg L^{-1}) within the linear range 10 - 800μg L^{-1} were used to examine the precision of the method. Twenty five chromatographs for each of the three selected terbutaline sulphate concentrations were obtained; five chromatographs each day for five days. Intra-day and inter-day variations, expressed as the coefficient of variation in peak height ratio, were calculated by dividing the standard deviation of the calculated concentrations by the mean concentration and multiplying by hundred. The mean (SD) intra-day assay variability, determined for the three standard concentrations of terbutaline sulphate on five occasions, was 2.8 (0.2) %. The inter-day assay variability, determined at the same three concentrations, using five replicate runs on different days was 4.5 (0.1) %. The results are shown in Table 3.2.

Table 3.2 Precision of the assay.

Nominal Concentration (μg L^{-1})	Intra-day Coefficient of variation (%)	Inter-day Coefficient of variation (%)
50 (n=25)	2.6	4.5
300 (n=25)	2.9	4.6
700 (n=25)	2.9	4.4
Mean±SD (n=75)	**2.8 ± 0.2**	**4.5 ± 0.1**

3.2.4 Accuracy

This is a measure of how close is the observed value to the actual value. The accuracy of the assay was calculated by comparison of the nominal terbutaline sulphate concentration to the actual concentration obtained from the linear regression line within the concentration range investigated (10 to 800μg L^{-1}). Twenty five chromatographs for each of the three

selected terbutaline sulphate concentrations were preformed; five chromatographs each day for five days. The results are shown in Table 3.3.

Table 3.3 Accuracy of the assay

Nominal Concentration (µg L^{-1})	Mean±SD µg L^{-1} of measured Concentration (n=5)	Mean±SD % of measured Concentration (Accuracy, n=5)
	Intra-assay variation	
50 (n=25)	51.3 ± 0.3	97.5 ± 0.7
300 (n=25)	285.8 ± 3.2	95.3 ± 1.1
700 (n=25)	676.5 ± 8.2	96.7 ± 1.2
Mean±SD (n=75)		**96.5 ± 1.1**
	Inter-assay variation	
50 (n=25)	50.1 ± 1.4	99.7 ± 2.8
300 (n=25)	293.7 ± 9.2	97.9 ± 3.1
700 (n=25)	660.9 ± 9.2	94.4 ± 1.3
Mean±SD (n=75)		**97.4 ± 2.7**

3.2.5 Detection and quantitation limits

According to the ICH and FDA guidelines, to determine the lower limit of quantitation (LLOQ) and the limit of detection (LOD) a method based on the signal-to-noise approach and a method based on the standard deviation of the response and the slope (liner regression line method) are used.

According to the method based on the signal-to-noise approach, the limit of detection (LOD) is the lowest concentration of analyte that can be detected but not quantified and it should be a value greater than 3:1 for the signal to noise ratio. The lower limit of quantitation (LLOQ) is the lowest concentration of analyte that can be measured with acceptable precision and accuracy by the assay and it should be a value greater than 10:1 for the signal to noise ratio. The limit of detection (LOD) and the lower limit of quantitation (LLOQ) can be calculated from the mean of the slope and SD of the intercept of five calibration curves using the linear regression line method. The LOD is equal to 3.3 multiplied by the SD of the intercept of the linear regression line divided by its slope. The LLOQ is equal to the 10 multiplied by SD of the intercept of the linear regression line

divided by its slope. The linear regression line method was used here to determine LOD and LLOQ. The LOD and LLOQ of terbutaline sulphate with a 50 µl injection volume were 10.9µg L^{-1} and 33.1µg L^{-1}, respectively.

3.2.6 Summary of the aqueous HPLC assays

The reversed-phase HPLC assay developed by Mazhar and Chrystyn (submitted for publication) for the determination of salbutamol in aqueous samples may also be used to determine the concentration of terbutaline sulphate in aqueous samples. The assay has acceptable limits for both accuracy and precision and has been successfully used to analyze samples from this study, and other subsequent studies. This assay is simple, sensitive (LLOQ is 33.1µg L^{-1}) and suitable for routine studies. Automatic injection allows up to 120 samples to be analyzed in one day.

3.3 Analysis of terbutaline in urine samples

Recently, Mazhar and Chrystyn (submitted for publication) also developed a reversed-phase ion-pair HPLC assay for salbutamol in urine, where terbutaline and bamethane were used as the internal standards. Bamethane and terbutaline have a similar chemical structure to salbutamol as shown in Figure 3.3. Bamethane does not have the CH_2OH on carbon number 3 of the phenyl group of salbutamol and has a different aliphatic group linked to the nitrogen atom. Terbutaline has an OH in place of the CH_2OH and the position of the other OH is different. These differences result in different retention times.

Figure 3.3 The chemical structure of terbutaline, salbutamol and bamethane.

The original method by Mazhar and Chrystyn has been used to develop a sensitive, robust and reliable HPLC assay for the determination of terbutaline concentrations in urine

samples following oral and inhaled administration. Hence, this determination could be used to study the relative deposition of terbutaline in the lung. The mobile phase was acetonitrile : methanol : tetrahydrofuran : ethyl acetate : buffer 5:5:5:5:80% v/v. The buffer was of 40mM phosphate buffer and 27.5mM sodium dodecyl sulphate adjusted to pH 6.75 using 10mM KOH. The mobile phase was filtered through a 45 mm membrane filter (Millipore) and degassed under vacuum in an ultrasonic bath for 10 minutes prior to use. A constant flow-rate of 1 ml min^{-1} was used with florescence detector was set at an excitation/emission of 267/313 nm. An operating temperature of 30^{o}C was used throughout the analysis using a column chiller model 7950 (Jones Chromatography, UK).

3.3.1 Materials

3.3.1.1 Standards

Terbutaline sulphate: HPLC grade; Sigma (UK).

Bamethane sulphate: HPLC grade; Sigma (UK).

Salbutamol sulphate: HPLC grade; Sigma (UK).

3.3.1.2 Mobile phase

Acetonitrile: HPLC grade; Fisher Scientific (UK).

Water: Highly purified double distilled water

Methanol: HPLC grade; Fisher Scientific (UK).

Tetrahydrofuran: HPLC grade; Fisher Scientific (UK).

Ethyl acetate: HPLC grade; Fisher Scientific (UK).

Potassium hydroxide: Analytical grade; Sigma (UK).

sodium dodecyl sulphate: Analytical grade; BDH (Poole, UK).

Potassium dihydrogen orthophosphate: Analytical grade; BDH (Poole, UK).

3.3.1.3 HPLC conditions

Column: ODS 5µm, 4.6 x 250mm, Zorbax C-18 (Agilent Technology, Phenomenex, UK).

Pre-column:	4mm x 3 mm; C-18 ODS (Phenomenex, UK)
Mobile phase:	acetonitrile : methanol : tetrahydrofuran : ethyl acetate : buffer 5:5:5:5:80% v/v. The buffer was of 40mM phosphate buffer and 27.5mM sodium dodecyl sulphate adjusted to pH 6.75 using 10mM KOH. The mobile phase was filtered through a 45mm membrane filter with a pore size of 0.45µm (Nylaflo, Pall corporation, UK) and degassed under vacuum in an ultrasonic bath for 10 minutes prior to use.
Flow rate:	1 ml min^{-1}
Pump:	Gilson model 307
Injector:	Shimadzu Corporation SIL-9A Liquid Chromatography Automatic Sample Injector fitted with a 200µl loop
Detector:	Shimadzu RF-551 fluorescence detector, set at an excitation/emission of 267/313 nm.
Integrator:	Shimadzu C-R6A Chromoatopac
Temperature:	30^{o}C using a column chiller model 7950 (Jones Chromatography, UK).

3.3.1.4 Standards

Aqueous stock solutions of terbutaline 1000 mg L^{-1} (w/v) equivalent to 1217.7 mg L^{-1} terbutaline sulphate were prepared and stored below -20^{o}C. From the terbutaline stock solution, working standards were prepared by serial dilution using pooled urine collected from five (two female) volunteers to yield nominal terbutaline concentrations of 10, 25, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 and 1200µg L^{-1} (w/v). Working solutions were stored below –20^{o}C prior to analysis. Stability studies over 15 days at room temperature, 21 days of storage at –20^{o}C, and through three thawing cycles showed no significant change in analyte concentration.

3.3.4 Solid phase extraction

HPLC is a good separation and quantification method, however urine cannot be directly injected into the HPLC column because it contains many endogenous compounds that can either block the column or may interfere with the chromatogram of the analytes. A pre treatment step is therefore required to remove these endogenous compounds. Solid Phase extraction (SPE) is a technique in which the biological liquid sample is passed through a sorbent bed to retain the analyte followed by flushing with different solvents to remove any interfering compounds. The method used is optimized so that the analyte remains on the column until the end when it is eluted free of endogenous compounds.

3.3.4.1 Extraction of unchanged terbutaline from urine samples - pre hydrolysis extraction

A solid-phase extraction method using Isolute HCX Cartridges (International Sorbent technology, U.K.) was developed by Mazhar and Chrystyn (submitted for publication) to extract salbutamol, terbutaline and bamethane from urine. The amino group of these three compounds is linked to the sulphonic acid group on the sorbent bed. A pretreated sample was prepared by adding 1 ml of urine, 1 ml of aqueous solution of 500μg L^{-1} (w/v) bamethane and/or 300μg L^{-1} (w/v) salbutamol as internal standards as appropriate and 2 ml of 30mM potassium dihydrogen phosphate. This was adjusted (if necessary) to pH 7 (using 10mM KOH) and mixed well for 30 seconds. Each Isolute Cartridge was conditioned with 1 ml methanol followed by 1 ml of 15mM potassium dihydrogen phosphate (pH 7). The pretreated sample was then applied to the cartridges followed by the addition of 1 ml of 15mM potassium dihydrogen phosphate (pH 7). The cartridge was then washed with 2 ml of 0.00001 N hydrochloric acid (pH 5) followed by a vacuum for 2 minutes, then 1 ml of 0.005 N hydrochloric acid (pH 2.5) was eluted through each cartridge using low vacuum (less than 3 bar) followed by drying for 5 minutes using vacuum drying. The cartridge was washed with 1.5 ml of 75% methanol and again dried by applying a full vacuum for 5

minutes. The interaction between the analytes and the sorbent bed was then broken by increasing the pH of the column. Hence, the analyte was eluted from the cartridge into a sample tube using 1 ml of 0.54 % (v/v) ammonia in methanol with the application of a low vacuum (less than 3 bars). After evaporation to dryness using a sample concentrator (Techne, USA) set at 65^{o}C with a stream of nitrogen for 15 min, the residue was reconstituted in 1 ml mobile phase and 100µl was injected into the HPLC system.

3.3.4.1.1 Pre hydrolysis sample extraction

Solid phase extraction cartridge: Isolute HCX 130mg 10ml XL cartridge, International Sorbent Technology, UK.

Nitrogen cylinder BOC gases, UK

Extraction station VAC-ELUT 10 manifold (Varian Limited, UK)

Sample concentrator DRI-BLOCK DB-3A, Temperature range ambient 5°C to 200°C. (Techne, USA).

Water: Highly purified double distilled water

Potassium dihydrogen orthophosphate: Analytical grade; BDH (Poole, UK).

Methanol: HPLC grade; Fisher Scientific (UK).

Hydrochloric acid: Analytical grade; BDH (Poole, UK).

Ammonia solution 33%: Analytical grade; BDH (Poole, UK).

Potassium hydroxide: Analytical grade; Sigma (UK).

3.3.4.2 Extraction of unchanged terbutaline and the terbutaline sulphate ester metabolite from urine samples - post hydrolysis extraction

Terbutaline is excreted in the urine as an unchanged molecule and its sulphate ester. The latter is not extracted by the solid phase method described above. To quantify the sulphate ester the conversion to terbutaline base by acid hydrolysis is required.

A solid-phase extraction method using Oasis HLB 30mg cartridges (Water Chromatography, UK) has been developed by Mazhar and Chrystyn (submitted for

publication) to extract salbutamol, terbutaline and bamethane from urine. A pretreated sample was prepared by adding 1 ml of urine, 1 ml of aqueous solution of 500µg L^{-1} (w/v) bamethane and/or 300µg L^{-1} (w/v) salbutamol as internal standards as appropriate and 8ml of 0.1N HCl in a test tube. The mixture was vortexed, covered with aluminium foil, boiled in a water bath at 100^{o}C for 1 hour and then cooled. 1 ml of 0.5M potassium dihydrogen phosphate pH 13.0 was then added to the mixture. This was adjusted (if necessary) to pH 6.5-7.2 using 10mM KOH. The final solution was mixed well for 30 seconds. Each HLB cartridge was conditioned with 2 ml methanol followed by 2 ml of 45mM potassium dihydrogen phosphate solution (pH 7). The pretreated sample was then applied to the cartridges followed by the addition of 2 ml of 15mM potassium dihydrogen phosphate (pH 7) followed by a vacuum drying for 2 minutes. The cartridge was then washed with 1 ml of 5% (v/v) methanol followed by vacuum drying for 1 minute, then 1 ml of 2% (v/v) acetonitrile followed by vacuum drying for another 1 minutes. The cartridge was then washed with 1 ml of 0.25% (v/v) tetrahydrofuran followed by vacuum drying for 1 minute. The analyte was then eluted from the cartridge into a sample tube using 2 ml of acetic acid 2% (v/v) with a low vacuum (less than 3 bars). After evaporation to dryness using a sample concentrator (Techne, USA) set at 125^{o}C with a stream of nitrogen for 30 min, the residue was reconstituted in 1 ml mobile phase and 100µl was injected into the HPLC system.

3.3.4.2.1 Post hydrolysis sample extraction

Solid phase extraction cartridge: Oasis HLB 30mg, Water Chromatography, UK.

Column reservoir: 25 ml column reservoir, IST Ltd., Wales, UK.

Sample concentrator DRI-BLOCK DB-3A, Temperature range ambient 5°C to 200°C. (Techne, USA).

Nitrogen cylinder BOC gases, UK

Extraction station VAC-ELUT 10 manifold (Varian Limited, UK)

Potassium dihydrogen orthophosphate: Analytical grade; BDH (Poole, UK).

Methanol: HPLC grade; Fisher Scientific (UK).

Tetrahydrofuran: HPLC grade; Fisher Scientific (UK).

Hydrochloric acid: Analytical grade; BDH (Poole, UK).

Acetic acid: Analytical grade; BDH (Poole, UK).

Acetonitrile: HPLC grade; Fisher Scientific (UK).

Water: Highly purified double distilled water

3.3.4.2.2 Acid hydrolysis of sulphate ester conjugates

Potassium hydroxide: Analytical grade; Sigma (UK).

Hydrochloric acid: Analytical grade; BDH (Poole, UK).

3.3.5 Calibration

Calibration curves were performed using fourteen urine standard samples containing terbutaline standards between 10µg L^{-1} and 1200µg L^{-1} with 300µg L^{-1} salbutamol and 500µg L^{-1} bamethane as the internal standards. Three injections were performed for each terbutaline standard. The peak height ratios of terbutaline and bamethane or salbutamol (internal standards) were plotted against the concentration of the terbutaline standards. A straight line was fitted to the data using linear regression. A representative plot, described by the equation $y = 0.003x + 0.1031$ (r^2=0.9983) was obtained using bamethane as the internal standard and $y = 0.0031x + 0.0643$ (r^2=0.9994) using salbutamol. The calibration curves are presented in Figure 3.4 and 3.5, respectively and representative chromatograms are shown in Figure 3.6 and 3.7. The detector response was shown to be linear over the urinary terbutaline concentration range of 50 to 1200µg L^{-1} using bamethane and salbutamol as internal standards with correlation coefficients of 0.9992 and 0.9997, respectively.

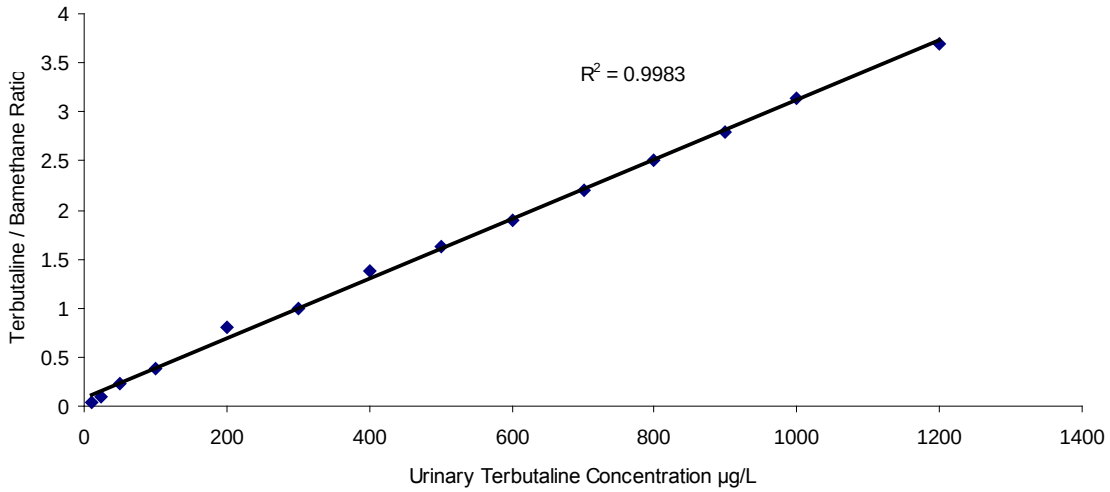


Figure 3.4 A representative calibration curve of the peak height ratio of terbutaline and bamethane against the concentration of terbutaline.

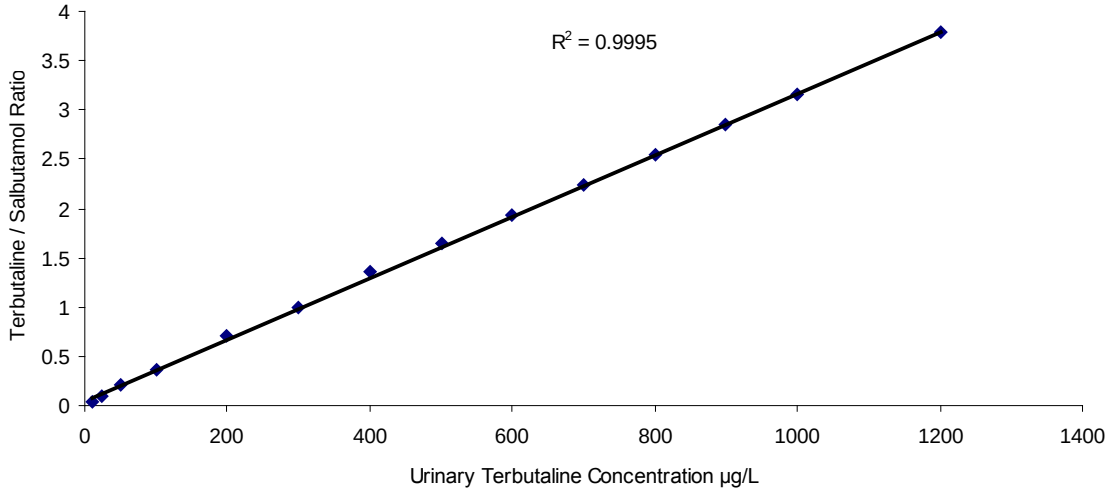


Figure 3.5 A representative calibration curve of the peak height ratio of terbutaline and salbutamol against the concentration of terbutaline.

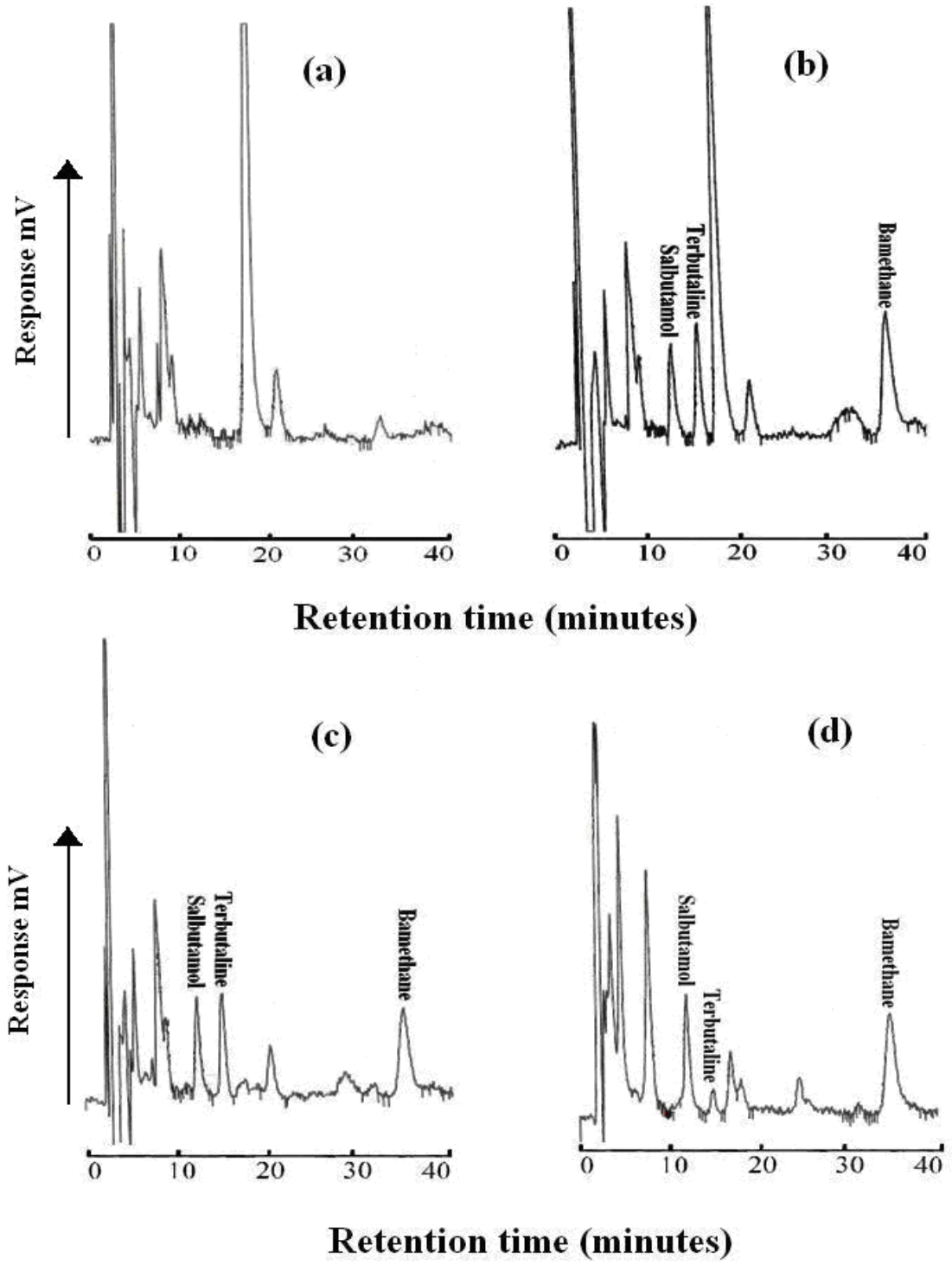


Figure 3.6 Pre-hydrolysis chromatograms obtained from the analysis of (a) an extracted blank urine sample (b) a standard urine sample containing 400µg L^{-1} terbutaline, 300µg L^{-1} salbutamol and 500µg L^{-1} bamethane (c) a volunteer urine sample 0–0.5 hour post inhalation of 2 doses of 250 µg terbutaline sulphate from Bricanyl MDI and (d) the same volunteers urine sample 0–0.5 hour post oral dose of 500 µg terbutaline sulphate using pre-hydrolysis sample extraction method.

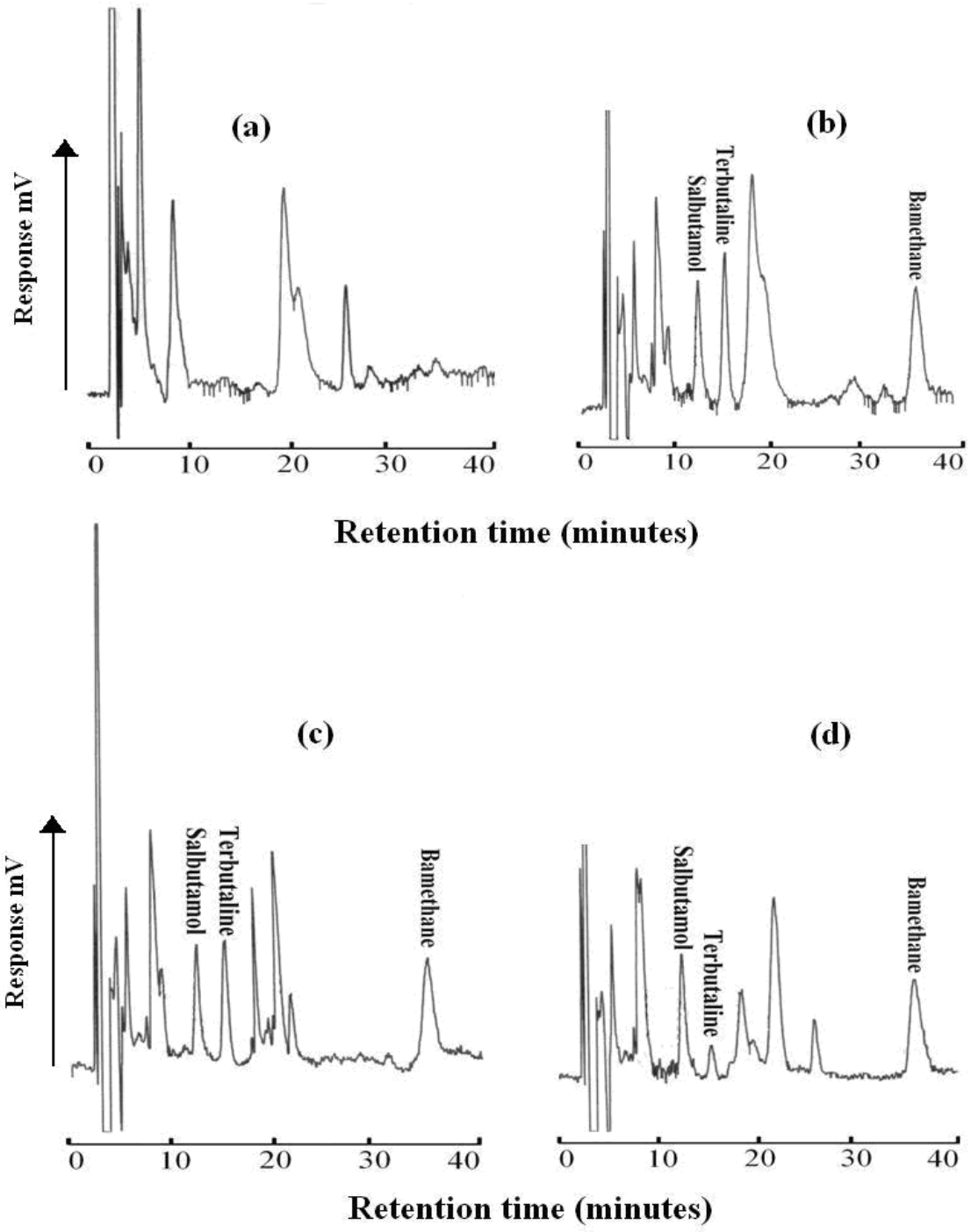


Figure 3.7 Post-hydrolysis chromatograms obtained from the analysis of (a) an extracted blank urine sample (b) a standard urine sample containing 400µg L^{-1} terbutaline, 300µg L^{-1} salbutamol and 500µg L^{-1} bamethane (c) a volunteer urine sample 0–0.5 hour post inhalation of 2 doses of 250 µg terbutaline sulphate from Bricanyl MDI and (d) the same volunteers urine sample 0–0.5 hour post oral dose of 500 µg terbutaline sulphate using post-hydrolysis sample extraction method.

Representative chromatographic parameters are shown in Table 3.4. The results are expressed as mean (SD) from 10 samples.

Table 3.4 Chromatographic parameters for the HPLC method, (n=10).

Parameter	Mean (SD)
R_t sal (min)	11.0 (0.1)
R_t ter (min)	14.0 (0.11)
R_t bam (min)	34.0 (0.2)
k' ter	5.3 (0.04)
k' ter	7.0 (0.13)
k' bam	18.3 (0.36)
α (ter/sal)	1.3 (0.1)
α (ter/bam)	2.4 (0.2)
R (ter/sal)	2.0 (0.2)
R (ter/bam)	10.5 (0.5)
N	5302.4(125.5)

Where R_t is retention time, *k*' is capacity factor, α is the separation factor, R is resolution and N is number of theoretical plates.

3.3.6 Recovery

The recovery was calculated by comparing the peak height of the extracted urine standards with the peak height of blank urine spiked with aqueous standards of terbutaline. Three concentrations (low 100, medium 500 and high 1000μg L^{-1}) over the linear range 50 - 1200μg L^{-1} were used to examine the recovery of the method. Four extractions from urine containing the above concentration of terbutaline provided a mean (SD) recovery of 92.7(1.5), 95.9(1.4) and 94.4(1.6) %, respectively. Overall combining all results the mean (SD) absolute recovery of terbutaline from urine, using this method, was 94.3 (1.6) %.

3.3.7 Precision

The same three concentration levels (low 100, medium 500 and high 1000μg L^{-1}) over the linear range 50 - 1200μg L^{-1} were used to examine the precision of the method. Twenty five chromatographs for each of the three selected terbutaline concentrations were preformed. Five chromatographs each day for five days. The intra-day and inter-day variation, expressed as the coefficient of variation in peak height ratio, were calculated by dividing the standard deviation of the calculated concentrations by the mean concentration and multiplying by hundred. The overall mean (SD) intra-day assay variability, determined for the three standard concentrations of terbutaline on five occasions using bamethane as the internal standard, was 7.1 (4.0) %. The inter-day assay variability, determined at the same three concentrations using five replicate runs on different days, was 12.0 (5.2) %. With salbutamol as the internal standard intra and inter-day assay variability was 6.0 (2.3) % and 9.9 (2.5) %, respectively. The results are shown in Table 3.5.

Table 3.5 Precision of the assay, (n=75).

Nominal Concentration (μg L^{-1})	Bamethane as internal standard		Salbutamol as internal standard	
	Intra-day Coefficient of variation (%)	Inter-day Coefficient of variation (%)	Intra-day Coefficient of variation (%)	Inter-day Coefficient of variation (%)
100 (n=25)	11.5	12.4	8.6	8.0
500 (n=25)	5.9	6.5	4.6	9.1
1000 (n=25)	3.8	16.9	4.7	12.7
Mean±SD (n=75)	**7.1 ± 4.0**	**12.0±5.2**	**6.0±2.3**	**9.9±2.5**

3.3.8 Accuracy

The accuracy of the assay was calculated by comparison of the nominal terbutaline concentration to the actual concentration obtained from the linear regression line over the concentration range investigated (50 to 1200μg L^{-1}). The results are shown in Table 3.6.

Table 3.6 Accuracy of the assay, (n=75).

Nominal Concentration (μg L^{-1})	Bamethane as internal standard		Salbutamol as internal standard	
	Mean±SD μg L^{-1} of measured Concentration	Mean±SD % of measured Concentration (Accuracy)	Mean±SD μg L^{-1} of measured Concentration	Mean±SD % of measured Concentration (Accuracy)
Intra-assay variation				
100 (n=25)	107.0 ± 3.5	93.0 ± 3.5	103.2 ± 2.9	96.8 ± 2.9
500 (n=25)	490.3 ± 11.4	98.1 ± 2.3	493.7 ± 7.3	98.7 ± 1.5
1000 (n=25)	1049.9 ± 27	95.0 ± 2.7	1028.3 ± 26.3	97.2 ± 2.6
Mean±SD (n=75)		**95.4 ± 2.5**		**97.6 ± 1.0**
Inter-assay variation				
100 (n=25)	108.2 ± 8.2	91.8 ± 8.2	99.3 ± 6	99.3 ± 6
500 (n=25)	515.9 ± 37.5	96.8 ± 7.5	511.3 ± 27.3	97.8 ± 5.5
1000 (n=25)	968.4 ± 48	96.8 ± 4.8	1044.9 ± 29.5	95.5 ± 3
Mean±SD (n=75)		**95.1 ± 3.0**		**97.5 ± 1.9**

3.3.9 Detection and quantitation limits

The limit of detection (LOD) and the lower limit of quantitation (LLOQ) were calculated from the mean of the slope and SD of the intercept of five calibration curves. The LOD and LLOQ for urine sample using bamethane as the internal standard were 24.2μg L^{-1} and 73.4μg L^{-1}, respectively and 20μg L^{-1} and 60.3μg L^{-1}, respectively, when salbutamol was used as the internal standard.

3.3.10 Summary of the urine HPLC assays

The reversed-phase ion-pair HPLC assay that was developed by Mazhar and Chrystyn (submitted for publication), for the determination of urinary salbutamol may also be used to determine the concentration of terbutaline in urine samples post dose. The clean-up stage using Isolute or HLB cartridges isolated terbutaline, salbutamol and bamethane from urinary endogenous substances that might interfere with the assay and gave a highly, reproducible absolute recovery of the three compounds. The assay has good accuracy and precision and has been successfully used to analyze samples from this study, and other subsequent clinical studies. Compared with other published methods (Polettini et al., 1995; Roig et al., 2002), this assay is simple, sensitive (LOD is 24.2μg L^{-1}) and suitable for routine clinical studies. Automatic injection allows up to 41 samples to be analyzed in one

day. Salbutamol can be used as the internal standard for all the volunteer samples, as it has shorter retention time than bamethane. For patient samples bamethane can be used as the internal standard as salbutamol is routinely inhaled by them. This enables routine therapy to be maintained when studying patients

3.3.10 Conclusions of the urine HPLC assays

The reversed-phase ion-pair HPLC assay developed by Mazhar and Chrystyn (submitted for publication), for the determination of urinary salbutamol may also be used to determine the concentration of terbutaline in urine samples post dose from both volunteers using salbutamol as the internal standard and patients using bamethane as the internal standard.

The assay has acceptable limits for both accuracy and precision and has been successfully used to analyze samples from this study, and other subsequent clinical studies. The assay is simple, sensitive (LOD is 24.2μg L^{-1}) and suitable for routine clinical studies. Automatic injection allows up to 41 samples to be analyzed in one day.

(Hindle and Chrystyn, 1992)

4.1 Introduction

Consistent and reliable dosage emission from an inhaler is essential for the management of patients (Tarsin et al., 2004), with respect to the aerodynamic particle size distribution, the fine particle dose and the emitted dose. These parameters are useful to provide some indication of therapeutic use (Weda et al., 2004).

The variation of the emitted dose is a very important factor that could affect the clinical response and side effects. The lung deposition for short acting β_2 agonist and budesonide dry powder inhalers can vary with the inhalation flow (Newman et al., 1991; Borgstrom et al., 1994). Gamma scintigraphy using radiolabelled drug in an inhalation device has shown different total lung deposition with inhalation flows that is consistent with separate in-vitro analysis of the emitted dose at different flows (Newman et al., 1991; Borgstrom et al., 1994). The Turbuhaler has previously been shown to be a device of high resistance, which also demonstrates in-vitro flow dependent dose emission (Ross and Schultz, 1996). Studies focus on inhalation flow of greater than 30 L min^{-1} through a DPI. However during routine use some patients do not achieve a minimum inhalation flow of less than 30 L min^{-1} required for the delivery of the dose (Pedersen et al., 1990; Calverley et al., 2003).

Therefore, the aim of this study was to investigate the in-vitro emitted dose and aerodynamic characteristics from the Bricanyl Turbuhaler at different inhalation flow from 10 to 60 L min^{-1}. Hence data below 30 L min^{-1} will be generated.

4.2 Methods

4.2.1 Equipment and inhalation devices

Equipment:

DPI sampling apparatus: Copley Scientific Ltd, UK

Andersen MKII Cascade Impactor: Copley Scientific Ltd, UK

A/E fibre glass filter discs: 47mm; Pall Corporation, USA

GF 50 filter: Copley Scientific Ltd, UK

GAST pump : Brook Crompton, UK

An electronic digital flow meter: MKS Instruments, USA

Parafilm M laboratory film: Pechiney Plastic Packaging, USA

Silicone fluid spray: Releasil B silicone spray, Dow Corning Limited, Barry, Glamorgan, UK.

Critical flow controller model TPK: Copley Scientific Ltd, UK

HPLC system: Previously described in section 3.2 in this thesis

Inhaler devices:

Bricanyl Turbuhaler: Labelled as a nominal dose of 500µg terbutaline sulphate per shot, AstraZeneca, UK

4.2.2 Procedure

4.2.2.1 Total Emitted Dose

The emitted dose from the Bricanyl Turbuhaler (labelled as a nominal dose of 500µg terbutaline sulphate per shot, AstraZeneca, UK) was measured using a DPI sampling apparatus with a critical flow controller model TPK (Copley Scientific Ltd, UK). The basic methodology is described in Appendix XII F of the British Pharmacopoeia (2005), 2.9.18 of the European Pharmacopeia (2001) and 601 of the United States Pharmacopeia (2005). The final filter was a 47 mm A/E fibre glass filter discs (Pall Corporation, USA). Vacuum flow through the apparatus was provided by a GAST pump (Brook Crompton, UK). The standard compendial methodology of the USP at an inhalation volume of 4 L and at pressure drop of 4 kPa was modified to allow determination at different flows and volumes. The inhalation flow through the mouthpiece of the Turbuhaler was set at 10, 20, 30, 40, 50 and 60 L min^{-1} with flow-duration of 24, 12, 8, 6, 4.8 and 4 sec. respectively to allow an inhaled volume of 4 L of air to be drawn through the inhaler (British Pharmacopoeia, 2005; United States Pharmacopeia, 2005). The flow was measured by an

electronic digital flow meter (MKS Instruments, USA). Parafilm M laboratory film (Pechiney Plastic Packaging, USA) was used to seal the apparatus.

Each inhaler was inserted tightly into the mouthpiece and aligned along the horizontal axis. The emitted dose from the Bricanyl Turbuhaler was measured by collecting one individual dose at different inhalation flows of 10, 20, 30, 40, 50 and 60 L min^{-1}. For each determination the Turbuhaler was loaded to deliver a dose, according to the instructions in the patient information leaflet. For each inhalation flow, the emitted dose of 10 separate, single doses, throughout the life of each inhaler, were determined. The randomisation schedule used is shown in Table 4.1. Doses not required were discharged to waste using a flow of 90 L min^{-1} for a 16 second vacuum period to clean the Turbuhaler.

Table 4.1 The randomization schedule for the dose numbers from the Turbuhaler inhaler for the dose emission determination (100 doses in the inhaler).

Flow (L min^{-1})	Dose Number from the inhaler
60	2, 10, 15, 24, 38, 47, 56, 72, 78, 95
50	13, 20, 23, 32, 40, 46, 55, 58, 73, 91,
40	3, 14, 31, 36, 48, 57, 64, 67, 77, 92
30	8, 16, 35, 42, 49, 59, 66, 71, 79, 93
20	5, 21, 30, 43, 53, 60, 68, 76, 80, 90
10	7, 17, 28, 39, 44, 54, 69, 74, 81, 89

For this procedure a switching system was used to produce sonic flow conditions, as recommended by the Pharmacopoeial Methods, (European Pharmacopeia, 2001; British Pharmacopoeia, 2005; United States Pharmacopeia, 2005). For each determination only one dose was discharged in the DPI sampling apparatus. For each flow ten separate determinations were made (n=10). Following dose emission into the apparatus, the sampling unit was washed with 25% acetonitrile and the filter was completely submerged in 25% acetonitrile and then sonicated for 3 minutes (preliminary analysis revealed that this procedure removes all drug entrained on the filter). All the solutions were collected and made up to a volume of 100, 250, 250, 250, 500 and 500ml, for the flow of 10, 20, 30,

40, 50 and 60 L min^{-1}, respectively. The amount of drug was determined by high performance liquid chromatography using the previously validated method described in section 3.2. The total dose emitted was the amount deposited in the plastic dose sampling apparatus and the final filter.

4.2.2.2 The Aerodynamic Particle Size Characterization

All the parts of the Andersen MKII Cascade Impactor (including the preseparator) were washed in methanol and allowed to dry. The ACI was assembled with the modification plates for a flow of 60 L min^{-1} hence stages 0 and 7 were replaced by -0 and -1 on the top of the impactor. The collection plates were then sprayed with silicone fluid (Releasil B silicone spray, Dow Corning Limited, Barry, Glamorgan, UK) and then allowed to dry for at least one hour prior to analysis. The cascade impactor was assembled with 10 ml of 25% acetonitrile placed in the preseparator and the final filter was a GF 50 (Copley Scientific Ltd, UK). The ACI was assembled with the mixing inlet as described in Figure 4.1. The mixing inlet allows the flow through the inhaler to be varied upto 60 L min^{-1} while a constant flow of 60 L min^{-1} is maintained through the ACI. That is achieved by supplementary air pumped through the side arm of the mixing inlet as shown in Figure 4.1. Figure 4.1 (b) describes how the air flow through the DPI is 10 L min^{-1} with 50 L min^{-1} air provided via the sidearm.

The flow through the mouthpiece of the Turbuhaler was set at 10, 20, 30, 40, 50 and 60 L min^{-1} with a flow-duration of 24, 12, 8, 6, 4.8 and 4 seconds respectively (corresponding for 4L through the inhaler) with a constant flow through the ACI of 60 L min^{-1}. The flow was measured using an electronic digital flow meter (MKS Instruments, USA) and a critical flow controller model TPK (Copley Scientific Ltd, UK). Parafilm M laboratory film (Pechiney Plastic Packaging, USA) was used to seal the apparatus.

Five determinations from each inhalation flow were made, according to the randomization procedure shown in Table 4.2.

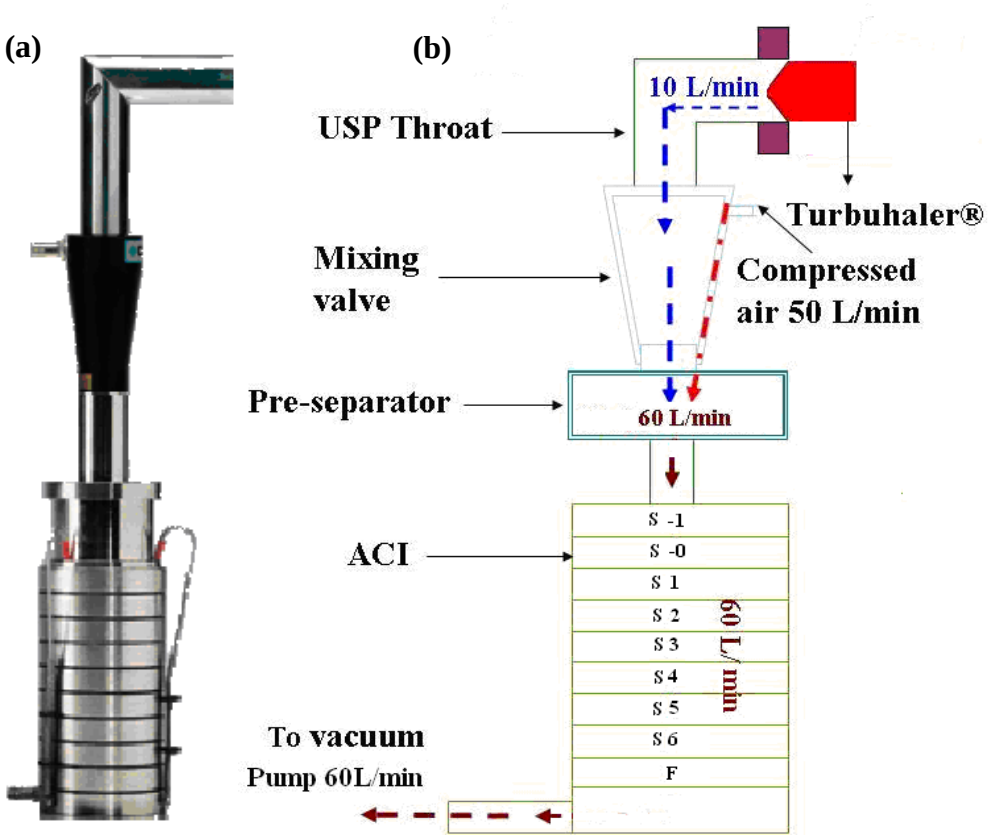


Figure 4.1 (a) The mixing inlet in the ACI with the preseparator. (b) Inhalation flow set up using the mixing inlet valve with S referring to stage.

For each determination, the inhaler was loaded to deliver one dose, according to the instructions in the patient information leaflet. It was placed into the mouthpiece. The pump (set for the required flow) was switched on for the previously mentioned inhalation time to allow a volume of 4 L of air, as recommended in Pharmacopoeial Methods, (European Pharmacopeia, 2001; British Pharmacopoeia, 2005; United States Pharmacopeia, 2005) to be withdrawn through the inhaler during each determination. For each determination only one dose was discharged into the ACI. For each flow five separate determinations were made (n=5). Each stage of the Cascade Impactor was rinsed with 25% acetonitrile and made up to the volume as described in Table 4.3. The washing procedure was the same as that described for the total dose emission. The amounts deposited on each stage were determined by high performance liquid chromatography using the previously validated method described in section 3.2.

Table 4.2 The randomization schedule for the dose numbers from the Turbuhaler inhaler for the aerodymanic charcteristics determination (100 doses in the inhaler).

Flow	Dose Number from the inhaler
60	9, 18, 33, 45, 85
50	4, 25, 52, 83, 94
40	6, 26, 61, 70, 96
30	12, 29, 50, 62, 86
20	11, 34, 41, 82, 97
10	13, 22, 65, 87, 98

Table 4.3 The washing volume of 25% acetonitrile for ACI stages in the Aerodynamic Characterization.

Stage	Volume of washing (ml)					
	10 L min^{-1}	20 L min^{-1}	30 L min^{-1}	40 L min^{-1}	50 L min^{-1}	60 L min^{-1}
Induction port	50	50	100	100	100	100
Preseparator	50	50	100	100	100	100
-1	10	10	10	10	10	10
-0	10	10	10	10	25	25
1	10	10	25	25	50	50
2	10	10	25	25	25	25
3	10	10	25	25	25	25
4	10	10	10	10	25	25
5	10	10	10	10	25	25
6	10	10	10	10	10	10
Filter	10	10	10	10	10	10

4.2.3. Data Analysis

The total dose emission was determined as the total amount of drug ex-mouthpiece. This has been reported with respect to the nominal emitted dose. Using the ACI with a flow of 60 L min^{-1} (after the mixing inlet unit) for the DPI, the effective cut-off diameter of each stage was fixed to 60 L min^{-1} flow (Copley, 2007; Van Oort, 1995). The fine particle dose (FPD) was the amount with particles that correspond to a size less than 5μm. The fine particle fraction % (FPF) was the FPD expressed as a percentage of the total amount deposited into the throat and stages of the cascade impactor (this is the dose exiting the mouthpiece). The mass median aerodynamic diameter (MMAD) was obtained from a plot of the logarithm of the percentage less than a stated size on a probability scale against the logarithm of the effective cut-off diameter of the stage (United States Pharmacopeia, 2005)

and this was done using Copley Inhaler Testing Data Analysis Software (CITDAS). The MMAD was the diameter corresponding to 50% undersize. The geometric standard deviation (GSD) was the square root for the size corresponding to 84.13% less than the stated size divided by the square root of the size for 15.87% (United States Pharmacopeia, 2005).

4.2.4 Statistical analysis

A two-way analysis of variance (ANOVA) test was used to compare the total emitted dose and aerodynamic particle size characterization at different flows using SPSS V15.0 (SPSS Inc., Chicago, USA).

4.3 Results

4.3.1 Total Emitted Dose

The individual doses and the mean (SD) emitted dose of terbutaline sulphate from the Turbuhaler are shown in Table 4.4 and Figures 4.1 and 4.2. These values are for each dose for which the nominal dose is 500μg terbutaline sulphate. The overall mean (SD) % of the nominal emitted dose (n = 10 doses), from the Turbuhaler at 10, 20, 30, 40, 50 and 60 L min^{-1} inhalation flows were 34.0(7.4), 48.0(8.3), 56.0(7.8), 64.8(5.7), 72.5(8.6) and 76.6(6.5) % of the emitted dose respectively.

The comparison of the dose emission results at different flows showed that the amount emitted increased significantly ($p< 0.001$) with the increase of the inhalation flow.

Table 4.4 Terbutaline sulphate emitted dose (µg) from the Turbuhaler (500 µg nominal dose) determined at different flows.

	Inhalation flow rate (L min^{-1})					
Dose	**10**	**20**	**30**	**40**	**50**	**60**
1	114.2	231.6	308.1	340.8	415.7	368.7
2	208.8	255.4	241.3	341.7	405.6	367.3
3	211.5	268.1	255.8	299.2	316.3	401.1
4	110.3	222.8	274.2	334.2	316.8	410.6
5	152.4	173.9	345.7	322.2	347.1	417.0
6	174.5	270.9	216.9	301.1	357.2	437.6
7	172.0	214.7	304.8	375.2	309.7	354.1
8	182.0	229.7	250.6	274.2	349.9	377.7
9	160.4	209.2	304.3	338.6	428.6	334.0
10	212.5	323.8	297.7	311.0	428.6	362.6
Mean	**169.8**	**240.0**	**279.9**	**323.8**	**362.2**	**383.1**
SD	**37.0**	**41.4**	**38.9**	**28.5**	**43**	**32.2**
RSD	**21.8**	**17.3**	**13.9**	**8.8**	**11.9**	**8.4**
% of nominal dose	**34.0 (7.4)**	**48.0 (8.3)**	**56.0 (7.8)**	**64.8 (5.7)**	**72.4 (8.6)**	**76.6 (6.4)**

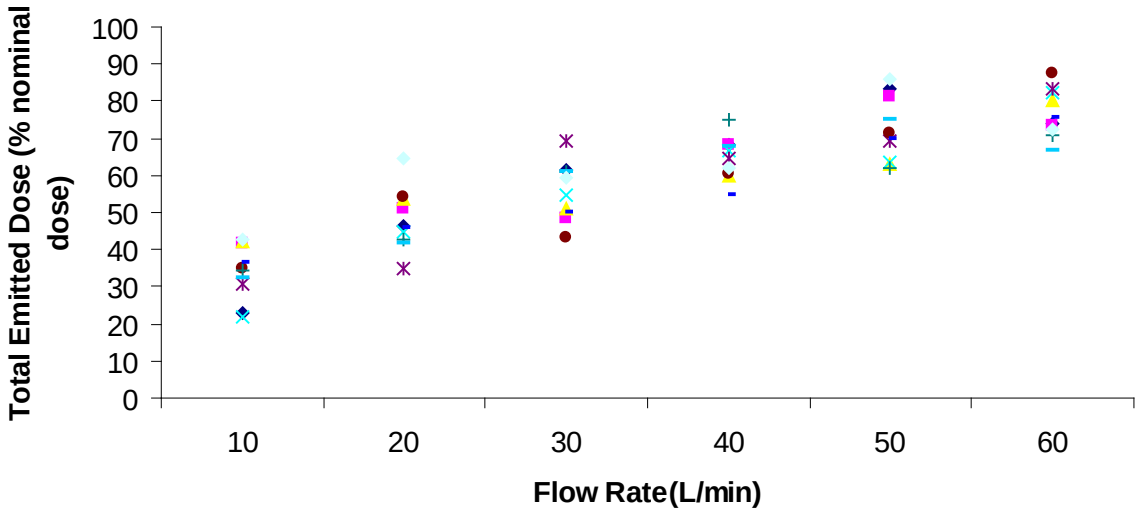


Figure 4.2 Terbutaline sulphate total emitted dose from the Turbuhaler, expressed as a percent of the nominal dose, at flows of 10, 20, 30, 40, 50 and 60 L min^{-1} (n=10 separate doses).

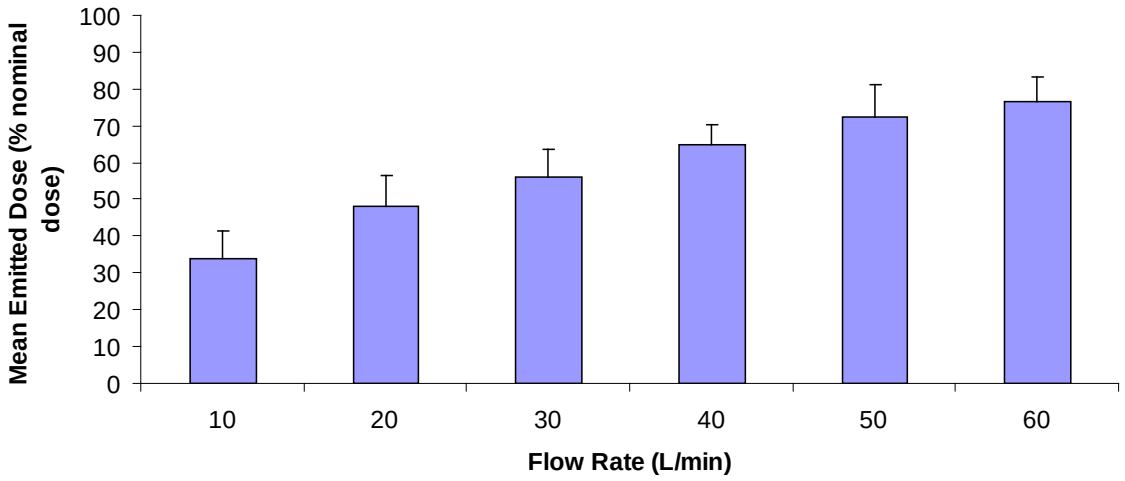


Figure 4.3 The mean (SD) total emitted dose of terbutaline sulphate from the Turbuhaler, expressed as a percent of the nominal dose, at each inhalation flow (n=10 separate doses).

4.3.2 The Aerodynamic Particle Size Characterization

A summary of the data obtained from the Andersen Cascade Impactor is shown in Table 4.5 together with Figures 4.4-4.6.

The majority of the emitted dose from the Turbuhaler is deposited in the induction port and the preseparator of the ACI. As the flow increases the deposition onto the induction port decreases while it increases in the preseparator. However, after 30 L min^{-1} the deposition increases in the induction port and decreases in the preseparator.

The comparison of the aerodynamic particle size characterization results for different flow showed that the total dose per shot, FPD and FPF significantly increases with the increase of the inhalation flow ($p < 0.001$).

4.3.3 Statistical analysis

A summary of the statistical comparison between flow 60 L min^{-1} and the other flows to highlight the differences is presented in Table 4.6.

Table 4.5 A summary of the data obtained from the ACI for the Turbuhaler (500 µg nominal dose). Mean values in µg are quoted except those in bold which are mean (SD).

	Stage Cut-off	**Amount in µg**					
		10 L min^{-1}	**20 L min^{-1}**	**30 L min^{-1}**	**40 L min^{-1}**	**50 L min^{-1}**	**60 L min^{-1}**
Induction port		91.4	68.5	38.9	91.6	105.4	146.8
Preseparator		2.8	110.1	130.3	109.5	104.7	81.4
-1	9.0	1.2	3.2	1.7	1.9	3.4	3.6
- 0	5.8	1.4	1.0	3.3	6.7	5.3	6.4
1	4.7	1.0	2.1	6.3	12.2	17.8	22.7
2	3.3	1.0	2.1	6.6	12.4	21.6	27.8
3	2.1	1.2	2.9	9.0	20.4	35.4	57.9
4	1.1	1.3	1.3	5.0	11.5	26.9	39.4
5	0.7	2.0	0.3	2.4	5.7	12.0	25.0
6	0.4	1.1	0.4	0.0	0.7	3.1	5.3
Filter	0.0	0.6	0.3	0.4	1.3	4.2	4.6
Total emitted dose (µg)		**105.0(7.5)**	**192.1(73.1)**	**203.9(15.4)**	**273.9(59.9)**	**339.8(72.7)**	**420.7(26.8)**
Total emitted dose (% of nominal dose)		**21.0(1.5)**	**38(20.7)**	**40.8(6.2)**	**54.8(13.8)**	**68.0(14.5)**	**84.1(5.4)**
FPD (µg)		**7.5(3.5)**	**7.9(0.4)**	**25.6(0.8)**	**56.4(14.3)**	**109.8(8.8)**	**168.6(23.3)**
FPF (% of nominal dose)		**1.5(0.7)**	**1.6(0.1)**	**5.1(0.3)**	**11.3(3.3)**	**22.0(1.8)**	**33.7(4.7)**
FPF% of the emitted dose		**7.0(3.1)**	**5.9(3.5)**	**12.8(1.3)**	**20.9(5.1)**	**33.2(5.6)**	**40.1(5.4)**
MMAD (µm)		**2.8(1.6)**	**4.4(0.8)**	**3.4(0.05)**	**3.2(0.3)**	**2.6(0.2)**	**2.5(0.03)**
GSD		**3.6(1.2)**	**2.3(0.6)**	**1.7(0.05)**	**1.8(0.1)**	**1.9(0.1)**	**1.9(0.03)**

Table 4.6 Mean difference (95% confidence interval) for the inhalation flow of 60 L min^{-1} compared to the other flows.

Comparator	FPD (µg)	FPF (%)	MMAD (µm)	Emitted dose (µg)
50 L min^{-1}	58.8 (43.0, 74.6)**	6.9 (1.3, 12.5)*	-0.1 (-1.2, 0.9)	20.8 (-13.5, 55.2)
40 L min^{-1}	112.1 (96.4, 127.9)**	19.2 (10.4, 28.0)**	-0.7 (-1.7, 0.4)	59.2 (24.9, 93.6)**
30 L min^{-1}	142.9 (127.2, 158.7)**	27.3 (18.5, 36.2)**	-0.9 (-2.0, 0.2)	103.1 (68.8, 137.5)**
20 L min^{-1}	160.7 (144.9, 176.4)**	34.2 (25.4, 43.0)**	-1.9 (-3.0, -0.9)**	143.1 (108.9, 177.4)**
10 L min^{-1}	161.0 (145.3, 176.8)**	33.1 (24.3, 41.9)**	-0.3 (-1.4, 0.8)	213.2 (178.9, 247.6)**

* $p<0.05$, ** <0.001 otherwise no significant difference.

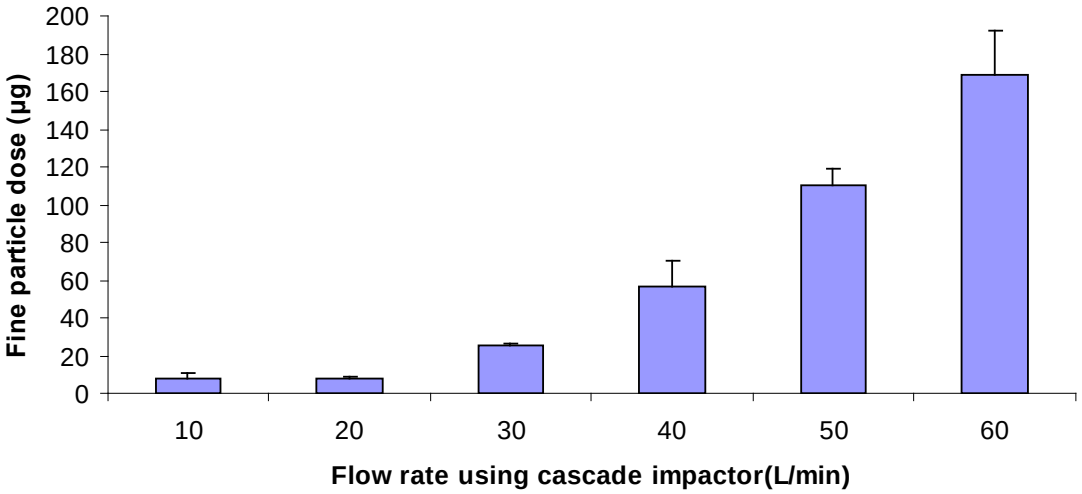


Figure 4.4 The mean (SD) fine particle dose of terbutaline sulphate from Turbuhaler, expressed as percent of nominal dose, at different flows (n=5).

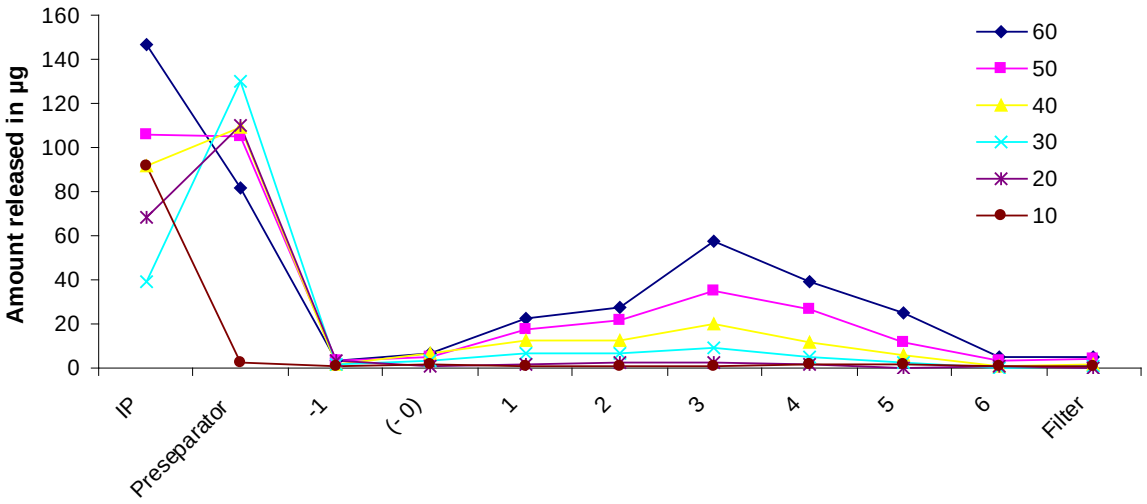


Figure 4.5 Mean (n=5) terbutaline sulphate (µg) deposited on each stage at each inhalation flow.

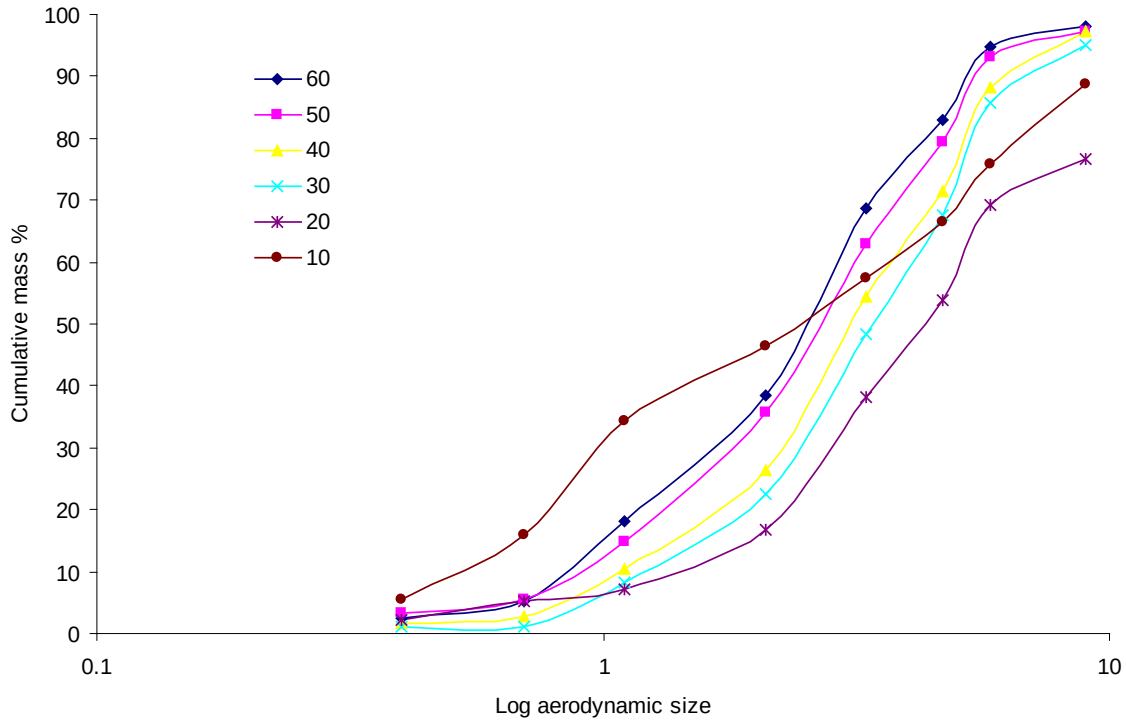


Figure 4.6 The mean (n=5) aerodynamic distribution of the dose emitted from the Turbuhaler at each inhalation flow.

4.4 Discussion

Total dose and fine particle dose emission together with the determination of the aerodynamic particle size distribution provide useful data about the inhalation method tested. Ross and Schultz (1996) and Palander et al. (2000) have demonstrated the property of flow dependent dose emission from dry powder devices. They showed that for some dry powder devices such as the Accuhaler and Easyhaler the effect of the inhalation flow is low whilst it is greater for the Turbuhaler. Hence an optimal inhalation flow may have to be achieved through DPIs to be used effectively.

Previous study have shown that children (Pedersen et al., 1990), adult asthmatic (Brown et al., 1995; Van der Palen, 2003) and COPD (Broeders et al., 2003; Tarsin et al., 2001) patients have problems achieving the recommended optimal (Borgstrom et al., 1994) inhalation flow of 60 L min^{-1} through a Turbuhaler.

Flow dependent fine particle dose characteristics have been reported from the Turbuhaler containing budesonide (Ross and Schultz, 1996; Hill and Slater, 1998), salbutamol (Prime et al., 1999), terbutaline (Meakin et al., 1995b; Meakin et al., 1995a) and a combination of budesonide and formoterol (Tarsin et al., 2004). However those studies were done using an inhalation flow above 30 L min^{-1}.

The results in this chapter show that the terbutaline sulphate from Turbuhaler has a flow dependent dose emission property and flow dependent fine particle dose characteristic. The dose emitted, FPD and FPF were significantly increased with the increase of the flow ($p<0.001$). The flow had a more pronounced effect on the FPD than the emitted dose, thus, a faster inhalation increases the respirable amount more than it increases the emitted dose. That might be due to the increase of the break down of the small particles to fine particles as the flow increase is higher than the increase of the ability of the air stream to carry large particles present in the mouthpiece of the Turbuhaler.

The results are consistent with the most desirable inhalation flow for use with the Turbuhaler which has been reported to be 60 L min^{-1} (Newman et al., 1991; Borgstrom et al., 1996) and the patient information leaflet which instructs the patients to inhale as deep and hard as they can during inhalation. All dry powder inhalers are designed with a resistance to airflow to facilitate particle de-aggregation during inhalation. When patients use the recommended inhalation technique for a Turbuhaler and inhale as deep and hard as they can, then their inhalation flow through this device will determine the amount deposited in their lung.

Tarsin et al. (2004) also measured the in-vitro dosage emission and the fine particle dose (FPD) from the 100/6 and 200/6 Symbicort Turbuhaler (budesonide and formoterol) at different flow rates (30, 60 and 90 L min^{-1}). The data also confirmed that the amounts of budesonide and formoterol emitted from the Symbicort 100/6 and Symbicort 200/6 inhalers were affected by the increased inhalation flow. Also the results demonstrated that

the average total dose emissions at 90 L min^{-1} were all greater than 100% (Tarsin et al., 2004). Each determination at 90 L min^{-1} was carried out after doses had been discharged to waste using a flow rate of 60 L min^{-1}. The data obtained suggest that at 60 L min^{-1} the total dose emission is not 100%. Thus total dose emission of >100% at 90 L min^{-1} suggests that at the higher flow some residual dose from the previous inhalation may also be inhaled (Tarsin et al., 2004). For this reason in this study doses were discharged to waste at 90 L min^{-1}.

Ross and Schultz (1996) compared the emitted dose delivery from a Diskhaler (Ventodisk 200µg), Rotahaler (Beclotide 100µg), and Turbuhaler (Pulmicort 200µg) at two different flows (30 and 60 L min^{-1}). The relative standard deviation (RSD) of the delivered dose for the DPI products ranged from 10% to 44%. In addition, the dose delivered from these DPI inhalers was generally less than the labelled amount and was dependent on the inhalation flow (Ross and Schultz, 1996).

The data represented in this chapter suggests that below 30 L min^{-1} although the MMAD increases it is still below 5µm. However the FPD falls off very sharply below this flow. This is the first data to study dose emission below 30 L min^{-1} and suggests that this is the minimum flow that should be used through the terbutaline Turbuhaler. Recently Nadarassan et al have shown that the pressure drop across the inhaler is the same with and without mixing valve at flows 10-60 L min^{-1} (Nadarassan et al., 2007).

The results in this chapter also showed that the inhalation flow has an effect on the amount deposited on the induction port and the preseparator, as seen in Table 3.5. The amount deposited on the induction port decreases and the amount deposited on the preseparator increase as the inhalation flow increase till the flow of 30 L min^{-1}. That might be because at an inhalation flow of 10 L min^{-1} the de-aggregation of the powder is low. This produces very big aggregates that are too heavy to be carried with the airstream through the bending of the induction port so they deposit more within the induction port. It is very unlikely that

patients will inhale as slow as this. However during acute exacerbation of severe asthma or COPD this could occur (Broeders et al., 2003). As the inhalation flow increases the ability to de-aggregate the powder increases hence decreasing the particle size, decreasing the amount deposited on the induction port and increasing the amount deposited on the preseparator till reaching an inhalation flow of 30 L min^{-1}. As the inhalation flow increases above more than 30 L min^{-1} the amount deposited on the induction port increases and the amount deposited on the preseparator decreases. This could be due to the increase of the velocity of the inhaled particles which increases the inertial impaction which is the dominant deposition mechanism for particle with large momentum [the product of velocity and mass] (Hillery et al., 2001).

4.5 Conclusions

In-vitro flow dependent dose emission has been demonstrated for terbutaline sulphate in the Turbuhaler. The effect of this was more pronounced for the fine particle dose especially below 30 L min^{-1}. The emitted dose at the higher flow was greater than at lower flows. The flow dependent dose emission results highlight the need for the Pharmacopoeias to use a variety of inhalation flows for in-vitro tests rather than one that is determined according to the resistance of the dry powder inhaler. The clinical relevance of the flow dependent and inconsistent dosage characteristics of terbutaline sulphate in the Turbuhaler requires investigation. The date suggests that the minimum inhalation flow through a terbutaline sulphate Turbuhaler is 30 L min^{-1}.

5.1 Introduction

Many patients have problems using their MDI with the recommended inhalation technique. Problems include not being able to co-ordinate the actuation of a dose with the start of an inhalation and the cold-freon effect which causes patients to stop inhaling (Crompton, 1982; Al-Showair et al., 2007). Another problem is that most of the MDI dose is deposited on the throat even if the patient has good co-ordination between the dose actuation and inhalation (Aswania and Chrystyn, 2001). Spacers are used to decrease oropharyngeal deposition and overcome the co-ordination problem (Aswania and Chrystyn, 2001; Fink, 2000; Terzano, 2001; Vaswani and Creticos, 1998). Also because the patient inhales from a static cloud and the dose has had some time to evaporate in the spacer then lung deposition is greater when using a spacer (Hirst et al., 2001; Richards et al., 2001).

Different spacers are commercially available for patient use. Some of them are prone to static charges (Newman, 2004). This static charge in addition to the shape and the type of material the spacer is formed of, can affect the amount inhaled by the patient. Some spacers have been designed to be antistatic (e.g. Nebuchamber, AstraZeneca; AeroChamber Max and Plus, Trudell Medical).

The use of in-vitro testing methodology for inhalation methods can give some information about the potential to deliver a dose to the lungs. The Dose Emission Unit gives information about the amount emitted from the inhaler while cascade impactors give information about the aerodynamic characteristics of the emitted dose.

Therefore, the aim of this study was to investigate the in-vitro emitted dose and aerodynamic characteristics from a Bricanyl MDI with and without different spacers.

5.2 Methods

5.2.1 Equipment and inhalation devices

Equipment:

MDI sampling apparatus: Copley Scientific Ltd, UK

Andersen MKII Cascade Impactor : Copley Scientific Ltd, UK

A/E fibre glass filter discs: 25 mm; Pall Corporation, USA

GF 50 filter: Copley Scientific Ltd, UK

GAST pump: Brook Crompton, UK

An electronic digital flow meter: MKS Instruments, USA

Parafilm M laboratory film: Pechiney Plastic Packaging, USA

Silicone fluid spray: Releasil B silicone spray, Dow Corning Limited, Barry, Glamorgan, U.K.

Critical flow controller model TPK: Copley Scientific Ltd, UK

HPLC system: Previously described in section 3.2 in this thesis

Inhaler and spacer devices:

Bricanyl Metered Dose Inhaler: Labelled as a nominal dose of 250µg terbutaline sulphate per puff, AstraZeneca, UK

Fisonair spacer: Fisons plc-Pharmaceuticals Division, UK

AeroChamber MAX spacer: Valved Holding Chambers; Trudell Medical International Europe Ltd, UK

AeroChamber Plus spacer: Valved Holding Chambers; Trudell Medical International Europe Ltd, UK

Nebuhaler: Valved Holding Chambers; AstraZeneca, UK

5.2.2 Procedure

5.2.2.1 Total Emitted Dose

The emitted dose from a Bricanyl MDI (labelled as a nominal dose of 250µg terbutaline sulphate per puff, AstraZeneca, UK) was determined. Determinations were made for the MDI and when the MDI was attached to each of the four different spacers. The spacers were the Fisonair spacer (Fisons plc-Pharmaceuticals Division, UK), the AeroChamber MAX [AMAX] and the AeroChamber Plus [APLUS] Valved Holding Chambers (Trudell

Medical International Europe Ltd, UK) and Nebuhaler (AstraZeneca, UK). The Fisonair was also washed with water and left to air dry [FISONAIRW]. The Fisonair without washing was primed using several single puffs from the MDI and was not washed after use [FISONAIR]. Also the AeroChamber MAX spacer was used with one puff with one inhalation volume [AMAX] and one puff with three inhalation volumes [AMAX3] through the spacer.

The MDI sampling apparatus (Copley Scientific Ltd, UK) with a critical flow controller model TPK (Copley Scientific Ltd, UK) was used. The final filter was a 25 mm A/E fibre glass filter (Pall Corporation, USA). Vacuum flow through the apparatus was provided by a GAST pump (Brook Crompton, UK). The flow through the MDI / MDI+Spacer was 28.3 L min^{-1} with a flow-duration of 8.5 sec such that the inhalation volume was 4 L. The flow was measured by an electronic digital flow meter (MKS Instruments, USA). Parafilm M laboratory film (Pechiney Plastic Packaging, USA) was used to seal the apparatus.

Each inhaler / spacer mouthpiece was inserted tightly into the mouthpiece adaptor of the dose sampling unit and aligned along the horizontal axis. The emitted dose from the MDI / MDI+Spacer was measured by collecting one individual dose at 28.3 L min^{-1} with an inhaled volume of 4 L. Ten determination were made for each dose emission (n=10). The MDI was shaken and primed by firing two doses to waste before use (Barry and O'Callaghan, 2003). The discharge of the dose was done in co-ordination with the switching on of the pump in case of the MDI without spacer. When using the spacer, the dose was discharged into the spacer and with the switching on of the pump within 1 second.

For each dose emission procedure the critical flow controller system was used to produce sonic flow conditions, as recommended by the Pharmacopoeial Methods, (European Pharmacopeia, 2001; British Pharmacopoeia, 2005; United States Pharmacopeia, 2005). Following dose emission into the apparatus, the sampling unit was washed with 25%

acetonitrile and the filter was completely submerged in 25% acetonitrile and then sonicated for 3 min (preliminary analysis revealed that this procedure removes all drug entrained on the filter). All the solutions were collected and made up to a volume of 250 ml for the MDI without spacer and 50 and 100 ml for MDI with a spacer (Dose emission unit and spacer, respectively). The amount of drug was determined by high performance liquid chromatography using the previously validated method descried in section 3.2. The total dose emitted was the amount deposited in the plastic dose sampling apparatus and the final filter.

5.2.2.2 The Aerodynamic Particle Size Characterization

All the parts of the Andersen MKII Cascade Impactor were washed in methanol and allowed to dry. The collection plates were then sprayed with silicone fluid (Releasil B silicone spray, Dow Corning Limited, Barry, Glamorgan, UK.) and allowed to dry for at least one hour prior to analysis. The cascade impactor was assembled and the final filter was a GF 50 (Copley Scientific Ltd, UK). The flow through the mouthpiece of the inhaler was 28.3 L min^{-1} with a flow-duration of 8.5 seconds (equivalent to 4 L inhalation volume). The flow was measured using an electronic digital flow meter (MKS Instruments, USA) and a critical flow controller model TPK (Copley Scientific Ltd, UK). Parafilm M laboratory film (Pechiney Plastic Packaging, USA) was used to seal the apparatus.

Five determinations from each spacer were made. For each determination, the discharge of the dose was done in co-ordination with the switching on of the pump in case of the MDI without spacer. When using the spacer, the dose was discharged into the spacer and with the switching on of the pump within 1 second. Each stage of the Cascade Impactor was rinsed with 25% acetonitrile and made up to the volume as described in Table 5.1.

The washing procedure of the filter was the same as that described for the total dose emission. The amounts deposited on each stage were determined by high performance liquid chromatography of the previously validated method described in section 3.2.

Table 5.1 The washing volume of 25% acetonitrile for ACI stages.

Stage	Volume of washing (ml)	
	MDI	MDI with spacers
Spacer	-	100
Induction port	100	25
0	10	10
1	25	25
2	25	25
3	25	25
4	25	25
5	10	10
6	10	10
7	10	10
Filter	10	10

5.2.3 Data Analysis

The total dose emission was determined as the total amount of drug ex-mouthpiece. This has been reported with respect to the nominal dose. Using the ACI at a flow of 28.3 L min^{-1} for the MDI the standard effective cut-off diameter of each stage at a flow 28.3 L min^{-1} was used (Copley, 2007). The fine particle dose (FPD) was the amount with particles that correspond to a size less than 5μm. The fine particle fraction % (FPF) was the FPD expressed as a percentage of the total amount deposited into the throat and stages of the cascade impactor (this is the dose exiting the mouthpiece). The mass median aerodynamic diameter (MMAD) was obtained from a plot of the logarithm of the percentage less than a stated size on a probability scale against the logarithm of the effective cut-off diameter of the stage (United States Pharmacopeia, 2005) and this was done using Copley Inhaler Testing Data Analysis Software (CITDAS). The MMAD was the diameter corresponding to 50% undersize. The geometric standard deviation (GSD) was the square root for the size corresponding to 84.13% less than the stated size divided by the square root of the size for 15.87% (United States Pharmacopeia, 2005).

5.2.4 Statistical analysis

A two-way analysis of variance (ANOVA) test was used to compare the total emitted dose and aerodynamic particle size characterization of the different spacer with the MDI using SPSS V15.0 (SPSS Inc., Chicago, USA).

5.3 Results

5.3.1 Total Emitted Dose

The individual doses and the mean (SD) emitted dose of terbutaline sulphate are shown in Table 5.2 and Figures 5.1-5.3.

The overall means (SD) amount of terbutaline sulphate (n=10 doses) when using the AeroChamber MAX spacer with three inhalations (AMAX3) were 91.7(8.9) and 107.4(15.9) µg for the dose emission unit and spacer. These correspond to a mean (SD) % of 36.7 (3.6) and 43.0 (6.4) % of the nominal dose (n = 10 doses), respectively for the total emitted dose and the spacer. These are similar to that of one inhalation with the AeroChamber MAX as shown in table 5.2 and Figures 5.1-5.3.

Table 5.2 Terbutaline sulphate emitted dose (µg) from the MDI (250µg nominal dose) determined at a flow 28.3 L min^{-1}.

Dose	Amount in µg									
	MDI	***FISONAIRW**		**FISONAIR**	**AMAX**		**APLUS**		**Nebuhaler**	
	ED	**ED**	**Spacer**	**ED**	**ED**	**Spacer**	**ED**	**Spacer**	**ED**	**Spacer**
1	208.8	46.4	130.7	107.8	84.3	108.4	80.9	184.3	72.1	140.2
2	204.2	56.2	163.2	73.6	91.4	97.1	87.5	122.2	62.4	186.3
3	213.1	47.8	127.0	90.7	99.0	121.3	81.5	153.2	67.1	140.0
4	173.9	58.3	166.4	98.3	95.9	129.1	65.8	139.0	71.1	120.8
5	144.4	50.6	136.2	112.9	98.5	128.1	89.3	121.5	72.6	155.6
6	152.8	58.9	156.4	84.1	88.0	100.9	65.1	158.0	65.8	156.2
7	216.3	63.7	162.6	99.2	98.6	94.0	69.2	112.1	75.8	157.3
8	212.1	40.8	104.1	100.0	82.5	93.9	91.2	124.3	75.4	135.3
9	206.9	45.4	116.3	95.5	82.7	89.7	73.0	116.2	74.9	165.9
10	194.8	53.9	119.9	90.8	79.9	95.8	67.8	146.4	74.3	161.9
Mean	**192.7**	**52.2**	**138.3**	**95.3**	**90.1**	**105.8**	**77.1**	**137.7**	**71.1**	**151.9**
STD	**26.3**	**7.2**	**22.4**	**11.3**	**7.5**	**15.0**	**10.1**	**22.9**	**4.6**	**18.4**
RSD	**13.6**	**13.8**	**16.2**	**11.9**	**8.4**	**14.2**	**13.1**	**16.6**	**6.4**	**12.1**
% of nominal dose	**77.1(10.5)**	**20.9(2.9)**	**55.3(8.9)**	**38.0(4.5)**	**35.1(3.0)**	**41.1(6.0)**	**30.9(4.1)**	**55.1(9.1)**	**28.5(1.8)**	**60.8(7.4)**

***** Fisonair spacer with washing of the spacer with water after use (FISONAIRW), Fisonair spacer without washing the spacer prior to use (FISONAIR), AeroChamber MAX spacer (AMAX), AeroChamber Plus spacer (APLUS), Emitted Dose (ED) and amount Left in Spacer (Spacer).

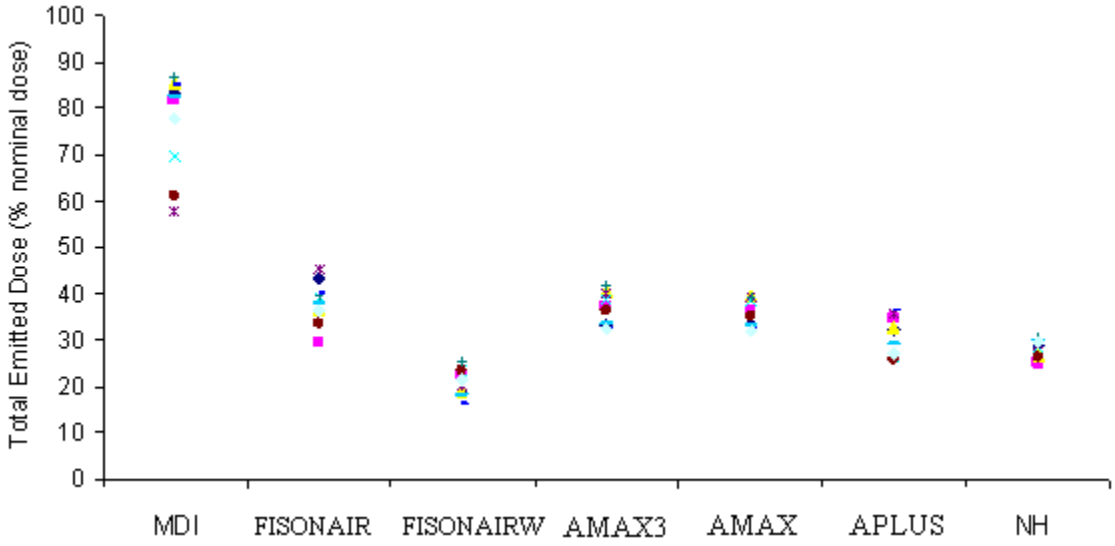


Figure 5.1 Terbutaline sulphate total emitted dose expressed as a percent of the nominal dose from the MDI at flow of 28.3 L min^{-1} (n=10 separate doses).

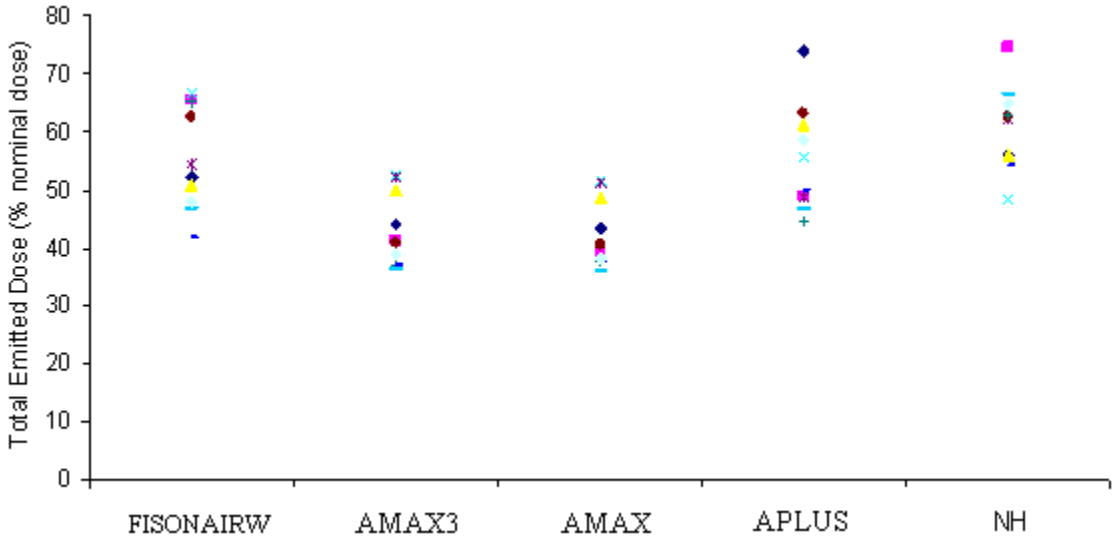


Figure 5.2 Terbutaline sulphate deposited in each spacer expressed as a percent of the nominal dose from the MDI at flow of 28.3 L min^{-1} (n=10 separate doses).

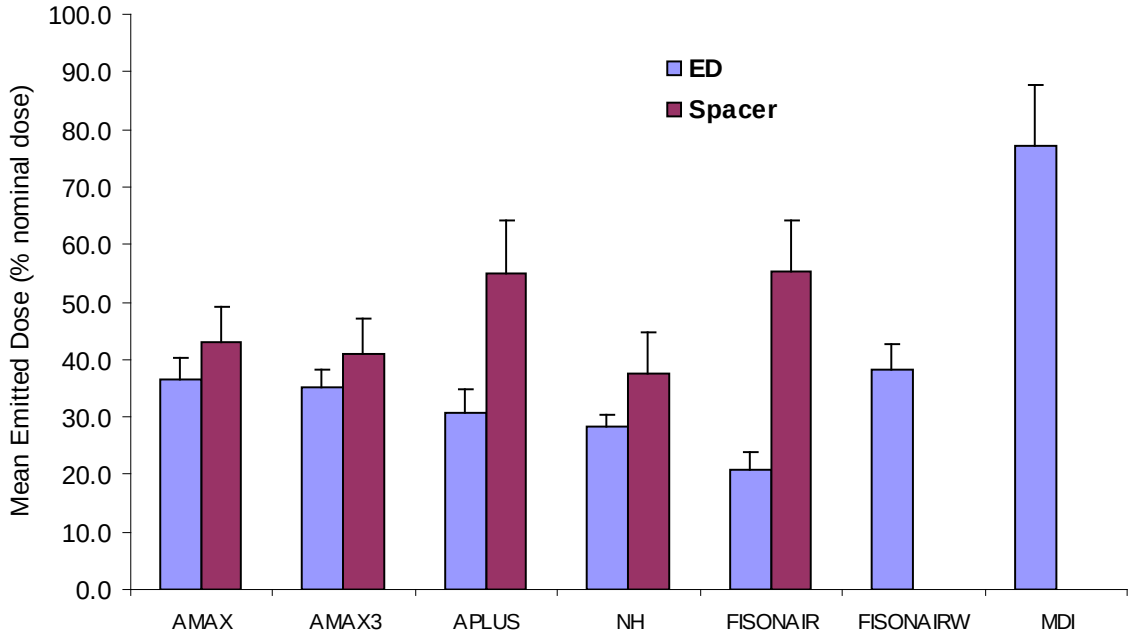


Figure 5.3 The mean (SD) total emitted dose (ED) and amount deposited in each spacer of terbutaline sulphate expressed as a percent of the nominal dose (n=10 separate doses).

5.3.2 The Aerodynamic Particle Size Characterization

A summary of the data obtained from the Andersen Cascade Impactor is shown in Table 5.3 and Figures 5.4-5.6.

The majority of the emitted dose is deposited in the induction port of the ACI, but when using the spacer the majority of the emitted dose is deposited in the spacer.

Table 5.3 A summary of the data obtained from the ACI for MDI (250 µg nominal dose). Mean values in µg are quoted except those bold which are mean (SD).

	Stage Cut-off	Amount in µg						
		MDI	**FISONAIRW**	**FISONAIR**	**AMAX**	**AMAX3**	**APLUS**	**Nebuhaler**
Spacer		--	104.5	--	96.3	86.4	140	158.3
Induction port		114.6	7.4	16.1	24.7	15.2	8.5	8.7
0	10	4.7	5	8.3	3.4	4.7	2.6	6.6
1	9	8.5	10	17.2	9.4	15.3	6.2	15.6
2	5.8	10.1	12.2	18.8	12.6	21.2	13.6	15.8
3	4.7	19	21	29.6	48.2	39.6	30.9	23.5
4	3.3	13.4	10.6	12.5	11.9	14.3	20.3	13.5
5	2.1	5.4	4.2	3.3	4	4.9	3	5
6	1.1	0	0	0	0	0	1.5	1.5
7	0.7	0	0	0	0	0	2.2	1.5
Filter	0.4	0	0	0	0	0	1.9	2.8
Total emitted dose (µg)*		**175.7(15.9)**	**70.4(10.9)**	**104.1(13.6)**	**114.1(10.4)**	**115.3(33.1)**	**90.6(9.0)**	**94.5(13.8)**
Total emitted dose (% of nominal dose)		**70.3(6.4)**	**28.2(4.4)**	**41.6(5.4)**	**45.6(4.2)**	**46.1(13.2)**	**36.2(3.6)**	**37.8(5.5)**
FPD (µg)		**41.1(7.9)**	**39.7(5.4)**	**39.7(5.4)**	**68.2(7.9)**	**65.9(19.1)**	**64.7(7.8)**	**52.9(9.6)**
FPF (of nominal dose)		**16.4(3.2)**	**15.9(2.2)**	**15.9(2.2)**	**27.3(3.2)**	**26.4(7.6)**	**25.9(2.1)**	**21.2(3.8)**
FPF% of the emitted dose		**23.4(3.9)**	**56.7(2.9)**	**48.7(5.9)**	**59.7(2.3)**	**57.0(2.7)**	**71.3(4.1)**	**55.8(3.6)**
MMAD (µm)		**4.1(0.2)**	**4.4(0.2)**	**4.7(0.3)**	**4.1(0.2)**	**4.3(0.2)**	**3.8(0.1)**	**4.4(0.2)**
GSD		**1.6(0.1)**	**1.6(0.05)**	**1.6(0.1)**	**1.4(0.2)**	**1.5(0.03)**	**1.4(0.05)**	**1.6(0.02)**

*Total is the total amount of terbutaline sulphate deposited in the ACI.

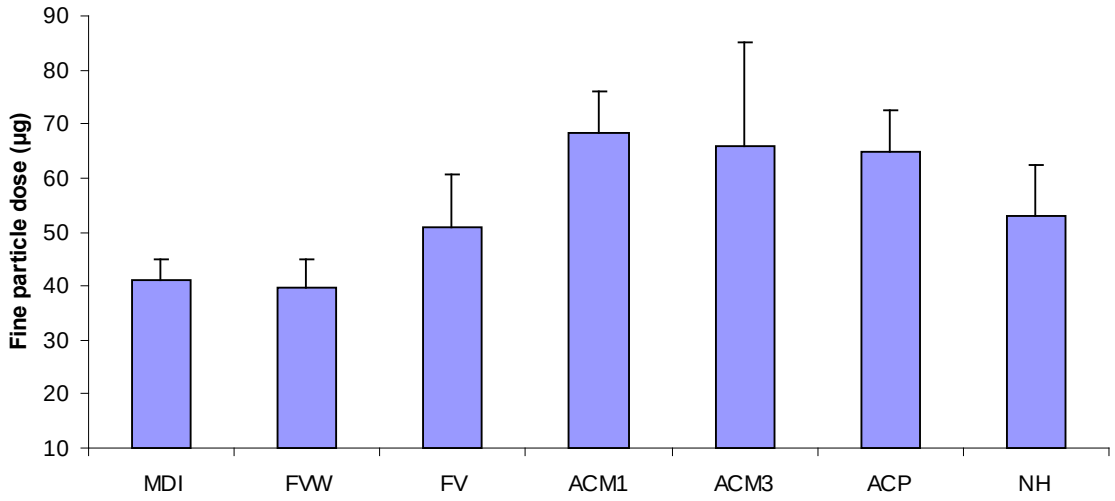


Figure 5.4 The mean (SD) fine particle dose of terbutaline sulphate expressed in µg (n=5) for each inhalation method.

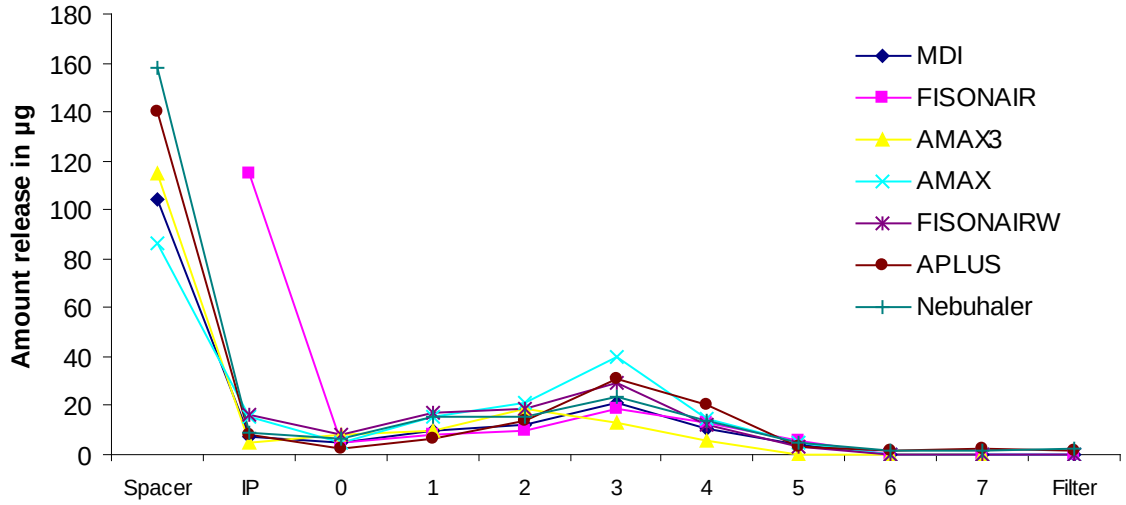


Figure 5.5 Mean terbutaline sulphate (µg) deposited on each stage of the ACI using an inhalation flow of 28.3 L min^{-1}.

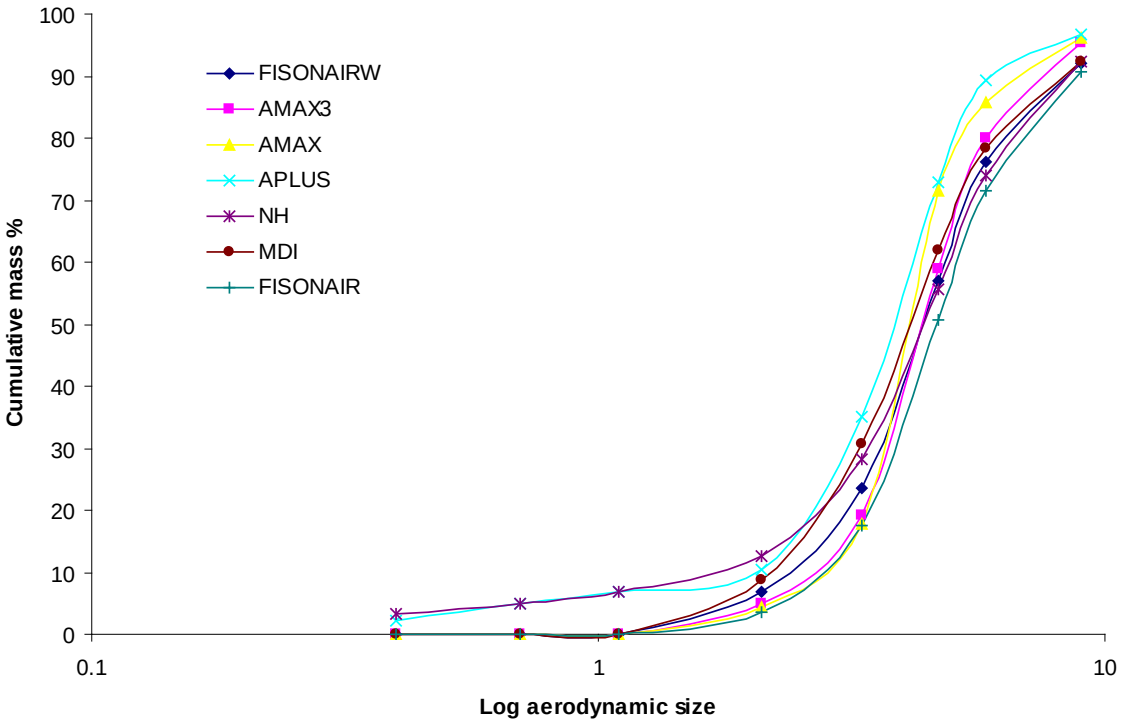


Figure 5.6 The mean (n=5) aerodynamic distribution of the dose emitted from the Bricanyl MDI with different spacers.

5.3.3 Statistical analysis

The comparison of the dose emission results from the MDI alone and the MDI with different spacers showed that the amount emitted from the MDI alone is significantly higher than with any of the MDI+spacers ($p>0.001$). The amount emitted increased in the following order, FISONAIRW, Nebuhaler, APLUS, AMAX, FISONAIR and MDI without spacer. However when statistically comparing the MDI+spacers with each other it was found that there was no significant difference between the Nebuhaler emitted dose and the APLUS emitted dose. Also, there was no significant difference between the AMAX and FISONAIR.

The amount deposited on wall of AMAX spacer is significantly lower than all other spacers ($p<0.001$)

The comparison of the aerodynamic particle size characterization results from the MDI alone and the MDI with different spacers showed that the FPD from the MDI alone was significantly lower than that from the AMAX and APLUS ($p< 0.01$). However, the total dose per shot was significantly higher than that of all MDI+Spacers ($p<0.001$). The FPD increased in the following order, FISONAIRW, MDI without spacer, FISONAIR, NH, APLUS and AMAX ($p< 0.05$). When comparing the MDI+spacers with each other it was found that there was no significant difference between them. However the FPD from FISONAIRW was significantly lower than that from the AMAX and APLUS ($p< 0.005$). When comparing the FPF (of the emitted dose), it was found that the APLUS had a significantly higher FPF than any of the other methods ($p<0.001$). The FPF from the MDI alone was significantly lower than all the methods ($p<0.001$).

Regarding the MMAD it was found that the APLUS had the smallest MMAD ($p<0.05$) and FISONAIR had the biggest MMAD ($p<0.05$).

A summary of the statistical comparison between the MDI and the MDI+Spacers to highlight the differences is presented in Table 5.4. Also a summary of the statistical comparison between the APLUS (as the gold standard) and the other MDI+Spacers combinations to highlight the differences is presented in Table 5.5.

Table 5.4 Mean difference (95% confidence interval) for MDI compared to the MDI+Spacers.

Comparator	FPD (µg)	FPF (%)	MMAD (µm)	Emitted dose (µg)
Nebuhaler	-11.7 (-27.4, 3.9)	-32.3 (-37.9, -26.8)***	-0.3 (-0.6, -0.02)*	121.6 (110.1, 133.1)***
APLUS	-23.6 (-39.2, -7.9)*	-47.9 (-53.4, -42.4)***	0.3 (0.004, 0.6)*	115.6 (104.1, 127.1)***
AMAX	-24.8 (-40.4, -9.5)*	-33.6 (-39.1, -28.1)***	-0.2 (-0.5, -0.04)	102.7 (91.2, 114.1)***
FISONAIRW	1.4(-14.2, 17.0)	-33.3 (-38.8, -27.7)***	-0.3 (-0.6, -0.003)*	140.6 (129.1, 152.0)***
FISONAIR	-9.7(-25.3, 6.0)	-25.3 (-30.8, -19.8)***	-0.6 (-0.9, -0.3)***	97.4 (86.0, 108.9)***

Table 5.5 Mean difference (95% confidence interval) for APLUS compared to the rest of MDI+Spacers.

Comparator	FPD (µg)	FPF (%)	MMAD (µm)	Emitted dose (µg)	Amount deposited in spacer (µg)
Nebuhaler	11.8 (-3.8, 27.5)	15.6 (10.0, 21.18)***	-0.6 (-0.9, -0.3)***	6.0 (-5.5, 17.5)	-14.2 (-31.2, 2.8)
AMAX	-1.2 (-16.8, 14.4)	14.3 (8.8, 19.8)***	-0.5 (-0.8, -0.2)***	-12.9 (-24.4, -1.5) *	31.9 (14.9, 48.9)***
FISONAIRW	25.0 (10.6, 41.8)**	14.6 (9.1, 20.2)***	-0.6 (-0.8, -0.3)***	25.0 (13.5, 36.4)***	30.4 (-17.5, 16.4)***
FISONAIR	13.9 (-0.5, 30.7)	22.6 (17.1, 28.1)***	-0.9 (-1.1, -0.6)***	-18.2 (-29.6, -6.7)***	NA

For both tables * $p<0.05$, ** <0.01, ***<0.001 otherwise no significant difference

5.4 Discussion

The results show that the total amount of terbutaline sulphate emitted from the MDI as well as the FPF from the same dose were significantly affected by the type of the spacer used ($p< 0.001$). The significantly higher emitted dose from the MDI alone ($p<0.001$) was due to the spacer which decreases the amount of large particles reaching the dose sampling unit. Therefore, as expected, the MDI without a spacer had the largest total emitted dose.

The AeroChamber Max is coated with an antistatic lining. The amount deposited on the walls of the AMAX spacer, therefore, is significantly lower than all other spacers ($p<0.001$). Also, there was no significant difference between the emitted dose from the FISONAIR and the AMAX spacer which is an antistatic spacer. This may account for the decrease of the static charge in the Fisonair spacer when used unwashed.

The static charge of polycarbonate spacers has been reported to vary greatly depending on the washing procedure (Wildhaber et al., 1996; Pierart et al., 1999). Also it have been reported that washing with light anionic detergent solution (most of the household detergents) decreases static charge (O'Callaghan and Barry, 2000; Chuffart et al., 2001). Static charge is produced from washing the Fisonair spacer with only water without detergent. It decreases the emitted dose as it increases the amount deposited in the spacer itself. The results here were consistent with the results reported by Kenyon et al (1998) and Wildhaber et al (2000). They had concluded that in-vivo lung deposition from plastic spacer devices will vary according to the electrostatic charge on the spacer walls (Kenyon et al., 1998; Wildhaber et al., 2000). The static charge can also account for the significantly lower amount deposited on walls of AMAX spacer ($p<0.001$).

The significantly lower FPF from the MDI alone ($p<0.001$) was consistent with the in-vivo studies by Hindle et al (1994) and Silkstone et al (2002). Both studies showed that the spacer significantly increased the relative lung bioavailability compared to the MDI alone ($p<0.01$). The result was also consistent with many in-vivo studies that have shown that the

spacers significantly increase lung bioavailability (Comis et al, 1993; Newman et al, 1998; Aswania and Chrystyn, 2001). This result might be due to the fact that patients inhaled from a static cloud because the spacer decreases the velocity of the aerosol particles and allows some evaporation of the droplets. This allows the propellant to evaporate thereby decreasing the particle size, hence decreasing the amount deposited on the induction port. In case of the MDI used alone, the aerosol particles travel at a high speed which enhances their deposition in the induction port and therefore, decreases the FPF.

It was also found that the FPF of the emitted dose from the Fisonair spacer without washing (FISONAIR) is higher than the FPF from the Fisonair spacer with washing (FISONAIRW). A study by Bisgaard et al (1995) have shown that priming the plastic spacers with repeated puffs from a budesonide MDI to minimise the electrostatic charge on the plastic prolonged the half life ($t_{1/2}$) of the aerosol in the Nebuhaler from nine to 32 seconds. A normal cleaning procedure reduced the aerosol $t_{1/2}$ back to baseline (Bisgaard et al., 1995). A short half-life increases the need for co-ordination between actuation and inhalation. Therefore non-electrostatic spacers deliver a significantly higher dose than plastic spacers.

Another study by Pierart et al (1999) showed that there was a three-fold increase in lung deposition from detergent-coated spacers [45.6(2.2) %] compared to static spacers [11.5(3.5) %]. The mean (range) gastrointestinal deposition was 2.4% (1.0±3.9) through a static spacer and 8.8% (4.1±11.2) through a non-static spacer ($p<0.001$). The mean (range) amount of salbutamol remaining in the static spacers was 76.7% (71.8±81.4) compared to 33.1% (26.0±40.8) in the detergent-coated spacers ($p<0.001$). Typical deposition patterns are shown in Figure 5.7.

There are many deposition studies involving the use of a plastic spacer, and the results are highly variable (Newman et al., 1989; Tal et al., 1996; Melchor et al., 1993). As the question of electrostatic charge affecting drug delivery from plastic spacers has not been

adequately addressed prior to the work of O'Callaghan et al. (1993) and Barry and O'Callaghan (1995), there is a strong possibility that a substantial part of the variability between studies using similar spacer devices and breathing patterns is due to differences in electrostatic charge. The effect of reducing electrostatic charge on the lung deposition from a spacer, highlighted in Figure 5.7, has not been adequately quantified.

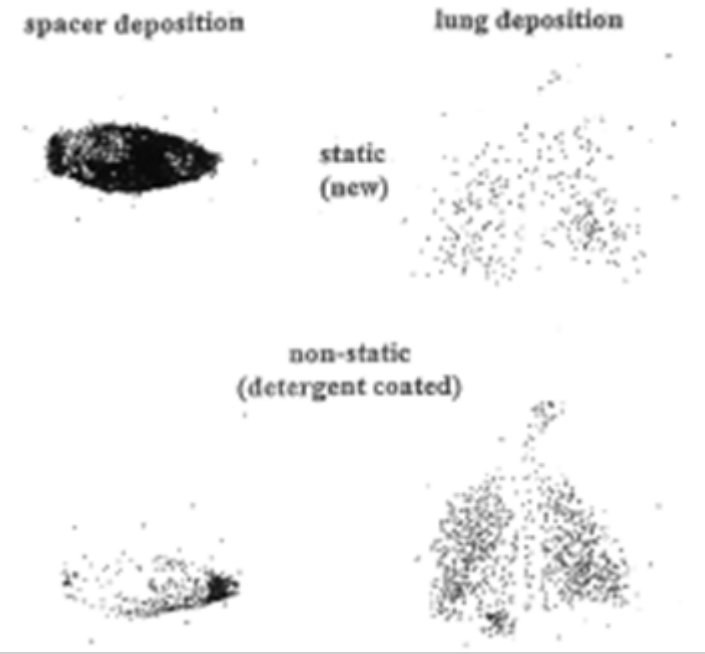


Figure 5.7 Typical deposition patterns of radioaerosol in the same subject after inhalation through a new and a detergent-coated spacer (posteroanterior view) [reproduced from Pierart et al. (1999)].

The static charge greatly affects the output from spacer. It was found that the drug output from a spacer lined with an antistatic agent may be increased by a factor of three or more (O'Callaghan and Barry, 2000). This was consistant with the result of the comparison between the FISONAIR and AeroChamber MAX spacer, the FPD for AeroChamber MAX spacer is higher than for FISONAIR, even though the emitted doses form FISONAIR and AeroChamber MAX spacers was not significantly different. This might be due to the AeroChamber MAX spacer, as antistatic spacer, having more ability to produce particles

with a smaller particle size than the FISONAIR spacer (as a primed spacer with repeatd puffs). This was also seen from the MMAD statistical comparison as the FISONAIR has a significantly higher MMAD than the AMAX ($p<0.02$).

When using the MDI without a spacer, the majority of the emitted dose is deposited in the induction port of the ACI, but when using the spacer the majority of the emitted dose is deposited in the spacer. Thus, the use of the spacer would decreases the amount deposited in the mouth.

For the AeroChamber MAX one inhalation and AeroChamber MAX three inhalations the emitted dose and FPD did not differ significantly, thus the use of the spacer with one inhalation or three inhalations does not affect the respirable fraction and the emitted dose. However the insignificant difference between 1 and 3 inhalations may be due to the large inhaled volume (4 L) used per inhalation.

The FPF of the AeroChamber Plus (small volume spacer) was significantly higher than all the other methods ($p<0.001$) and the MMAD was significantly lower than all the other methods ($p<0.05$). However the AeroChamber MAX have the largest emitted dose and FPD of all the used spacers. Which are more important FPF and MMAD or emitted dose and FPD, needs further investigation.

5.5 Conclusions

When the MDI is attached to a spacer there is an increase in the FPD and FPF and a decrease in the amount deposited in the induction port. The dose emitted and the FPD from plastic spacer devices will vary according to the electrostatic charge on the spacer walls. Thus, using a spacer theoretically increases the amount that reaches the respiratory tract and decreases the amount deposited in the mouth. The AeroChamber MAX had the largest FPD. However the AeroChamber Plus has the best FPF and MMAD.

6.1 Introduction

The choice of a nebulisation system for a patient usually depends on the equipment available as there is only limited guidance on the selection and use of a nebuliser. In-vitro determination of the droplet size distribution, the fine particle dose and the emitted dose are necessary to compare nebulisers because they are important parameters that have the potential to indicate differences in the clinical response and side effects. Hence the European Respiratory Society Nebuliser Guidelines were published to standardise nebuliser performance and therapy (Boe et al., 2001). These guidelines recommend the Comité Européen Normalisation (CEN) method for the in-vitro measurement of the aerodynamic particle size of nebulisers (Boe et al., 2001; Comité-Européen-Normalisation., 2001). This method uses the Marple 298X Cascade Impactor. The volumetric flow leaving the nebuliser is set at a constant flow of 15 L min^{-1} with 2 L min^{-1}, of this flow, drawn through the impactor. The 15 L min^{-1} flow was chosen for the CEN standard to represent a typical (adult) mid-point flow for tidal breathing (Dennis and Pieron, 2004). The aerosol is sampled anisokinetically by the low flow impactor from the main flow exiting the nebuliser. This could be a significant limitation of the CEN methodology as anisokinetic sampling may introduce size-related bias that thus far has not been quantified. Furthermore the sampling of a limited fraction of the emitted dose (2/15), the limited loading capacity of the cascade stages, filter fitting (and availability plus their cost), desorption problems and its suitability for operation with drug formulations may each affect the accuracy and precision of assessments (Jauernig et al., 2002; Jauernig et al., 2004) .

The Next Generation Impactor (NGI) was originally introduced for the determination of the aerodynamic particle size distribution of the dose emitted from metered dose and dry powder inhalers using compendial procedures (British Pharmacopoeia, 2005; United States Pharmacopeia, 2005) and calibrated within the flow range of 30–100 L min^{-1}. Later, this

archival NGI was calibrated at 15 L min^{-1} for use in the evaluation of nebulisers. Unlike the CEN approach the NGI allows sampling of all of the flow from the nebuliser by the impactor (Marple et al., 2004b). Several studies have since assessed the use of the NGI without the preseparator, with a filter in or after the Micro-Orifice Collector (MOC) to collect any extra-fine particles that would bypass this component (Marple et al., 2004a; Berg et al., 2007b) and with cooling the NGI either in water bath (Jauernig et al., 2003) or refrigerator at 5^{o}C for 90 minutes before use (Berg and Asking, 2004; Berg et al., 2007b). A consortium of pharmaceutical companies 'The NGI Consortium' was formed in 2002 to investigate the NGI at a flow of 15 L min^{-1} in compliance with the CEN recommendations for nebulisers, and sampling the whole air stream leaving the nebuliser. In 2003 the EPAG (European Pharmaceutical Aerosol Group) took over technical management of the NGI. Its Nebuliser Sub-Team was formed in 2005 to evaluate specific aspects of nebuliser testing in relation to the development of a new monograph, 2.9.44 "Preparations for Nebulisation", proposed for the European Pharmacopeia. One of the tasks of this group has been an inter-laboratory evaluation of the need to chill the NGI to avoid heat transfer-related evaporation of aqueous droplets from nebulisers (Berg et al., 2007b; Dennis et al., 2008).

Recently evaporation effects during aerodynamic characterisation of the aerosolised dose from nebulised salbutamol (5mg/ml) solutions has been noted with cooling the NGI for 90 minutes in a refrigerator immediately before use when using a flow of 15 L min^{-1} (Berg et al., 2007b).

The aim of this Chapter was to further investigate the aerodynamic characteristics of aerosolised doses from nebuliser systems using different operating conditions with the NGI and to determine similar properties using the CEN methodology to identify if evaporation effects occur. To address this aim the NGI has been operated at inhalation flows of 15 and 30 L min^{-1} with and without cooling. The CEN method was modified to analyse the drug rather than alter the formulation by adding a fluoride tracer (Silkstone et al., 2002). Two

different nebulisers operate by different principles have been used in this chapter to examine the robustness of the new approach. The Sidestream is a jet nebuliser and therefore the aerosolised dose will be cooler than ambient room conditions (Fink et al., 2001). The Aeroneb Professional is a vibrating mesh nebuliser and thus the aerosolised dose that is emitted will be similar to ambient room temperature (Fink et al., 2001).

6.2. Material and Method

6.2.1 Equipment and inhalation devices

Equipment:

Next Generation Impactor (NGI):	Copley Scientific Ltd, UK
Cascade Impactor	Marple Series 298X, Low flow cascade impactor (Graseby Ltd., UK)
Breathing simulation machine:	Compass breathing simulator, Pari GmbH, Germany
GAST pump :	Brook Crompton, UK
An electronic digital flow meter:	MKS Instruments, USA
Parafilm M laboratory film:	Pechiney Plastic Packaging, USA
Critical flow controller model TPK:	Copley Scientific Ltd, UK
Electrostatic filter:	An electrostatic filter (Pari GmbH, Germany) enclosed in filter holder (Pari GmbH, Germany)
Marple stages filters	Glass fibre filters with radial slits for Marple Impactor, Diameter 34mm, (SKC Inc., USA)
Marple back filters	Glass fibre filters for the bottom stage of Marple Impactor, without radial slits, Diameter 34mm (SKC Inc., USA)
Data analysis software :	Copley Inhaler Testing Data Analysis Software (CITDAS), Copley Scientific Ltd, UK
HPLC system:	Previously described in section 3.2 in this thesis

Inhalation devices:

Normal saline: Sodium chloride 0.9% respiratory solution, Teva, UK

Sidestream jet nebuliser: Intersurgical Ltd, UK; attached to a Porta Neb compressor, Respironics, UK

Porta Neb compressor: Respironics, UK

The Aeroneb Professional Nebuliser: Vibrational mesh nebuliser system; Aerogen,Inc,USA

Inhalation solution: Bricanyl Respules labelled as a nominal dose of 5mg terbutaline sulphate 2.5mg/ml, AstraZeneca, UK

6.2.2 Procedure

6.2.2.1 The Aerodynamic Particle Size Characterization using the NGI

The NGI method was identical to that described by Berg et al (Berg et al., 2007b) except a standard T-mouthpiece, routinely used with each nebulised system, was fitted tightly to the USP/Ph.Eur induction port of the NGI using a standard rubber mouthpiece adapter. Any remaining air flow not delivered from the nebulised system was thus provided through the open (inlet) end of the T-mouthpiece as shown in Figure 6.1.

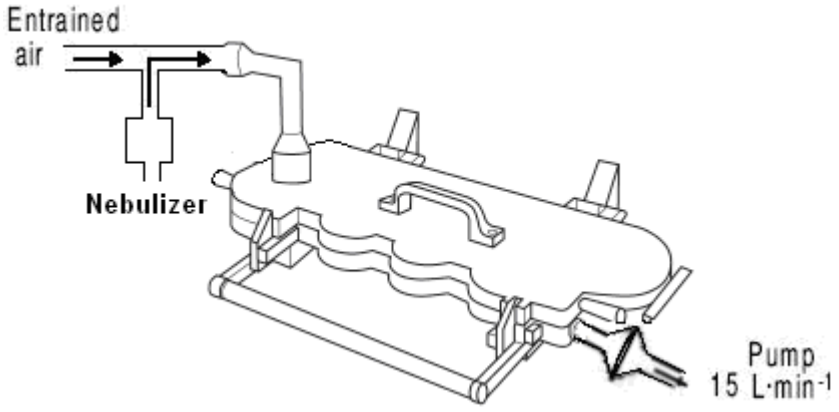


Figure 6.1 Schematic of NGI methodology to measure nebulised aerosol droplet size. A constant inhalation of 15 L min^{-1} is drawn over (and through) the nebuliser to the NGI.

All the parts of the Next Generation Impactor were washed in methanol and allowed to dry. The NGI collection cups were not pre-coated with an agent to provide a tacky surface. It has been recommended recently by the European Pharmaceutical Aerosol Group (EPAG) that the NGI collection cups do not require coating for nebuliser aerosol assessments (Berg et al., 2007a; Berg et al., 2008). The micro orifice collector (MOC) is ineffective to hold the very small aerosol droplets when the NGI is operated at 15 L min^{-1} (Marple et al., 2004a) therefore a back-up filter (Pari GmbH, Germany) was placed after the MOC. The vacuum flow through the apparatus was provided by a GAST pump (Brook Crompton, UK). The flow was measured using an electronic digital flow meter (MKS Instruments, USA) and a critical flow controller model TPK (Copley Scientific Ltd, UK). Parafilm M laboratory film (Pechiney Plastic Packaging, USA) was used to seal the apparatus.

Determinations were made using an inhalation flows of 15 and 30 L min^{-1} through the system at room ambient conditions; temperature=25^{o}C and relative humidity=55% (ROOM). Also these experiments were repeated after the NGI (with its collection plate in place) and the throat had been placed in a refrigerator set at 5^{o}C for 90 minutes (COLD). For this procedure nebulisation of the dose started within 5 minutes of removal from the fridge and the nebulisation process was <5 minutes in accordance with the published recommendations (Finlay and Stapleton, 1999; Marple et al., 2004a; Berg et al., 2007b; Berg et al., 2008; Dennis et al., 2008). For each determination the dose was nebulised to dryness when using the Aeroneb Pro and to sputtering with the Sidestream.

For each set of operating conditions five separate determinations were made for each nebulised system. Following dosage emission the pump was switched off. Each plate of the NGI was rinsed with 25% acetonitrile and made up to fixed pre-determined volumes for optimal assay sensitivity. Similarly amounts left in the nebuliser system (chamber and tubing) were also recovered by rinsing. The amounts of terbutaline sulphate deposited on

each plate and remaining in the nebuliser system were determined by high performance liquid chromatography of the previously validated method described in section 3.2.

6.2.2.2 CEN (prEN13544-1) methodology

6.2.2.2.1 Aerosol output according to (prEN13544-1) –CEN

The total output of each system was determined according to the draft European Standard (Boe et al., 2001; Comité-Européen-Normalisation., 2001) except that amounts of terbutaline sulphate were determined rather than the use of a fluoride tracer (Silkstone et al., 2002). Each of the two nebuliser systems (described above) was assembled according to Figure 6.2.

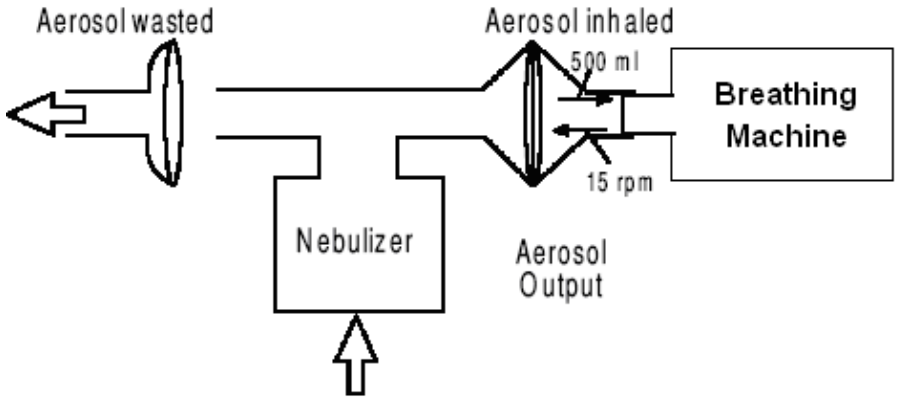


Figure 6.2 Schematic of Comité European de Normalisation methodology to measure nebulised aerosol output. Reproduced from Boe et al (2001).

Two ml of terbutaline sulphate solution (2.5mg/ml; Bricanyl respiratory solution) was added to the chamber of each nebuliser. In case of the Sidestream jet nebuliser the determination was done twice with and without the addition of 2 ml normal saline to the terbutaline sulphate solution. A breathing simulation machine (Pari GmbH, Germany) was set at a sinus flow of 15 breaths per minute with an inspiration : expiration ratio of 1:1 and a tidal volume of 500 ml. An electrostatic filter (A) was attached between the mouthpiece of the nebuliser and the breathing machine. This filter, therefore, would collect all the aerosol produced during the inspiration phase of a breathing cycle and thus provides a

good measure of the total inhaled aerosol dose (referred to as the in-vitro emitted dose available for inspiration). Another electrostatic filter (B) was attached to the vacuum pump. The vacuum pump was set at a flow of 25 L min^{-1} to scavenge any exhaled aerosol that would be released into the immediate ambient environment during expiration. Preliminary analysis revealed that this gross flow of 25 L min^{-1} collect all exhaled aerosols from the exhalation phase and did not affect the collection of the aerosol output that was inhaled by the breathing machine. The B filter was positioned close to the open end (4 cm away) of the nebuliser mouthpiece of each system to entrain drug emitted during the expiration phase. The amount collected on this filter would be that which would be exhaled during the nebulisation process. The breathing machine and vacuum pump were switched on 30 seconds before the nebuliser compressor. The Sidestream nebulisers were allowed to operate until sputtering occurred, consistent with the NGI-based determinations. Likewise the Aeroneb Pro devices were operated until dryness had been reached. For each nebuliser system ten determinations were made (n=10). Amounts trapped on the filters and left in the chamber and T-piece were determined by high performance liquid chromatography of the previously validated method described in section 3.2 in this thesis.

6.2.2.2.2 Aerodynamic characterization of the respirable dose according to (prEN13544-1) –CEN

A Marple Series 298 low flow cascade impactor (Graseby Ltd., UK) was used according to the draft European Standard (Boe et al., 2001; Comité-Européen-Normalisation., 2001) except that amounts of terbutaline sulphate were determined rather than the use of a fluoride tracer (Silkstone et al., 2002). This impactor has 8 stages and a final filter. A marple stage filter was placed on each stage except the final stage where it had a Marple back filter. The stages have effective cut-off diameters of 21.3, 14.8, 9.8, 6.0, 3.5, 1.55, 0.93 and 0.52 µm respectively, at the recommended flow of 2 L min^{-1}.

Figure 6.3 shows that the airflow through the Marple 298X was set at a continuous flow of 2 L min^{-1} with a further 13 L min^{-1} drawn from a supplementary pump connected between the cascade impactor and the nebuliser. Hence a constant flow of 15 L min^{-1} was drawn across the outlet of the nebuliser. The vacuum pump operating the impactor was switched on 30 seconds before each nebuliser system. To prevent sample overload on the filter on each stage of the impactor, preliminary experimentation (using congo red dye added to each formulation) indicated that the optimal sampling time for the Sidestream was 130 seconds and for the Aeroneb Pro system was 100 seconds. Amounts of terbutaline sulphate on each stage were obtained by desorption and determined by high performance liquid chromatography, the previously validated method described in section 3.2 in this thesis. Ten determinations were made (n=10) for each nebuliser system.

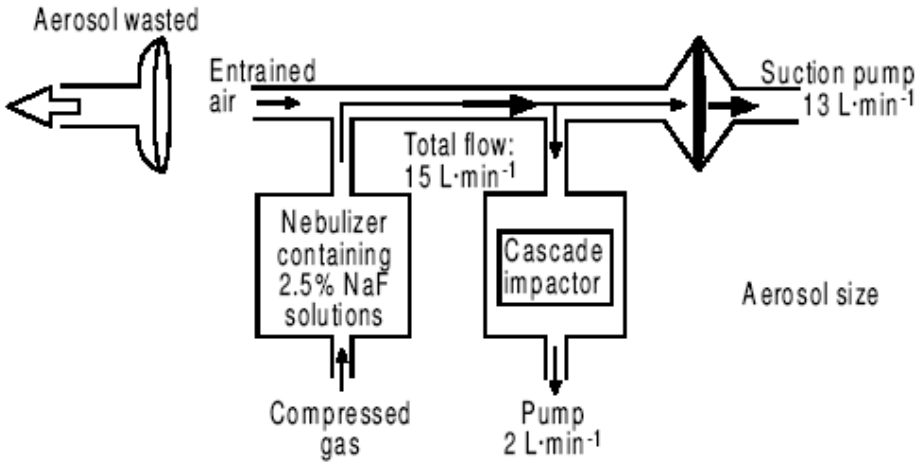


Figure 6.3 Schematic diagram of the Comité European de Normalisation methodology to measure nebulised aerosol droplet size. A constant inhalation of 15 L min^{-1} is drawn over (or through) the nebuliser. Reproduced from Boe et al (2001).

6.2.3 Data Analysis

The fine particle dose (FPD) was the amount of terbutaline sulphate that contained droplets < 5 µm aerodynamic diameter. The fine particle fraction % (FPF) was the FPD divided by the total amount that deposited into the throat and onto the stages of the cascade impactor

for the NGI (this is the dose exiting the mouthpiece). For the CEN method the total amount was that recovered from stage 0 to the final filter of the cascade impactor. The mass median aerodynamic diameter (MMAD) was obtained from a plot of the percentage less than a stated size on a probability scale against the logarithm of the effective cut-off diameter of the stage (United States Pharmacopeia, 2005). Copley Inhaler Testing Data Analysis Software (CITDAS, Copley Scientific, Nottingham, UK) impactor data analysis software was used for assessing the data. The MMAD was the diameter corresponding to 50% undersize. The geometric standard deviation (GSD) was the square root for the size corresponding to 84.13% less than the stated size divided by the square root of the size for 15.87 % (United States Pharmacopeia, 2005).

6.2.4. Statistical analysis

A Two Way analysis of variance (ANOVA) was used to compare the 5 different operating conditions (15COLD, 15ROOM, 30COLD, 30ROOM and CEN) with respect to the FPF, MMAD and GSD using SPSS V15.0 (SPSS Inc., Chicago, USA). From this analysis the mean difference (95% confidence limits) were obtained for each operating condition compared to 15COLD. This comparator was used as the benchmark because of the recommendation to cool the NGI for characterising nebulised doses. A paired T-test was used to compare the results of Sidestream jet nebuliser and Aeroneb Pro at different conditions.

6.3 Results

6.3.1 Aerodynamic Particle Size Characterization using the NGI

A summary of the data obtained from the NGI methodology is shown in Tables 6.1 and 6.2 and Figures 6.4-6.11. The amount remaining in the Aeroneb Pro chamber is about one third of the amount remaining in the Sidestream jet nebuliser chamber.

The FPD when using the Aeroneb Pro is significantly higher then the FPD when using the Sidestream jet nebuliser at the different flows tested ($p<0.001$).

Table 6.1 A summary of the data obtained from the NGI for the terbutaline sulphate Respules (5 mg nominal dose) nebulised using Aeroneb Pro nebuliser. Mean values in µg are quoted except those bold which are mean (SD).

	Stage Cut-off	**Amount in µg**			
		15 ROOM	15 COLD	30 ROOM	30 COLD
Remaining		1220.1	1559.2	1344.2	1459.3
Throat		75.2	113.7	135.7	194.4
1	14.1	264.9	278.6	273.9	308.4
2	8.61	444.7	637.0	520.3	702.5
3	5.39	552.9	692.6	284.3	694.3
4	3.3	650.8	859.9	431.8	776.6
5	2.08	660.8	642.4	987.8	497.0
6	1.36	413.9	232.7	627.3	77.3
7	0.98	175.3	76.3	251.2	42.1
MOC	0	100.2	33.4	136.3	26.0
Total emitted dose (µg)		**3338.8(198.9)**	**3566.7(151.5)**	**3648.6(119.1)**	**3318.5(363.7)**
Total % of nominal dose		**66.8(4.0)**	**71.1(3.4)**	**72.6(2.6)**	**67.6(7.8)**
FPD (µg)		**1918.7 (151.0)**	**1716.0(109.7)**	**2572.7(123.2)**	**1758.5(143.2)**
FPF % of nominal dose		**38.4(3.0)**	**34.5(2.5)**	**51.1(2.7)**	**35.6(3.1)**
FPF(% of the emitted dose)		**57.5(2.7)**	**48.1 (1.5)**	**70.5 (3.4)**	**53.0 (1.9)**
MMAD (µm)		**4.1(0.3)**	**5.0(0.1)**	**2.0(0.3)**	**4.4(0.2)**
GSD		**2.5(0.1)**	**2.2(0.03)**	**4.0(0.4)**	**2.1(0.04)**

Table 6.2 A summary of the data obtained from the NGI for the terbutaline sulphate Respules (5 mg nominal dose) nebulised using Sidestream jet nebuliser. Mean values in µg are quoted except those bold which are mean (SD).

	Stage Cut-off	**Amount in µg**			
		15 ROOM	15 COLD	30 ROOM	30 COLD
Remaining		3441.6	3282.1	3477.0	3433.6
Throat		68.7	34.2	26.9	48.9
1	14.1	61.9	73.3	52.8	112.2
2	8.61	102.8	194.0	77.8	334.3
3	5.39	160.3	304.6	59.1	330.5
4	3.3	208.8	358.7	290.4	406.2
5	2.08	290.6	285.2	451.6	299.3
6	1.36	261.6	157.6	288.2	140.9
7	0.98	156.9	80.2	153.9	73.9
MOC	0	123.3	43.1	113.7	50.9
Total emitted dose (µg)		**1434.9(155.1)**	**1530.8(255.9)**	**1514.4(302.3)**	**1797.1(103.8)**
Total % of nominal dose		**28.0(3.1)**	**30.3(5.9)**	**29.8(6.9)**	**36.8(1.0)**
FPD (µg)		**1009.4(74.7)**	**877.7(102.3)**	**1329.4(.4)**	**1138.9(80.0)**
FPF % of nominal dose		**20.0(1.7)**	**17.1(2.0)**	**25.8(4.9)**	**23.4(0.8)**
FPF(% of the emitted dose)		**70.3 (5.7)**	**57.3 (4.6)**	**87.8 (5.2)**	**63.4 (1.1)**
MMAD (µm)		**2.6(0.4)**	**4.2(0.4)**	**1.7(0.1)**	**3.5(0.1)**
GSD		**2.8(0.14)**	**2.2(0.05)**	**2.1(0.6)**	**2.3(0.1)**

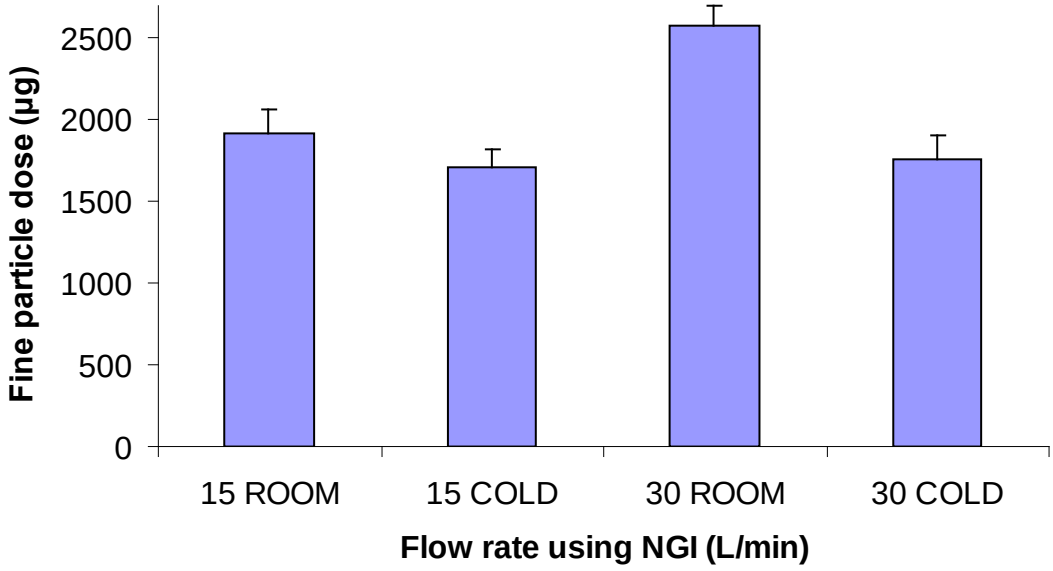


Figure 6.4 The mean (SD) fine particle dose of terbutaline sulphate, expressed in µg, determined using the NGI for Bricanyl Respules (5 mg nominal dose) nebulised using the Aeroneb Pro nebulisers.

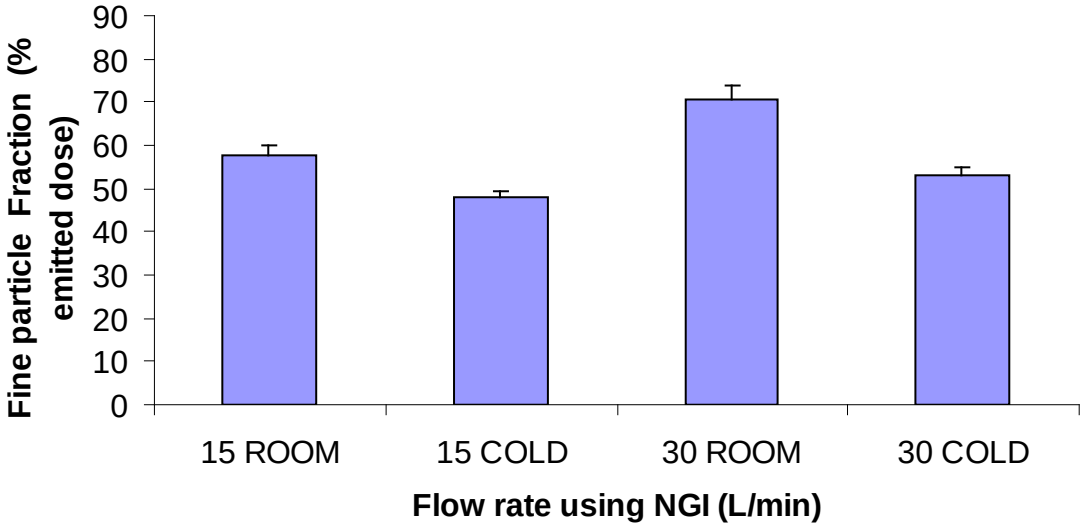


Figure 6.5 The mean (SD) fine particle fraction of terbutaline sulphate, expressed as percent of the emitted dose, determined using the NGI for Bricanyl Respules (5 mg nominal dose using the Aeroneb Pro nebulisers.

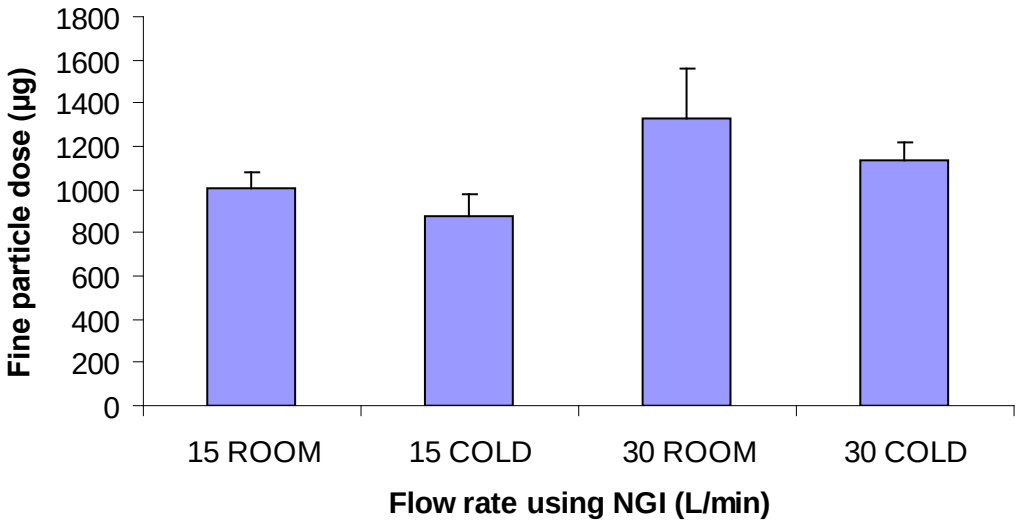


Figure 6.6 The mean (SD) fine particle dose of terbutaline sulphate, expressed in µg, determined using NGI for Bricanyl Respules (5 mg nominal dose) nebulised using the Sidestream jet nebulisers.

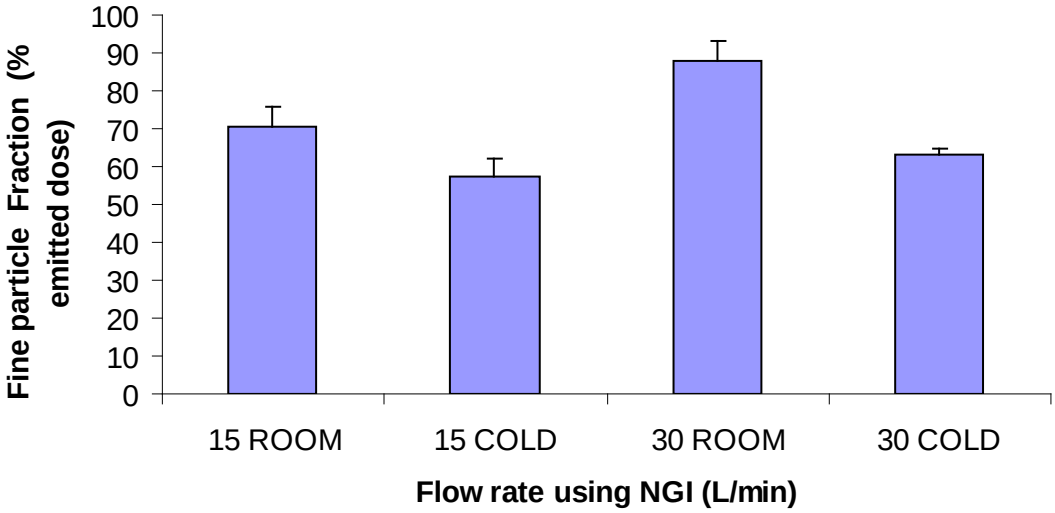


Figure 6.7 The mean (SD) fine particle fraction of terbutaline sulphate, expressed as percent of the emitted dose, determined using the NGI for Bricanyl Respules (5 mg nominal dose using the Sidestream jet nebulisers.

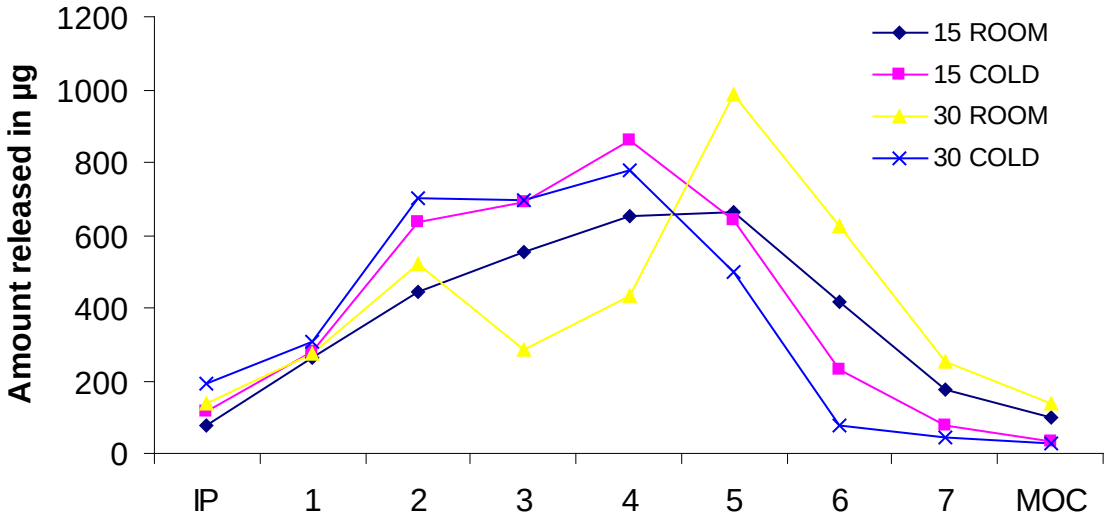


Figure 6.8 The mean (n=5) amount of terbutaline sulphate captured on each stage of the NGI following the nebulisation of 5mg in 2ml by the Aeroneb Pro nebuliser using different NGI operating conditions.

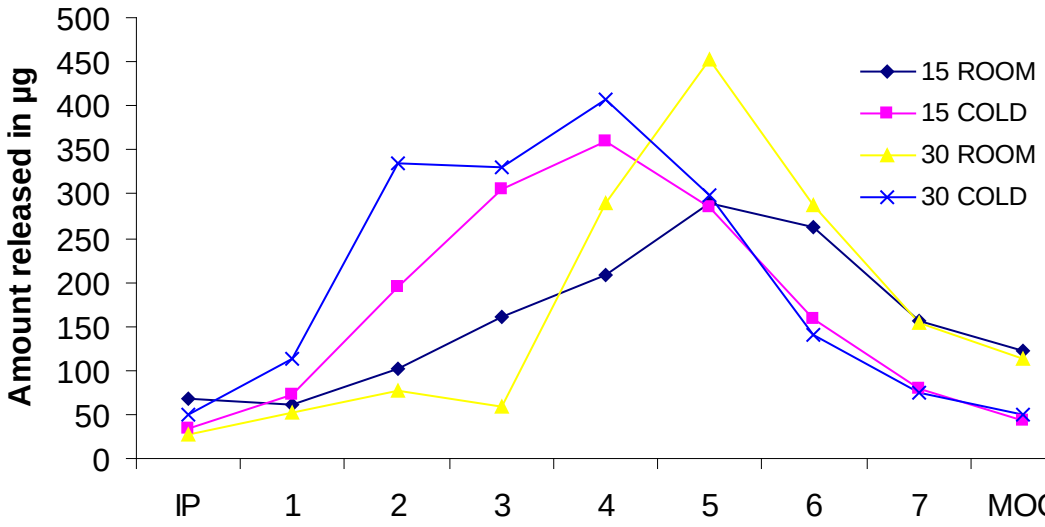


Figure 6.9 The mean (n=5) amount of terbutaline sulphate captured on each stage of the NGI following the nebulisation of 5mg in 2ml by the Sidestream jet nebuliser using different NGI operating conditions.

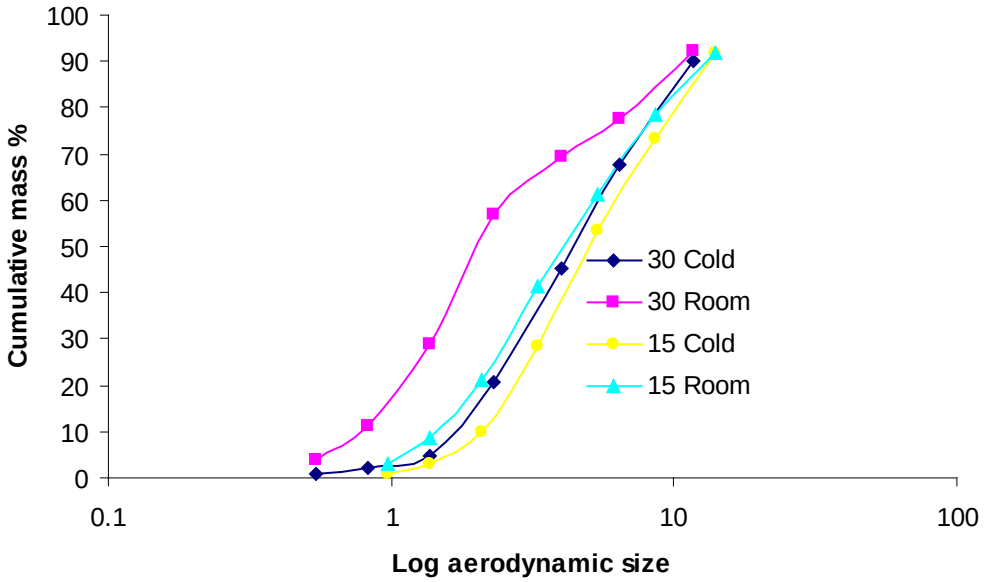


Figure 6.10 The mean (n=5) aerodynamic distribution of the dose nebulised from the Aeroneb Pro using the different NGI operating conditions.

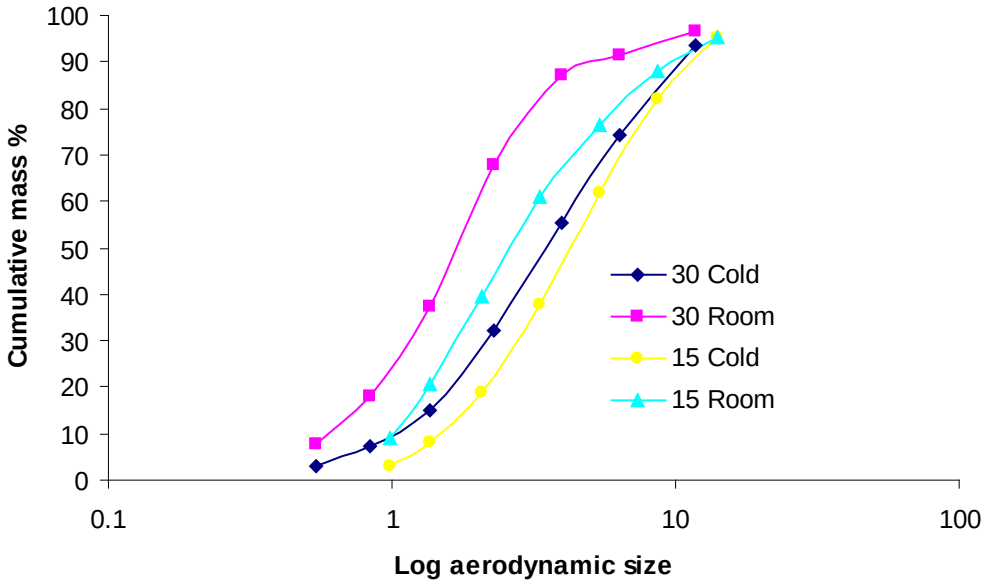


Figure 6.11 The mean (n=5) aerodynamic distribution of the dose nebulised from the Sidestream using the different NGI operating conditions.

The mean difference (95% confidence interval) for FPD between the Aeroneb Pro and the Sidestream jet nebuliser was 162.3 (637.8, 1040.8) µg for the 15COLD methodology.

6.3.2 CEN (prEN13544-1) methodology

6.3.2.1 Aerosol output according to (prEN13544-1) –CEN

The individual doses and the mean (SD) total aerosol output using the CEN methodology for the Aeroneb Pro are presented in Tables 6.3 and Figure 6.12 whereas Table 6.4 and Figure 6.13 present the data for the Sidestream jet nebuliser. The data of the CEN methodology for the Sidestream jet nebuliser with the addition of 2 ml saline is presented in Table 6.5 and Figure 6.14. The mean (SD) emitted dose of terbutaline sulphate expressed as a percent of the nominal dose from the nebulisers is presented in Table 6.6 and Figure 6.15. The data labeled 'total' in the previously mentioned Tables and Figure is the total amount deposited on the T-piece, the inhalation filter and the exhalation filter.

Table 6.3 Terbutaline sulphate emitted dose (µg) for the 2 ml Bricanyl Respules (5 mg nominal dose of Terbutaline sulphate in 2 ml) nebulised using the Aeroneb Pro nebuliser.

	Amount in µg				
Dose	**Inhalation filter**	**Exhalation Filter**	**T-piece**	**Nebuliser**	**Total**
1	969.8	1389.5	949.3	870.5	3308.6
2	849.9	1078.8	1284.5	638.4	3213.1
3	861.5	1158.4	1226.4	976.7	3246.2
4	836.2	1241.3	706.7	1068.4	2784.2
5	876.2	1211.8	851.7	822.3	2939.6
6	907.2	958.0	1048.3	773.6	2913.5
7	938.5	1232.2	937.0	1037.1	3107.6
8	986.1	1306.9	1016.8	841.8	3309.8
9	1079.1	1379.1	936.1	911.6	3394.3
10	1189.8	1257.3	1172.7	678.7	3619.8
Mean	**949.4**	**1221.3**	**1012.9**	**861.9**	**3183.7**
STD	**112.6**	**131.7**	**176.9**	**142.3**	**251.4**
RSD	**11.9**	**10.8**	**17.5**	**16.5**	**7.9**

Table 6.4 Terbutaline sulphate emitted dose (µg) for the 2 ml Bricanyl Respules (5 mg nominal dose of Terbutaline sulphate in 2 ml) nebulised using the Sidestream jet nebuliser.

	Amount in µg				
Dose	**Inhalation filter**	**Exhalation Filter**	**T-piece**	**Nebuliser**	**Total**
1	384.8	291.0	59.9	2982.6	735.7
2	206.0	167.2	54.4	3250.6	427.6
3	439.6	149.6	69.7	1994.4	658.9
4	196.3	127.1	48.8	3175.8	372.1
5	192.6	262.2	49.2	3031.9	504.0
6	347.3	324.4	59.1	2670.4	730.8
7	320.3	277.6	50.9	2386.0	648.8
8	230.6	200.5	58.0	3601.8	489.1
9	350.4	224.7	55.2	3573.5	630.3
10	391.7	240.4	61.8	3506.9	693.9
Mean	**306.0**	**219.3**	**56.3**	**3012.2**	**589.1**
STD	**92.9**	**64.4**	**6.7**	**563.0**	**130.4**
RSD	**30.4**	**29.6**	**11.5**	**18.7**	**22.14**

Table 6.5 Terbutaline sulphate emitted dose (µg) for the 2 ml Bricanyl Respules (5 mg nominal dose of Terbutaline sulphate in 2 ml) nebulised using the Sidestream jet nebuliser with the addition of 2 ml saline.

	Amount in µg				
Dose	**Inhalation filter**	**Exhalation Filter**	**T-piece**	**Nebuliser**	**Total**
1	781.2	523.1	61.9	2692.8	1366.2
2	606.0	473.1	78.7	2726.8	1157.8
3	515.9	449.1	69.0	2724.7	1034.1
4	626.9	536.9	60.7	2372.4	1224.5
5	565.4	376.9	58.9	2935.5	1001.3
6	517.8	709.7	75.6	2862.7	1303.1
7	622.1	562.0	72.6	1972.4	1256.7
8	600.9	570.4	70.1	2887.9	1241.4
9	587.3	547.8	51.1	2864.1	1186.2
10	555.3	659.6	63.0	2702.6	1277.8
Mean	**597.9**	**540.9**	**66.2**	**2674.2**	**1204.9**
STD	**75.4**	**96.7**	**8.5**	**293.6**	**114.8**
RSD	**12.6**	**17.9**	**12.9**	**11.0**	**9.5**

Table 6.6 The mean (SD) amount of terbutaline sulphate deposited on each part expressed as a percent of the nominal dose from different nebuliser (n=10 separate doses).

	Inhalation filter	Exhalation Filter	T-piece	Nebuliser	Total
▪ Aeroneb Pro	19.0(2.3)	24.4(2.6)	20.3(3.5)	17.2(2.8)	63.7(5.0)
▪ Sidestream jet nebuliser	6.1(1.8)	4.4(1.3)	1.1(0.1)	60.4(10.6)	11.8(2.6)
▪ Sidestream jet nebuliser with 2 ml saline	12.0(1.5)	10.8(1.9)	1.3(0.2)	53.5(5.9)	24.1(2.3)

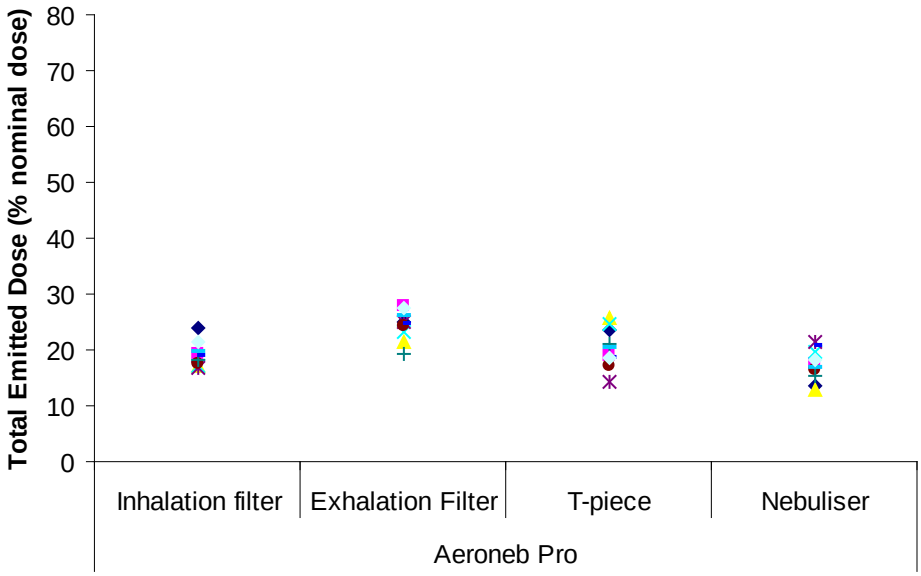


Figure 6.12 Terbutaline sulphate emitted dose, expressed as a percent of the nominal dose, when 2 ml Bricanyl respiratory solutions were nebulised using the Aeroneb Pro nebuliser (n=10 separate doses).

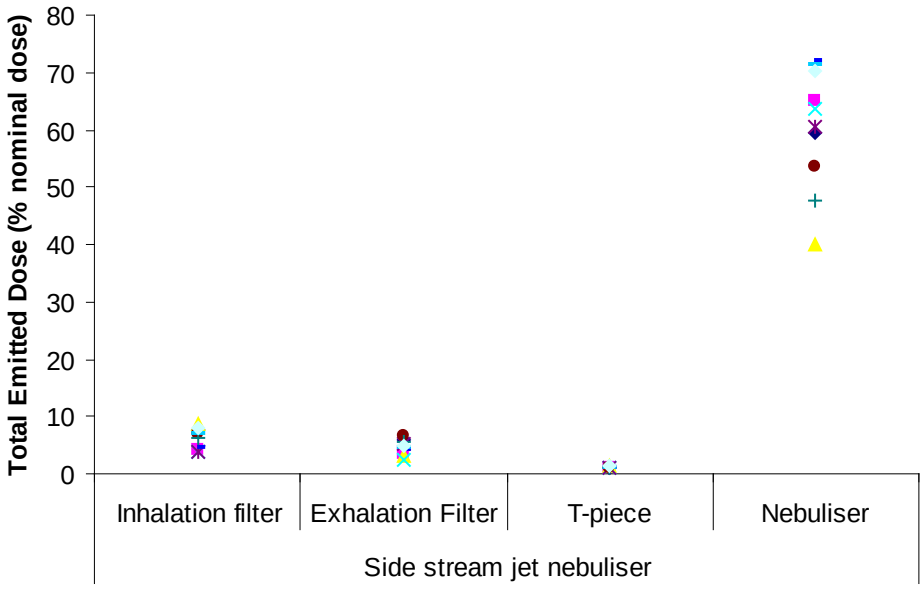


Figure 6.13 Terbutaline sulphate emitted dose, expressed as a percent of the nominal dose, when 2 ml Bricanyl respiratory solutions were nebulised using the Sidestream jet nebuliser (n=10 separate doses).

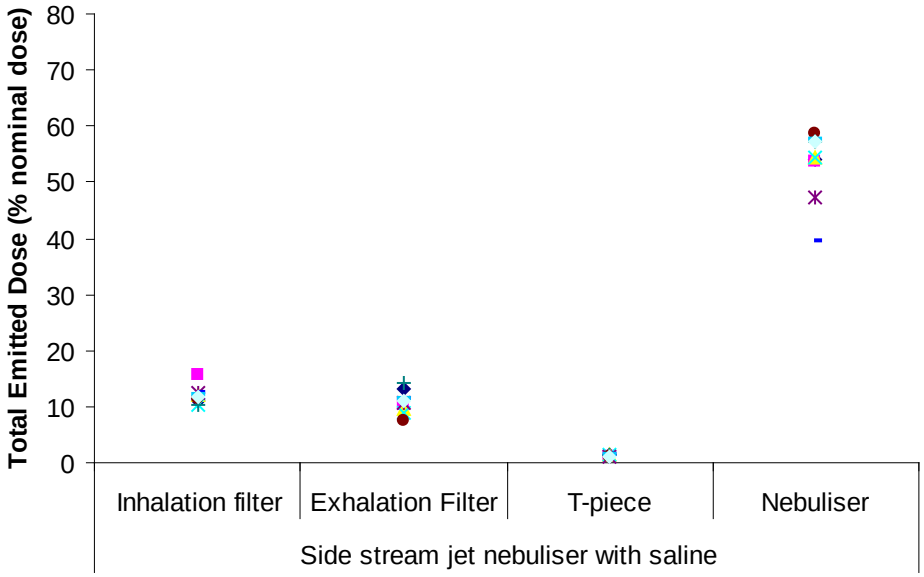


Figure 6.14 Terbutaline sulphate emitted dose, expressed as of the nominal dose, when 2 ml Bricanyl respiratory solutions with the addition of 2 ml saline were nebulised using the Sidestream jet nebuliser (n=10 separate doses).

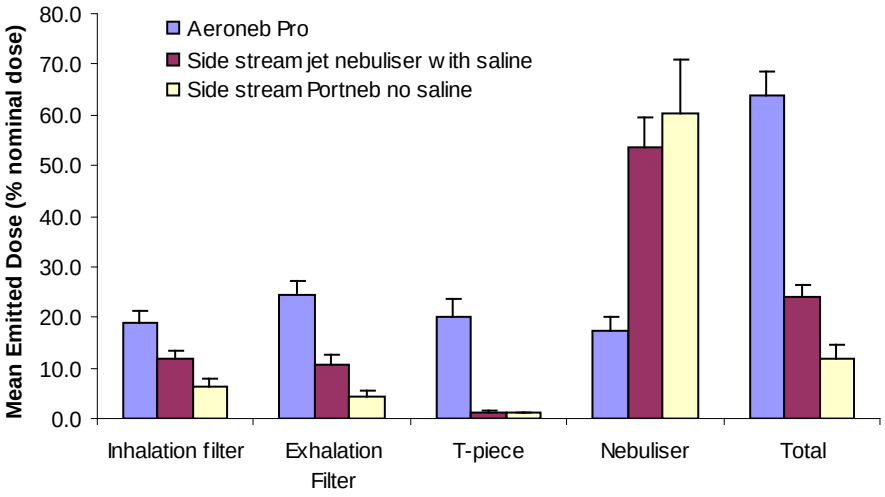


Figure 6.15 The mean emitted dose of terbutaline sulphate expressed as a percent of the nominal dose from different nebulisers (n=10 separate doses).

The overall mean (SD) total output, the amount deposited on the inhalation filter, from the Aeroneb Pro and the Sidestream nebuliser with and without the addition of 2ml saline (n = 10 doses), were 949.4(112.6), 597.9(75.4) and 306.0(92.9) μg respectively. The amount emitted from the Aeroneb Pro is significantly higher ($p < 0.001$) than from the amount emitted from the Sidestream jet nebuliser. The mean difference (95% confidence interval) for the amount emitted between Aeroneb Pro and Sidestream jet nebuliser was 351.6 (255.1, 448.0).

6.3.2.2 Aerodynamic characterization of the respirable dose according to (prEN13544-1) –CEN

A summary of the data obtained from the Marple Series 298 low flow cascade impactor using the Aeroneb Pro and the Sidestream jet nebuliser is shown in Table 6.7 and Figures 6.16 - 6.18.

Table 6.7 A summary of the data obtained from the CEN method for the 2 ml Bricanyl Respules (5 mg nominal dose of Terbutaline sulphate in 2 ml) nebulised using the two different nebuliser. Mean values in µg are quoted except those bold which are mean (SD).

		Amount of Terbutaline sulphate in µg	
		Aeroneb Pro	**Sidestream jet nebuliser**
Inhalation filter	**Stage Cut-off**	186.9	954.0
Exhalation filter		0.3	0.3
T-piece		150.8	37.7
Top filter	>50	0.5	1.4
1	21.3	0.7	1.4
2	14.8	1.1	2.6
3	9.8	4.6	12.7
4	6	4.6	18.0
5	3.5	8.4	22.9
6	1.55	26.1	37.9
7	0.93	4.5	13.9
8	0.52	3.1	10.7
Filter	0.25	0.6	3.0
Total amount emitted in the impactor(µg)		**54.2(1.6)**	**124.4(9.3)**
FPD(µg)		**39.4(9.1)**	**79.2(6.8)**
FPF% of amount emitted in the impactor		**72.5(15.7)**	**63.6(3.6)**
MMAD(µm)		**3.0(0.5)**	**3.2(0.3)**
GSD		**2.3(0.5)**	**2.9(0.4)**

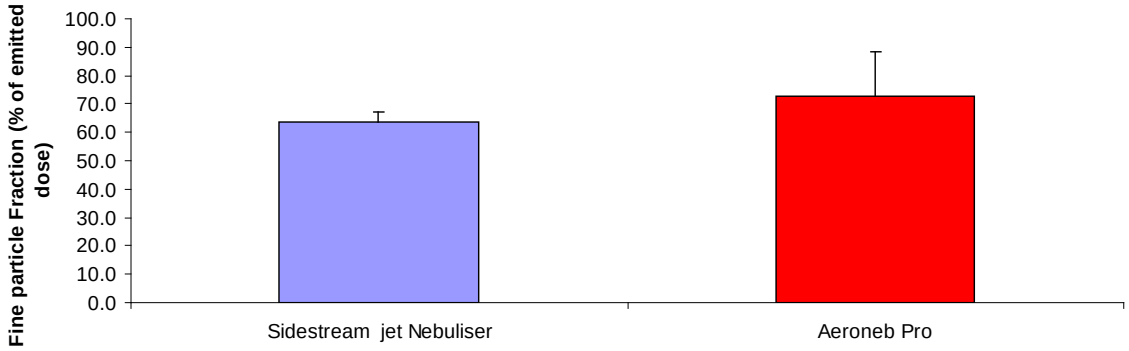


Figure 6.16 The mean (SD) fine particle fraction of terbutaline sulphate, expressed as percent of the emitted dose captured in the cascade impactor, using the CEN method for the Bricanyl Respules (5 mg in 2ml of terbutaline sulphate as nominal dose).

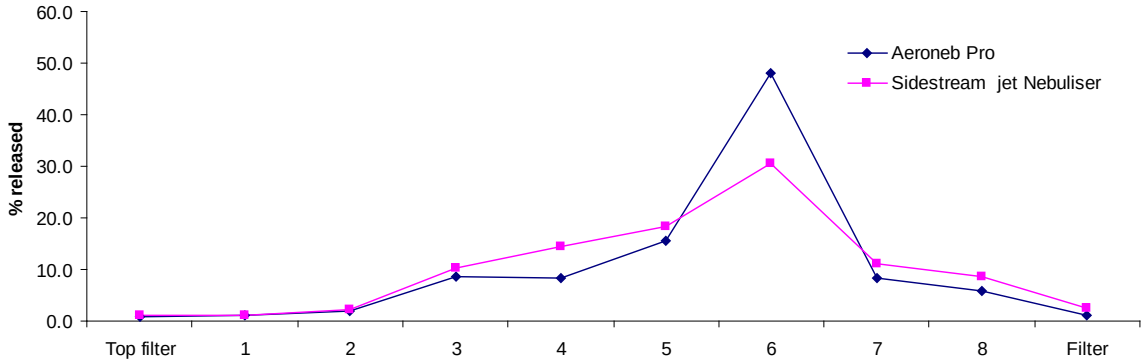


Figure 6.17 Terbutaline sulphate (µg) deposited on each stage of the cascade impactor using the CEN method for the Bricanyl Respules (5 mg in 2ml of terbutaline sulphate as nominal dose).

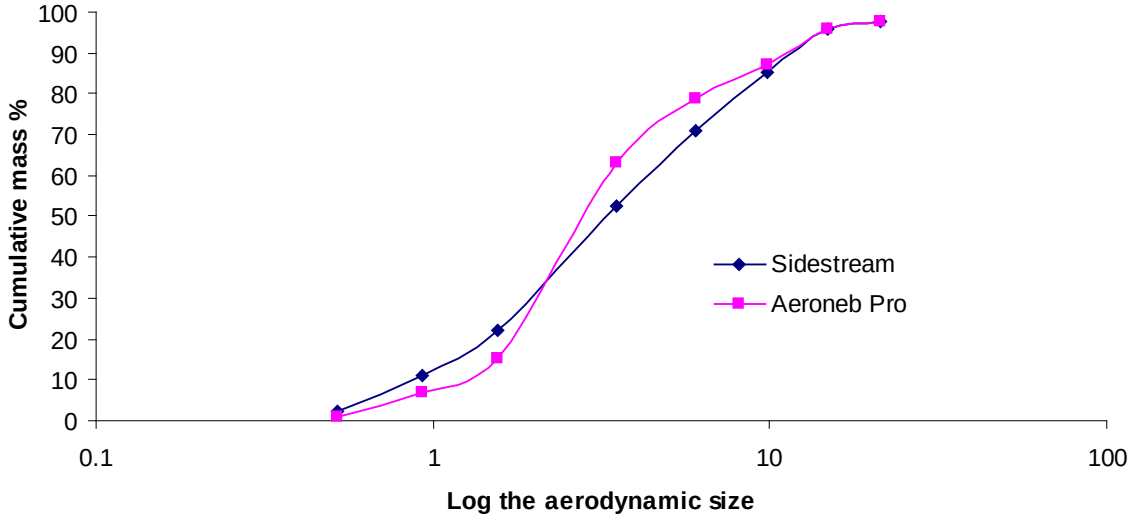


Figure 6.18 The mean (n=5) aerodynamic distribution of the dose nebulised from the Aeroneb Pro and the Sidestream measured using the Marple 298X cascade Impactor (CEN method).

The aerodynamic particle size characterization of the emitted dose from the Sidestream jet nebuliser and Aeroneb Pro nebuliser (n=10 doses) revealed a mean fine particle fraction (FPF) of 63.6 (3.6) and 72.5 (15.7) % of the emitted dose respectively captured in the cascade impactor and MMADs of 3.0 (0.5) and 3.2 (0.3) µm, respectively. The majority of

the emitted dose is deposited on the inhalation filter as shown in Table 6.7. The FPF and the MMAD of the Aeroneb Pro and the Sidestream jet nebuliser were similars.

The data in Table 6.7 highlight that the mean (SD) amount of terbutaline sulphate captured in the Marple 298X cascade impactor was 54.2 (1.6) µg from the Aeroneb pro and 124.4 (9.3) µg from the Sidestream nebuliser. In addition to the total amount deposited in the cascade impactor a further 150.8 (111.8) µg was recovered from the T-mouthpiece and 186.9 (56.5) µg on the filter fitted to the vacuum pump when using the Aeroneb Pro. For the Sidestream these amounts were 37.7 (8.0) and 945.0 (141.3) µg, respectively. This leads to a total 392.0 and 1116.4µg emitted from the Aeroneb Pro and the Sidestream nebulisers respectively. The amounts sampled in the Marple therefore represent 13.8 and 11.2% of the amount emitted from the Aeroneb Pro and the Sidestream nebuliser and are much lesser than the amounts sampled in the total aerosol output using the CEN methodology. From the prievous section (6.3.2.2) it was found that the overall mean (SD) total output from the Aeroneb Pro and the Sidestream nebuliser systems captured on the inhalation filter were 949.4 (112.6) and 306.0 (92.9) µg respectively. Hence the amount captured in the Marple Cascade Impactor represents only 5.7% and 40.6% of the dose available to be inhaled (also equivalent to 1.1 and 2.5% of the nominal dose).

6.3.2.3 Statistical analysis

A summary of the statistical comparison between the Aeroneb Pro and the Sidestream using the NGI and the CEN methodology is shown in table 6.8. It shows that the Aeroneb Pro resulted in a significantly higher emitted dose, FPD ($p<0.001$) and MMAD ($p<0.01$) and a lower FPF ($p<0.01$) using NGI methodology. When using the CEN method there was no significant difference.

A summary of the statistical comparison between 15COLD (as it is the benchmark condition) and the other operating conditions to highlight the very large differences is presented in Table 6.9. Statistical analysis revealed that there was a highly significant

difference ($p<0.001$) between the FPF and the MMAD for each nebuliser system with respect to the 4 operating conditions of the NGI and that of the CEN method. Similar statistical analysis of the GSD data between the operating conditions (including the CEN method) also revealed highly significant differences, ($p<0.001$) for the Aeroneb Pro and ($p=0.002$) for the Sidestream.

Table 6.8 Mean difference (95% confidence interval) for Aeroneb Pro *vs* Sidestream.

Comparator	**Aeroneb Pro *vs* Sidestream**
Input output	328.7 (230.8, 426.6; p<0.001)***
NGI 15 COLD	
FPD	839.3 (637.8, 1040.7; p<0.001)***
FPF	-9.7 (-15.6, -3.8; p=0.01)**
MMAD	0.8 (0.4, 1.3; p=0.008)**
GSD	0.2 (-0.04, 0.07; p=0.445)
CEN	
FPF	7.8 (-5.6, 21.4; p=0.181)
MMAD	-0.2 (-0.8, 0.4; p=0.408)
GSD	-0.5 (-1.1, 0.1; p=0.072)

* p<0.05, ** <0.01, *** <0.001, otherwise N/S

Table 6.9 Mean difference (95% confidence interval) for 15COLD compared to the other operating conditions

Comparator	Aeroneb Pro		Sidestream	
	FPF (%)	MMAD (μm)	FPF (%)	MMAD (μm)
15ROOM	-9.4 (-13.7, -5.0)**	1.0 (0.5, 1.4)**	-13.0 (-18.3, -7.7)***	1.6 (1.1, 2.1)***
30COLD	-5.0 (-9.4, -0.7)*	0.6 (0.2, 1.1)**	-5.6 (-10.8, -0.3)*	0.7 (0.2, 1.3)*
30ROOM	-22.4 (-26.8, -18.1)***	3.0 (2.5, 3.4)***	-30.5 (-35.7, -25.2)***	2.5 (2.0, 3.0)***
CEN	-24.4 (-32.1, -16.8)***	2.0 (1.4, 2.6)***	-6.9 (-12.2, -1.5)***	1.1 (0.7, 1.5)***

* $p<0.05$, ** <0.01, *** <0.001, otherwise no significance difference

6.4 Discussion

The results obtained using the NGI provide further evidence of the evaporation effects shown by Berg et al for a jet nebuliser (Berg et al., 2007b) and their finding that the NGI should be cooled prior to use with nebulisers using a low flow of 15 L min^{-1}. The data presented for a flow of 30 L min^{-1} adds further evidence of evaporation and shows that at this flow even cooling does not prevent this effect. Although a slight modification has been made to the method of Berg et al (2007) in that the exit of the mouthpiece was a tight seal with the NGI throat using a standard rubber adapter the MMAD values for the two nebuliser systems are in line with those previously reported (Berg et al., 2007b; Berg et al., 2008).

Two different nebuliser systems were studied. Although the relative humidity of their aerosolised doses would be expected to be similar, the temperature would be different. The aerosolised dose emitted from the Sidestream (jet nebuliser) would be about 10^{o}C cooler than ambient room temperature whilst that from the Aeroneb Pro will be similar to room temperature (Fink et al., 2001; Zhou et al., 2005). The magnitude of the different between 15ROOM and 15 COLD NGI operating conditions, summarised in Table 6.9, for the two systems were comparable with those for the Sidestream being slightly bigger. This would be due to the lower temperature of the aerosol emitted from the Sidestream compared to ambient room conditions (Berg et al., 2007b; Berg et al., 2008). Hence evaporation of the aerosol solvent due to thermal transfer from the NGI (when operated at room temperature) will be greater with Sidestream. These evaporation effects in the NGI are comparable to those previously reported for the Andersen Cascade Impactor for nebulised salbutamol and budesonide (Finlay and Stapleton, 1999; Zhou et al., 2005).

At higher flows more relatively warm and dry room air is entrained in the equipment used and this will cause the particles to evaporate and shrink. Evaporation effects will be offset when cooling the NGI because the lower temperatures will raise the relative humidity and

decrease the evaporation. Using lower flows and limiting the distance between the nebuliser output and the NGI would minimise evaporation effects. Therefore, the distance between the nebuliser output and NGI here was limited and a tight seal (akin to the mouth sealed round the mouthpiece) was used. Hence this mimicked the situation during normal patient use. Inhalation flows lower than 15 L min^{-1} were not used because the NGI has not been calibrated for flows below this value. The Marple 298X Cascade Impactor samples air at 2 L min^{-1} and thus, theoretically could minimise evaporation effects. For this method a constant flow of 15 L min^{-1} is drawn across the output of the nebuliser. Comparing the CEN results to the equivalent operating conditions of 15ROOM with the NGI suggest greater evaporation effects occur with the CEN method. Thus lowering the vacuum flow through the Marple 298X cascade impactor did not have any effect on minimising evaporation.

The different results (very small MMAD and high FPF) using the CEN methodology warrant further investigation.

Two abstract studies have previously compared the NGI and CEN methods. One concluded encouraging equivalence between the NGI and the Marple 298X impactor for the nebuliser system (Pieron et al., 2007b) when nebulising a commercially available budesonide suspension. The other indicated that the two impactors have poor equivalence when measuring the droplet size distributions from the same nebuliser system (Pieron et al., 2007a). For the later study they used the Omron VVT nebuliser and NE C30 compressor with 2ml 2.5% w/v sodium fluoride and they placed the NGI in a fridge at 5^{o}C for only 1 hour prior to sizing. The first study used budesonide suspension rather than a clear solution like that of all bronchodilator respiratory solutions. The equivalence between the CEN and the NGI methods in this study may be because the evaporation has less effect on the suspension than on the solution. Thus there was no significance difference between the NGI results and the CEN results. The results of the second study are consistent with the

data presented here in that the aerodynamic characteristics of the emitted dose from different nebuliser systems are not the same.

Pieron et al (2007) reported that the standard CEN methods embodied in EN13544-1:2001 to size nebulised aerosol using the Marple 298X impactor are highly robust, repeatable and easily adapt to sizing budesonide suspension. However the effect of evaporation is not addressed and, as described above, may be less for particles suspended rather than drug dissolved in the emitted droplets (Pieron et al., 2007c).

Although these in-vitro methods have been introduced to provide a comparison between different nebuliser systems the clinical meaning of these results needs to be investigated. It has been shown that lung deposition and physiologic response was greater when using a nebuliser that emits small (MMAD 3.3μm) compared to large (MMAD 7.7μm) particles (Johnson et al., 1989). Further evidence of a link between fine particle dose and relative lung deposition has been reported as well as the total emitted dose and relative systemic delivery (Silkstone et al., 2002). Doubts have recently been raised about the clinical meaning of the CEN results for nebulised solutions in that the reported relative lung bioavailability of nebulised salbutamol in patients with asthma and COPD was less than that for a metered dose inhaler attached to a spacer yet the aerodynamic characteristics of the nebulised method indicated more favourable lung deposition (Mazhar et al., 2008). In this clinical study the relative lung bioavailability for the two inhalation methods was similar but the fine particle dose was thirteen times greater for the nebuliser method. The FPF of 80.1% for the nebulised method suggest that the CEN method may be producing misleading information due to an evaporation effect. The effect of evaporation with respect to the CEN method requires investigation. Hence further method validation of the CEN method and also for the NGI method is required so that meaningful data can be obtained especially when the differences in the aerodynamic characteristics of the nebulised doses for the different operating conditions were huge. Correlation to the clinical effect (efficacy

and safety) would also be required to highlight what the in-vitro results mean and help in the recommendation of the most appropriate operating conditions.

No large scale robust attempt has been made to investigate in-vitro / in-vivo correlations. Although this is not easy it will help understand the clinical significance of different in-vitro results. Conditions that mimic those present in the airways were not used so in the future aerodynamic studies should investigate such conditions. Without a robust in-vitro in-vivo examination the proposed 'European Respiratory Society Guidelines on the use of nebulisers' (Boe et al., 2001) will remain a meaningless issue. The results here and that of others comparing many more nebulising systems (Smith et al., 1995) confirm that their in-vitro aerosolised dose properties are different. In clinical practice these differences become insignificant due to the use of very high doses that are above that required to reach the plateau of the dose response relationship (Smith et al., 1995). Although this provides the necessary clinical response the greater systemic availability should be considered during routine use in stable patients.

One drawback of the cooled NGI method is that realistically the nebulisation process has to be completed within 5 minutes. This limits the volume of solution to be nebulised. However studies have shown that the aerodynamic properties of an aerosolised dose from a nebuliser are not affected by the fill volume (Clay et al., 1983a; Hess et al., 1996; Silkstone et al., 2002) with the only difference being a larger emitted dose and hence fine particle dose as the fill volume increases (Clay et al., 1983b; Hess et al., 1996).

When using jet nebulisers due to the large residual volume (as shown in this chapter) in clinical practice the solution is diluted with an equal volume of saline nebuliser solution (Clay et al., 1983b; Clay and Clarke, 1987). To add 2ml saline nebuliser solution to the 2ml terbutaline sulphate solution in the Sidestream would have prolonged the nebuliser time to over 5 minutes (Silkstone et al., 2002). The residual volumes were much higher and the emitted dose was lower than if 2ml saline had been added to the terbutaline nebuliser

solution. Hence the solution routinely used in clinical practice with these nebulisers cannot be characterised by the NGI method unless a cooling system or jacket is included or do the test for part of the solution instead of the whole volume. This problem did not appear with the Aeroneb Pro because it is recommended that the nebuliser solution is not diluted and only 0.3 ml is left in the nebulisation chamber as a residual volume (as mentioned in the Aeroneb Pro Data Sheet).

The aerosol output results show that the amount of terbutaline sulphate deposited on the inhalation filter from the Aeroneb Pro is significantly higher then that from the Sidestream jet nebuliser ($p < 0.001$). This was as expected because as the results show the residual volume of Aeroneb Pro (Table 6.6) is much lower in accordance to previous results reported by Fink and Schmidt (2002). The Aeroneb Pro nebuliser system delivered greater than four times more drug than the other pneumatic small volume nebulisers when delivering 3 ml doses of salbutamol (Fink and Schmidt, 2002).

The inhalation laboratory results also show that dilution with 2ml approximately doubles the output from the Sidestream. However even with this increased emitted dose when using saline with the Sidestream, the Aeroneb Pro emitted dose was still almost double that of the Sidestream. The fine particle dose mirrored the amount emitted but whether the higher MMAD, for the Aeroneb Pro, would have any effect on influencing the amount and distribution in the lung is not known. However the higher FPD from Aeroneb Pro does suggest a higher total lung deposition. The relatively high amounts that were deposited onto the T-mouthpiece of the Aeroneb Pro could be due to the downward delivery of the emitted dose.

6.5 Conclusions

The NGI method is an easy, reliable and reproducible method for the determination of the aerodynamic particle size distribution but evaporation of the droplets from the nebulised dose of a solution is possible. Using two different nebulising systems it has been shown

that the use of cooling and operation at an inhalation flow of 15 L min^{-1} is very important in limiting the evaporation effect when using the NGI method based on Berg et al (2007). The 5 minutes set up time and 5 minute nebulisation time of the cooled system do provide constraints and thus adaptations to introduce a cooling jacket may be necessary to characterise the doses routine used in clinical practice. The CEN method needs a more through validation to highlight the optimal operating conditions, the evaporation effect and the interpretations of the results. Whether the sampling of the emitted dose also introduces errors needs to be addressed. The large differences in the FPF, MMAD and GSD for the different methodologies highlight that a through validation is required with correlation linking to clinical effects so that an optimised method can be recommended that produces quality control and clinically relevant data.

7.1 Introduction

Pulmonary drug delivery by inhalation is primarily used to treat conditions of the airway by delivering locally acting drugs to their site of action. This reduces the dose needed to produce a pharmacological effect in the lungs with minimal systemic effects (Dhand, 2000; Hillery et al., 2001; Kondili and Georgopoulos, 2002). Following inhalation, most of the dose is swallowed while less than 20% is deposited into the airways (Newman et al., 1981). The pulmonary absorbed fraction is responsible for the pharmacological effect while the swallowed part is responsible for systemic side effects (Davies, 1975).

The assessment of pulmonary drug deposition is becoming increasingly important (Mobley and Hochhaus, 2001). Lung deposition can be identified by gamma scintigraphy and pharmacokinetic methods. Gamma scintigraphy provides direct measurements of lung deposition and highlights the zones of the lungs where the deposition occurs. However the formulation has to be modified to incorporate a radio- isotope (Chrystyn, 2000; Chrystyn, 2001). The application of traditional pharmacokinetic methods to lung deposition studies is difficult, because the doses administered are small and together with the volume of distribution means systemic concentrations are low. Thus these concentrations are difficult to assay accurately and reproducibly (Rogers and Ganderton, 1995). Also the resulting systemic drug concentrations are due to the swallowed and inhaled fractions (Newman et al., 1981).

Pharmacokinetic methods to evaluate the relative lung bioavailability of inhaled drugs have used plasma or urine concentration of the drug in the absorption lag time phase of the orally swallowed portion. The amounts delivered to the systemic circulation that are cleared by the renal route during the lag phase will represent the drug absorbed from the lung after inhalation. The proposed sampling times for plasma are 5, 10, 20 minutes post inhalation (Anhoj et al., 1999; Lipworth and Clark, 1997b; Mobley and Hochhaus, 2001).

Urinary terbutaline excretion with the administration of oral charcoal to block oral absorption using 36 hours post dose collection periods (Borgstrom and Nilsson, 1990; Derom et al., 2001; Borgstrom et al., 2000) and the amount of salbutamol excreted in the urine in the first 30 minutes post inhalation (Hindle and Chrystyn, 1992) are as a urinary pharmacokinetic methods for inhaled drugs. The charcoal block method can identify total lung deposition but cannot be used with patients because it will block all the absorption of orally administered drugs. The urinary salbutamol pharmacokinetic method demonstrated that following oral administration of salbutamol, negligible amounts of salbutamol are excreted in the urine during the first 30 minutes post dose, and that significantly greater amounts ($p<0.001$) are excreted 30 minutes post inhalation. Thus, the urinary excretion of salbutamol during the 30 minutes post inhalation period is a representative of the amount delivered to the lungs. This study also demonstrated that urinary excretion of salbutamol over the 24 hours post dose period reflects the total systemic bioavailability. A dose response relationship and the reproducibility of the Hindle and Chrystyn method have been reported (Tomlinson et al., 2003). Another study has shown the method to be more sensitive to identify a difference between inhalation techniques than bronchoprovacation challenge testing using methacholine (Tomlinson et al., 2005). This urinary pharmacokinetic method has been used to compare different inhalation methods and products (Hindle et al., 1995; Hindle et al., 1994; Chege et al., 1996a; Chege and Chrystyn, 1996), to determine an optimal inhalation technique for MDIs (Hindle et al., 1993), to determine the effect of inhalation flow and formulation (Chege and Chrystyn, 2000; Chege et al., 1996b), when administration is prolonged (Silkstone et al., 2000), to provide useful information on the use of large volume spacers (Chege and Chrystyn, 1994), and nebulisers (Silkstone et al., 2002). This technique has been extended to identify the relative lung deposition of sodium cromoglycate (Aswania et al., 1997; Aswania and Chrystyn,

2002), nedocromil (Aswania et al., 1998), gentamicin (Al-Amoud et al., 2002), tobramycin (Barber, 2002) and formoterol (Nadarassan et al., 2007).

Similar to the principle of the urinary salbutamol pharmacokinetic method (Hindle and Chrystyn, 1992) the urinary excretion of terbutaline at 30 minutes following inhalation could be used to indicate the relative amount of drug reaching the respiratory tract and thus can be used to determine the relative bioavailability to the lungs following inhalation. Also the 24 hours renal excretion could be an index for the relative bioavailability of terbutaline to the body following an inhalation. A bioanalytical method to measure terbutaline from urine samples using a reversed phase ion-pair high performance liquid chromatography (HPLC) assay has been previously described in section 3.3 of this thesis. This analysis method will be used to determine the amount of terbutaline excreted in urine post administration.

7.2 Validation of relative bioavailability of terbutaline to the lung following inhalation using urinary excretion

7.2.1 Methods

The aim of this section was to identify if the urinary pharmacokinetic method developed for the relative lung bioavailability of salbutamol post inhalation can be applied to terbutaline post inhalation.

7.2.1.1 Equipment and inhalation devices

Inhaler device:	Bricanyl Metered Dose Inhaler labelled as a nominal dose of 250μg terbutaline sulphate per shot (AstraZeneca, UK)
Charcoal	Carbomix, Meadon Laborties Limited, UK
HPLC condition:	Previously described in section 3.3 in this thesis

7.2.1.2 Subjects

Ethical approval was obtained from the University of Bradford. Twelve healthy, non-smoking volunteers (six females) with a FEV_1>90% of predicted gave written informed

consent (University of Bradford Ethics Committee Approval obtained) to take part in this study. On separate occasions subjects received the following

(a) The oral administration of 500μg terbutaline sulphate in 20 ml water [O].

(b) The oral administration of 500μg terbutaline sulphate in 20 ml water with 20g activated charcoal (10g in 50 ml water before and after dosing) [OC].

(c) Two 250μg doses inhaled from a terbutaline sulphate metered dose inhaler (Bricanyl, AstraZeneca, UK) [I].

(d) Two 250μg doses inhaled from a terbutaline sulphate metered dose inhaler with 20g activated charcoal (10g in 50 ml water before and after dosing) [IC].

The use of charcoal in this dose has been proven to be enough to block the gastrointestinal absorption of the swallowed terbutaline sulphate (Borgstrom and Nilsson, 1990). All subjects were trained on how to use a MDI according to the patient information leaflet and the MDI was shaken and primed by firing two doses to waste before use (Barry and O'Callaghan, 2003). Subjects were trained to shake the MDI, remove the cap, exhale slowly as far as comfortable, put the MDI into their mouth and seal their lips round the mouthpiece. They were then instructed to start a slow inhalation through their mouth and activate the MDI immediately after the start of this slow inhalation. This slow inhalation continued until their lungs were full of air (total lung capacity). After inhalation they held their breath for 10 seconds and for the second dose this was repeated 30 seconds later (Hindle et al., 1993). Subjects voided their urine pre-dosing and provide urine samples at 0, 0.5, 1, 2, 4, 6, 12, and 24 hours post study dose. The volume of urine excreted was recorded and aliquots of each sample were frozen at -20° C prior to analysis. The order of the four study doses was randomized with a 7-day washout period between administrations. The HPLC method with solid phase extraction developed and validated for the assay of terbutaline from urine samples (previously described in section 3.3 in this thesis) was used to identify urinary terbutaline concentration.

7.2.2 Statistical analysis

The urine samples were compared using two way analysis of variance (ANOVA) using SPSS V15.0 (SPSS Inc., Chicago, USA). The mean differences with 95% confidence interval were calculated.

7.2.4 Results

Twelve healthy volunteers (six females), whose mean (SD) age, weight and height, were 29.1(4.6) years, 65.6(12.7) kg and 166.8(5.1) cm, respectively, with a FEV_1 of 96.1(3.7) % of predicted completed the study. Table 7.1 describe the demographic data of the subjects. No terbutaline was detected in all urine samples following oral dosing with concomitant administration of oral charcoal [OC]. Each individual's and the mean (SD) urinary excretion post I, IC and O dosing is shown in Tables 7.2-7.7. A summary of the mean (SD) amounts excreted during each collection period is shown in Table 7.8. Table 7.9 and Figure 7.2 describe the cumulative urinary excretion of terbutaline post study dosing. The mean (SD) urinary terbutaline excretion rates post study doses are shown in Table 7.10 and Figure 7.1. These show that negligible amounts of terbutaline were excreted in the urine following oral administration during the first 0.5 hour urine collection period. There was no terbutaline detected from the urine samples of five subjects during the first 30 minutes post oral dosing. A mean (SD) of 0.2 (0.2) µg and 167.7 (68.1) µg of the oral dose was excreted, in the urine, during the 0.5 and 24 hours post dose collection periods, respectively. Following the inhaled dose, the terbutaline renal excretion during the 0.5 and 24 hours post dose collection periods were 7.4 (2.2) µg and 230.0 (86.2) µg excreted, respectively. The terbutaline excreted at the 0.5 and 24 hours collection periods following IC dosing were 6.5 (2.1) µg and 65.8 (32.6) µg, respectively.

Table 7.1 Demographic data of the patients that participated in the study

Patient code	Gender	Age	Height	Weight	FEV_1
1	Female	25.0	161.0	50.0	99.0
2	Male	25.0	168.0	68.0	95.0
3	Male	31.0	163.0	95.0	98.0
4	Male	36.0	163.0	65.0	90.0
5	Male	32.0	170.0	80.0	99.0
6	Female	32.0	165.0	54.0	98.0
7	Female	36.0	175.0	57.0	97.0
8	Female	24.0	172.0	62.0	92.0
9	Female	24.0	160.0	54.0	99.0
10	Female	25.0	162.0	60.0	89.0
11	Male	32.0	169.0	70.0	99.0
12	Male	27.0	173.0	72.0	98.0
Mean		**29.1**	**166.8**	**65.6**	**96.1**
SD		**4.6**	**5.1**	**12.7**	**3.7**

Table 7.2 Individual and mean (SD) urinary terbutaline excretion rates post inhaled 500µg terbutaline sulphate dosing via a MDI (I) expressed in µg/hour, (n=12).

Mid Point	1	2	3	4	5	6	7	8	9	10	11	12	Mean	SD
0.25	14.7	19.3	7.0	14.7	18.0	21.0	11.9	14.8	8.3	15.3	19.5	12.5	**14.7**	**4.3**
0.75	18.6	46.0	6.1	5.4	16.9	12.4	14.9	3.8	0.7	46.0	11.6	14.7	**16.4**	**14.9**
1.5	37.9	45.2	17.8	0.0	41.4	18.8	47.2	0.4	19.0	44.7	18.7	49.9	**28.4**	**18.1**
3	22.4	41.5	28.2	14.0	24.5	57.8	15.4	13.8	28.9	41.3	50.3	14.1	**29.3**	**15.1**
5	23.9	31.3	22.4	11.9	24.8	26.3	19.1	10.5	17.9	34.6	32.2	23.8	**23.2**	**7.5**
9	5.2	13.0	5.4	7.7	4.0	13.2	1.6	7.4	5.0	13.7	11.9	3.6	**7.6**	**4.2**
18	9.2	0.2	1.5	0.9	9.5	4.5	0.0	0.7	1.3	0.4	3.9	3.8	**3.0**	**3.3**

Table 7.3 Individual and mean (SD) urinary terbutaline excretion rates post inhaled with charcoal 500µg terbutaline sulphate dosing via a MDI (IC) expressed in µg/hour, (n=12).

Mid Point	1	2	3	4	5	6	7	8	9	10	11	12	Mean	SD
0.25	8.3	6.5	10.2	9.6	9.3	18.3	14.6	12.8	14.5	14.3	20.4	16.6	**13.0**	**4.3**
0.75	4.7	0.5	0.0	1.5	4.1	1.4	1.1	1.0	1.1	1.1	1.6	1.3	**1.6**	**1.4**
1.5	0.9	1.2	0.6	8.7	0.5	0.0	0.0	2.5	0.0	2.9	0.0	0.0	**1.4**	**2.5**
3	4.5	9.5	11.7	2.5	0.0	0.0	1.7	1.7	12.1	9.2	0.0	1.2	**4.5**	**4.7**
5	4.7	8.0	0.0	0.5	3.1	6.4	4.4	10.2	0.0	10.7	1.2	9.3	**4.9**	**4.0**
9	0.4	5.6	1.7	0.9	0.9	4.4	4.1	5.2	1.8	5.6	5.1	4.5	**3.4**	**2.0**
18	0.8	0.0	0.0	1.9	0.1	2.2	1.3	3.2	0.0	0.0	7.9	0.8	**1.5**	**2.3**

Table 7.4 Individual and mean (SD) urinary terbutaline excretion rates post oral ingestion 500µg terbutaline sulphate dosing (O) expressed in µg/hour, (n=12).

Mid Point	1	2	3	4	5	6	7	8	9	10	11	12	Mean	SD
0.25	0.0	0.9	0.4	0.6	0.0	0.0	0.6	0.5	0.4	0.0	0.0	0.7	**0.3**	**0.3**
0.75	4.4	9.7	7.0	11.1	5.1	15.1	13.0	10.3	7.0	9.6	15.1	30.2	**11.5**	**6.9**
1.5	9.5	7.7	37.8	10.2	12.7	45.7	14.5	10.8	18.6	7.1	43.8	14.3	**19.4**	**14.4**
3	24.0	20.6	13.4	14.5	30.9	18.6	34.3	13.4	7.1	20.6	16.3	40.2	**21.2**	**9.7**
5	30.3	13.1	15.1	3.6	38.3	25.7	15.1	5.1	8.1	12.4	20.1	13.6	**16.7**	**10.3**
9	8.2	3.6	4.3	1.7	10.7	14.6	6.5	2.4	2.2	2.5	15.5	6.9	**6.6**	**4.8**
18	1.0	0.0	2.6	4.5	1.6	4.2	0.5	5.5	1.4	0.0	5.1	0.7	**2.3**	**2.0**

Table 7.5 Cumulative urinary excretion of terbutaline post inhaled 500µg terbutaline sulphate dosing via a MDI (I) expressed in µg, (n=12).

Time	1	2	3	4	5	6	7	8	9	10	11	12	mean	SD
0.5	7.4	9.6	3.5	7.3	9.0	10.5	6.0	7.4	4.1	7.6	9.8	6.2	**7.4**	**2.2**
1	16.6	32.6	6.5	10.0	17.5	16.7	13.4	9.3	4.5	30.6	15.5	13.6	**15.6**	**8.6**
2	54.5	77.8	24.3	10.0	58.8	35.5	60.7	9.8	23.5	75.3	34.2	63.5	**44.0**	**24.1**
4	99.4	160.8	80.7	38.1	107.8	151.1	91.4	37.4	81.3	157.9	134.8	91.6	**102.7**	**42.1**
6	147.1	223.4	125.4	61.8	157.3	203.7	129.6	58.3	117.1	227.1	199.2	139.3	**149.1**	**56.5**
12	178.3	301.3	157.7	108.3	181.1	282.6	139.1	102.5	147.4	309.3	270.3	160.9	**194.9**	**75.2**
24	288.4	304.2	176.0	119.2	295.1	336.7	139.1	111.0	163.4	313.9	316.9	206.3	**230.8**	**86.2**

Table 7.6 Cumulative urinary excretion of terbutaline post inhaled with charcoal 500µg terbutaline sulphate dosing via a MDI (IC) expressed in µg, (n=12).

Time	1	2	3	4	5	6	7	8	9	10	11	12	mean	SD
0.5	4.2	3.2	5.1	4.8	4.7	9.2	7.3	6.4	7.3	7.2	10.2	8.3	**6.5**	**2.1**
1	6.5	3.5	5.1	5.6	6.7	9.9	7.9	6.9	7.8	7.7	11.0	9.0	**7.3**	**2.1**
2	7.4	4.7	5.7	14.3	7.2	9.9	7.9	9.4	7.8	10.6	11.0	9.0	**8.7**	**2.6**
4	16.5	23.6	29.1	19.3	7.2	9.9	11.3	12.7	31.9	29.0	11.0	11.3	**17.7**	**8.6**
6	25.8	39.7	29.1	20.3	13.4	22.6	20.1	33.2	31.9	50.3	13.5	29.9	**27.5**	**10.7**
12	28.5	73.1	39.6	25.4	19.1	48.9	44.9	64.5	42.9	83.8	44.3	57.1	**47.7**	**19.3**
24	37.8	73.1	39.6	48.3	19.8	75.7	60.2	103.2	42.9	83.8	138.8	66.6	**65.8**	**32.6**

Table 7.7 Cumulative urinary excretion of terbutaline post oral ingestion of 500µg terbutaline sulphate dosing (O) expressed in µg, (n=12).

Time	1	2	3	4	5	6	7	8	9	10	11	12	mean	SD
0.5	0	0.4	0.2	0.3	0	0	0.3	0.2	0.2	0	0	0.4	**0.2**	**0.2**
1	2.2	5.3	3.7	5.9	2.6	7.6	6.8	5.4	3.7	4.8	7.5	15.5	**5.9**	**3.5**
2	11.7	12.9	41.5	16.1	15.2	53.3	21.3	16.2	22.3	11.9	51.3	29.7	**25.3**	**15.2**
4	59.7	54.2	68.3	45.1	77.1	90.5	89.9	43.1	36.4	53.1	83.9	110.2	**67.6**	**22.8**
6	120.3	80.4	98.6	52.2	153.7	141.9	120.1	53.3	52.7	77.9	124.1	137.4	**101.0**	**36.9**
12	169.2	102.3	124.2	62.6	217.7	229.7	158.9	67.4	66.0	93.0	216.9	178.8	**140.6**	**62.8**
24	181.1	102.3	155.8	117.1	237.3	279.9	164.7	133.1	83.2	93.0	277.5	187.1	**167.7**	**68.1**

Table 7.8 The mean (SD) amounts of urinary terbutaline excreted, expressed in µg, during each collection period post 500µg terbutaline sulphate dosing via a MDI (I), a MDI with simultaneous oral administration of 20g activated charcoal (IC) and an oral solution of 500µg terbutaline sulphate (O).

Time in Hour	I	IC	O
0.0-0.5	7.4(2.2)	6.5(2.1)	0.2(0.2)
0.5-1	8.2(7.4)	0.8(0.7)	5.7(3.4)
1-2	28.4(18.1)	1.4(2.5)	19.4(14.4)
2-4	58.4(30.2)	9.0(9.5)	42.3(19.4)
4-6	46.4(15.1)	9.8(8.0)	33.4(20.6)
6-12	45.8(25.5)	20.2(12.1)	39.5(28.8)
12-24	35.9(40.0)	18.1(27.2)	27.1(24.5)

Table 7.9 The mean (SD) cumulative urinary excretion of terbutaline excreted, expressed in µg, post 500µg terbutaline sulphate dosing via a MDI (I), a MDI with simultaneous oral administration of 20g activated charcoal (IC) and an oral solution of 500µg terbutaline sulphate (O).

Time in Hour	I	IC	O
0.5	7.4(2.2)	6.5(2.1)	0.2(0.2)
1	15.6(8.6)	7.3(2.1)	5.9(3.5)
2	44.0(24.1)	8.7(2.6)	25.3(15.2)
4	102.7(42.1)	17.7(8.6)	67.6(22.8)
6	149.1(56.5)	27.5(10.7)	101.0(36.9)
12	194.9(75.2)	47.7(19.3)	140.6(62.8)
24	230.8(86.2)	65.8(32.6)	167.7(68.1)

Table 7.10 Mean (SD) urinary terbutaline excretion rates post 500µg terbutaline sulphate dosing via a MDI (I), a MDI with simultaneous oral administration of 20g activated charcoal (IC) and an oral solution of 500µg terbutaline sulphate (O) expressed as µg/hour, (n=12).

Mid Point	I	IC	O
0.25	14.7(4.3)	13.0(4.3)	0.3(0.3)
0.75	16.4 (14.9)	1.6 (1.4)	11.5 (6.9)
1.5	28.4 (18.1)	1.4 (2.5)	19.4 (14.4)
3	29.3 (15.1)	4.5 (4.7)	21.2 (9.7)
5	23.2 (7.5)	4.9 (4.0)	16.7 (10.3)
9	7.6 (4.2)	3.4 (2.0)	6.6 (4.8)
18	3.0 (3.3)	1.5 (2.3)	2.3 (2.0)

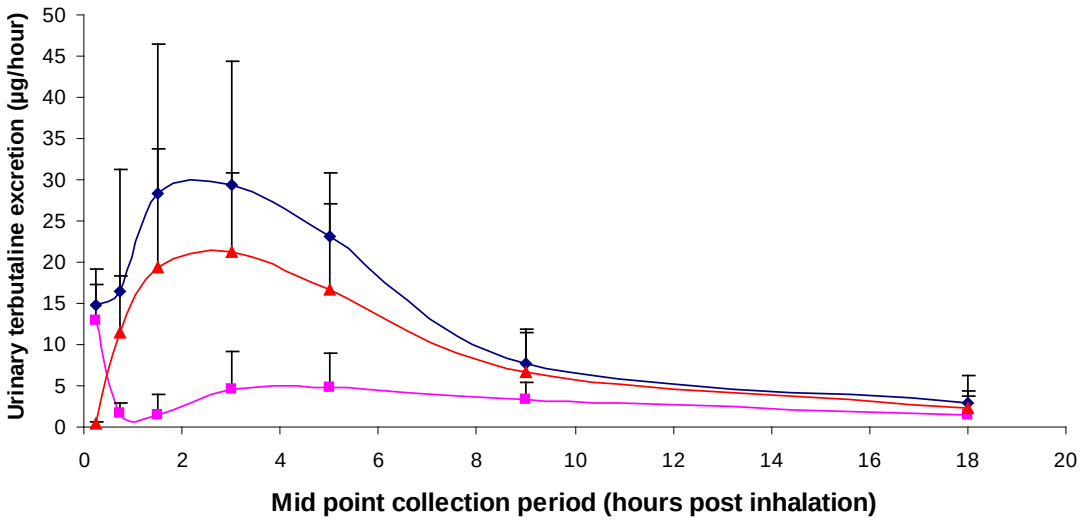


Figure 7.1 Mean (SD) urinary terbutaline excretion rates post study doses.

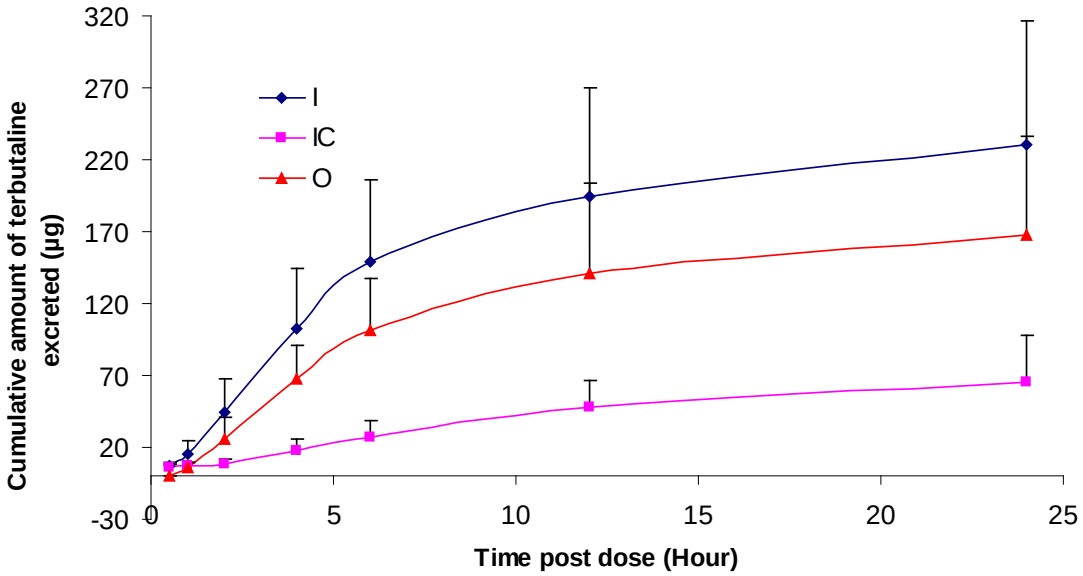
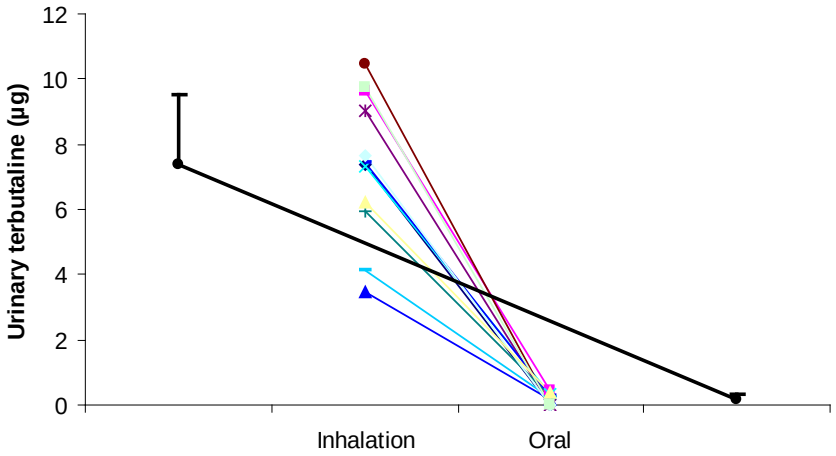
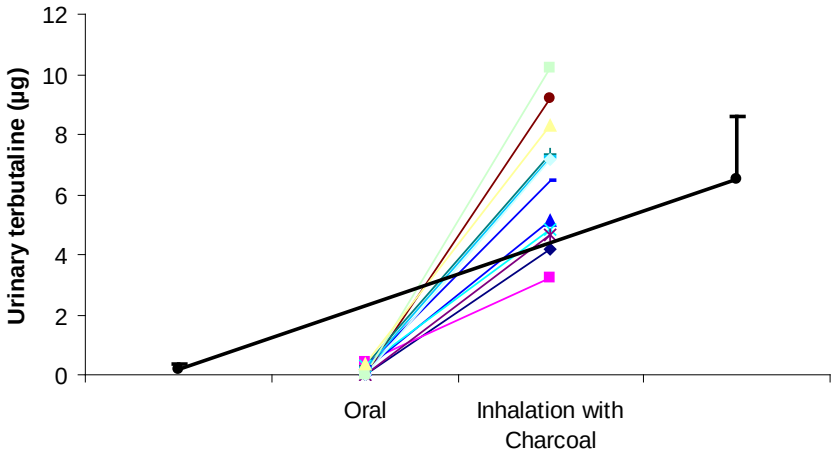


Figure 7.2 Mean (SD) cumulative urinary excretion of terbutaline, expressed in µg, post 500µg terbutaline sulphate dosing via a MDI (Inhalation), a MDI with simultaneous oral administration of 20g activated charcoal and an oral solution of 500µg terbutaline.

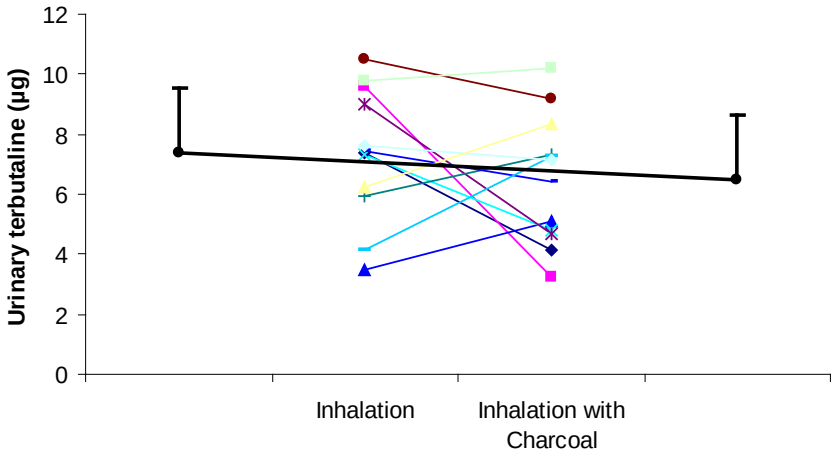
Figure 7.3 shows that, the amount of terbutaline recovered at 30 minutes post dose was significantly higher after inhaled and IC compared with oral ($p<0.001$). The mean difference (95% confidence intervals) for the 0.0–0.5 hour urinary drug excretion post inhaled and IC administration compared with oral administration were 7.2 (5.7, 8.7) µg and 6.3 (4.9, 7.8) µg, respectively ($p<0.001$).



(a)



(b)



(c)

Figure 7.3 Individual (n=12) and mean (SD) amounts of terbutaline expressed in µg recovered post 500µg terbutaline sulphate dosing via (a) MDI [I] vs the oral solution [O], (b) a MDI and 20g oral activated charcoal [IC] *vs* the oral solution [O] and (c) MDI [I] vs a MDI and 20g oral activated charcoal [IC].

A summary of the statistical comparison between I, IC and O is presented in Table 7.11. No significant difference was found between the amount of terbutaline excreted in the urine samples 0.0–0.5 hour post dose following inhaled and IC administration as shown in Figure 7.3c and Table 7.11.

Table 7.11 Mean difference (95% confidence interval) between the cumulative amount (in µg) excreted post different times for I *vs* O, IC *vs* O and I *vs* IC.

Time	I *vs* O (µg)	IC *vs* O (µg)	I *vs* IC (µg)
0.5 hour	7.2 (5.7, 8.7)***	6.3 (4.9, 7.8)***	0.9 (-0.7, 2.4)
1 hour	9.7 (5.0, 14.3)***	1.4 (-3.3, 6.0)	8.3 (3.6, 12.9)***
2 hours	18.7 (3.6, 33.8)**	-16.6 (-31.7, -1.5)*	35.3 (20.2, 50.4)***
4 hours	35.1 (12.1, 58.0)***	-49.9 (-72.8, -27.0)***	85.0 (62.0, 107.9)***
6 hours	48.1 (17.9, 78.3)**	-73.6 (-103.8, -43.3)***	121.6 (91.4, 151.8)***
12 hours	54.3 (10.6, 98.0)**	-92.9 (-136.6, -103.5)***	147.2 (103.5, 190.9)***
24 hours	69.3 (23.8, 114.7)**	-101.9 (-147.3, -56.424)***	171.1 (125.7, 216.6)***

* $p<0.05$, ** <0.01, ***<0.001 otherwise no significant difference.

Table 7.11 shows that when comparing the cumulative amount excreted in the urine following inhaled and oral dosing, a significant difference ($p<0.01$) was found at 0.0-2, 0.0-6, 0.0-12 and 0.0-24 hours urine collection intervals investigated and the difference was found more significant ($p<0.001$) at 0.0-0.5, 0.0-1 and 0.0-4 hours urine collection interval. When comparing the cumulative amount excreted in the urine following IC and oral dosing, a significant difference ($p<0.001$) was found at all time intervals investigated, except for the cumulative excretion over the first hour where no significant difference was found and ($p<0.05$) over the first 2 hours.

The mean (SD) urinary excretion of terbutaline over the 24 hours period post dosing of inhaled (I) dosing, inhaled plus charcoal (IC) and oral (O) as 230.0 (86.2), 65.8 (32.6) and 167.0 (68.1)µg, respectively. The 0-24 hours urinary terbutaline excretion following IC was a mean (SD) of 16.0 (7.9) % of the nominal dose. This represents the total lung bioavailability for the MDI.

7.2.5 Discussion

Statistically significantly more amounts of terbutaline were excreted in the urine following inhalation compared with oral administration during the first 30 minutes post dose. The ratios of the amounts of terbutaline recovered in the urine following oral and inhaled administration were 1:43.1 and 1:1.37 for the 0.5 and 24 hours urinary collection periods post dose, respectively. The high 0.5 hour ratio is due to the rapid and complete absorption of the terbutaline fraction deposited in the lungs, and the slow and negligible early absorption from the gastrointestinal tract that highlights the lag time for drug absorption from the gastrointestinal tract. This is similar to the salbutamol urinary excretion data following inhaled and oral administration originally presented by Hindle & Chrystyn (Hindle and Chrystyn, 1992). In this original study the mean ratio (SD) of inhaled to oral excretion at 0.5 hour and 24 hours was 8.3 (2.2) and 0.92 (0.25) respectively. As expected since the activated charcoal effectively blocks the gastrointestinal absorption of terbutaline then the amount excreted in the urine following inhaled administration with oral charcoal did not differ significantly from that following inhaled dosing during the first 0.5 hour urine collection period. The significant difference between the amounts of urinary terbutaline in samples taken more than 0.5 hour post inhalation from the inhaled and IC highlights the contribution of the orally absorbed fraction.

Thus the amount of terbutaline excreted in the urine during the first 0.5 h following inhalation can be used as an index of the relative bioavailability of terbutaline to the lungs

following inhalation. This index could be, therefore, used to compare the in-vivo lung deposition of inhaled terbutaline products or methods of inhalation.

The low ratios (1:1.37) of the amounts of terbutaline recovered in the urine following oral and inhaled administration confirms that this index identifies the relative total systemic bioavailability of terbutaline post inhalation. Hence the amount of terbutaline delivered to the body and excreted in the urine over the 24 hours post inhalation period could be used to compare the systemic delivery following inhalation of different products or by different techniques. This index represents the relative bioavailability to the body following an inhalation. The 24 hours terbutaline excreted post oral dosing was relatively lower than that post inhalation even though the doses are the same. This might be due to the incomplete absorption of the oral dose compared to the inhaled dose. This was consistent with the previously reported relative bioavailability of oral terbutaline [14.8%] (Martindale, 2002) compared to the relative bioavailability of inhaled terbutaline [16.51%] (Borgstrom and Nilsson, 1990). Finally this 16.5% lung deposition for the MDI with oral charcoal reported by Borgstrom and Nilsson (1990) is very similar to the 16.0% reported in this study.

7.3 Intra- and inter-subject variability

7.3.1 Methods

7.3.1.1 Equipment and inhalation devices

Inhaler device: Bricanyl Metered Dose Inhaler labelled as a nominal dose of 250μg terbutaline sulphate per shot (AstraZeneca, UK)

HPLC condition: Previously described in section 3.3 in this thesis

7.3.1.2 Procedure

Ethical approval was obtained from the University of Bradford and all volunteers gave signed informed consent. Twelve non-smoking volunteers (six females), older than 18 years with a FEV_1> 90% of predicted, agreed to repeat the MDI inhalation study dose

(described as [I] in section 7.2) on five separate occasions to determine the reproducibility of the 30 minutes urinary terbutaline. They all received training on how to use a metered dose inhaler (MDI) and the MDI was shaken and primed by firing two doses to waste before use (Barry and O'Callaghan, 2003). Subjects were trained to shake the MDI, remove the cap, exhale slowly as far as comfortable, put the MDI into their mouth and seal their lips round the mouthpiece. They were then instructed to start a slow inhalation through their mouth and activate the MDI immediately after the start of this slow inhalation. This slow inhalation continued until total lung capacity. After inhalation they held their breath for 10 seconds and for the second dose this was repeated 30 seconds later (Hindle et al., 1993).

Following a light breakfast and no caffeine or alcohol containing drinks for at least 12 hours, each subject inhaled two doses from a Bricanyl MDI (labelled 250µg terbutaline sulphate nominal dose, AstraZeneca, UK). Immediately before each study dose(s) each subject voided their urine and then provided a urine sample 30 minutes after the start of the first dose. The volume was measured and the urinary terbutaline assayed using the previously validated HPLC method described in section 3.3 in this thesis.

7.3.2 Results

Twelve (six females) healthy non-smoking subjects with a mean (SD) age, weight and height of 29.2(4.7) years, 66.8(13.3) kg and 168.2(6.2) cm, respectively and FEV_1 96.8(3.2) % of predicted completed the reproducibility study. Table 7.12 describe the demographic data of the subjects. The mean 30 minutes post inhalation urinary terbutaline excretion and coefficient of variation is shown in Table 7.13 The mean (SD) urinary terbutaline in the 30 minutes post inhalation was 10.4(2.9) µg following the two doses and the mean (SD) intra-subject coefficient of variation was 9.2 (2.1) %. The mean (SD) inter-subject coefficient of variation was 28.8 (1.0) %.

Table 7.12 Demographic data of the patients that participated in the study

Patient code	Gender	Age	Height	Weight	FEV_1
1	Female	25.0	161.0	50.0	99.0
2	Male	25.0	168.0	68.0	95.0
3	Male	31.0	163.0	95.0	98.0
4	Male	32.0	170.0	80.0	99.0
5	Male	27.0	173.0	72.0	99.0
6	Male	37.0	180.0	80.0	98.0
7	Female	24.0	160.0	54.0	99.0
8	Female	25.0	162.0	60.0	89.0
9	Male	32.0	169.0	70.0	99.0
10	Female	32.0	165.0	54.0	98.0
11	Female	36.0	175.0	57.0	97.0
12	Female	24.0	172.0	62.0	92.0
Mean		**29.2**	**168.2**	**66.8**	**96.8**
SD		**4.7**	**6.2**	**13.3**	**3.2**

Table 7.13 Urinary excretion of terbutaline 30 minutes post inhalation of two 250µg terbutaline sulphate doses from the MDI for the 12 individuals on five different occasions.

Occasion	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**Mean**	**SD**	**inter-subject C.V.%**
1	10.3	8.5	5.1	13.9	10.0	7.7	9.2	12.7	7.1	12.6	9.7	15.8	10.2	3.1	30.0
2	10.2	10.3	4.2	11.5	9.9	8.0	10.1	12.5	7.2	13.7	10.9	15.4	10.3	3.0	28.6
3	8.6	8.2	5.8	12.2	10.2	9.7	9.4	15.9	8.2	14.6	9.9	12.4	10.4	2.9	27.5
4	8.0	9.0	5.5	12.6	8.4	9.6	10.1	14.5	9.4	14.5	9.8	15.2	10.5	3.0	28.6
5	9.5	9.1	5.3	13.4	8.4	9.5	10.0	14.8	7.8	12.7	8.7	15.5	10.4	3.1	29.4
Mean	**9.3**	**9.0**	**5.2**	**12.7**	**9.4**	**8.9**	**9.8**	**14.1**	**7.9**	**13.6**	**9.8**	**14.8**	**10.4**	**2.9**	**28.8 (1.0)**
SD	**1.0**	**0.8**	**0.6**	**1.0**	**0.9**	**1.0**	**0.4**	**1.4**	**0.9**	**1.0**	**0.8**	**1.4**			
intra-subject C.V.%	**10.7**	**9.0**	**11.9**	**7.6**	**10.0**	**10.8**	**4.6**	**10.2**	**11.8**	**7.0**	**8.1**	**9.3**	**9.2 (2.1)**		

7.3.3 Discussion

Healthy volunteers were used because lung deposition is affected by the airway calibre and the renal function and those should remain stable in healthy volunteers (Lipworth and Clark, 1997a). The inter-subject variability was high [28.8 (1.0) % after two dose] due to between subject variability of lung deposition together with their renal excretion. This variability between subjects is consistent with previous reports (Chege and Chrystyn, 1994; Hindle and Chrystyn, 1992; Hindle et al., 1995; Hindle et al., 1994; Tomlinson et al., 2003). However the intra-subject variability was much [9.2 (2.1) %] lower and similar to that reported by Hindle and Chrystyn (1992) when volunteers inhaled four doses (Hindle and Chrystyn, 1992; Tomlinson et al., 2003). Hence the method is suitable for cross over studies.

7.4 Dose response relationship

7.4.1 Methods

7.4.1.1 Equipment and inhalation devices

As section 7.3.1.1

7.4.1.2 Procedure

Ethical approval was obtained from the University of Bradford and all volunteers gave signed informed consent. The twelve subjects (six females) were healthy, non-smoking volunteers older than 18 years with a FEV_1> 90% of predicted. They all received training on how to use a metered dose inhaler (MDI). The MDI was shaken and primed by firing two doses to waste before use (Barry and O'Callaghan, 2003). Subjects were trained to shake the MDI, remove the cap, exhale slowly as far as comfortable, put the MDI into their mouth and seal their lips round the mouthpiece. They were then instructed to start a slow inhalation through their mouth and actuate the MDI immediately after the start of this slow inhalation. This slow inhalation continued until total lung capacity. After inhalation they held their breath for 10 seconds and if another dose was scheduled it was inhaled 30

seconds later (Hindle et al., 1993). On each of the four occasions, following a light breakfast and no caffeine or alcohol containing drinks for at least 12 hours, each subject randomly inhaled either one, two, three or four doses from a Bricanyl MDI (labelled 250µg terbutaline sulphate nominal dose, AstraZeneca, UK). Immediately before each study dose(s) each subject voided their urine and then provided a urine sample 30 minutes after the start of the first dose and cumulatively collected their urine for the rest of the 24 hours. The volume was measured and the urinary terbutaline assayed using the previously validated HPLC method described in section 3.3 in this thesis.

Statistical analysis looked at the linear regression to identify correlation coefficient (*r*), intercept and slope for each subject together with an inspection to determine if there was a systematic deviation from the overall line of linear regression using the residuals.

7.4.2 Results

Twelve (six females) healthy subjects with a mean (SD) age, weight and height of 28.5(4.0) years 67.3(13.9) kg, 168.2(6.2) cm, respectively and FEV_1 96.8(3.2) % predicted completed all four inhalations. Table 7.14 describe the demographic data of the subjects. Table 7.15 and Figure 7.4-7.7 show the mean (SD) and individual cumulative urinary excretion of terbutaline following inhaled one, two, three and four dose(s) of 250µg via a MDI expressed as µg, (n=12).

Although there was an inter-subject variability the amount of terbutaline excreted in the urine during the first 30 minutes and 24 hours post dosing were linear ($p< 0.002$) with the dose. The mean (SD) for the *r* values was 0.9858(0.0202) with a range of 0.9255 to 0.9996. The regression slopes were significantly above zero. The mean (SD) terbutaline excreted after one, two, three and four doses during the 30 minutes post start of the inhalation was 4.23(2.13), 8.94(2.74), 13.05(3.1) and 18.27(4.68) µg, respectively and the mean (SD) 24 hours urinary terbutaline was 103.9(52.2), 219.6(67.4), 320.6(76.1) and 448.6(115) µg, respectively.

Table 7.14 Demographic data of the patients that participated in the study

Patient code	Gender	Age	Height	Weight	FEV_1
1	Female	25.0	161.0	50.0	99.0
2	Male	25.0	168.0	68.0	95.0
3	Male	31.0	163.0	95.0	98.0
4	Male	29.0	180.0	85.0	98.0
5	Male	32.0	170.0	80.0	99.0
6	Female	32.0	165.0	54.0	98.0
7	Male	32.0	169.0	70.0	99.0
8	Male	27.0	173.0	72.0	98.0
9	Female	36.0	175.0	57.0	97.0
10	Female	24.0	172.0	62.0	92.0
11	Female	24.0	160.0	54.0	99.0
12	Female	25.0	162.0	60.0	89.0
Mean		**28.5**	**168.2**	**67.3**	**96.8**
SD		**4.0**	**6.2**	**13.9**	**3.2**

Table 7.15 Urinary excretion of terbutaline post MDI inhalation expressed in µg, (n=12).

Number of doses	Time	1	2	3	4	5	6	7	8	9	10	11	12	Mean	SD
One dose	**0.5**	3.6	3.5	2.3	4.0	2.3	5.4	4.6	1.7	3.0	4.0	7.5	8.8	**4.2**	**2.1**
	24	88.3	85.5	57.5	97.2	56.9	132.3	112.5	42.5	73.9	98.0	185.1	217.2	**103.9**	**52.2**
Two doses	**0.5**	8.5	11.6	3.5	7.3	9.2	10.5	10.1	5.1	9.7	7.8	10.4	13.6	**8.9**	**2.7**
	24	209.9	285.5	85.8	180.2	225.3	257.4	248.4	126.2	237.0	192.0	254.5	332.8	**219.6**	**67.4**
Three doses	**0.5**	12.9	14.7	6.3	10.4	15.0	15.3	13.1	12.4	11.0	13.6	13.0	19.1	**13.1**	**3.1**
	24	316.4	360.2	154.2	255.6	368.0	375.7	321.3	305.5	270.1	332.9	318.9	468.9	**320.6**	**76.1**
Four doses	**0.5**	20.5	22.5	10.1	13.0	19.7	19.8	16.6	19.1	12.7	21.7	16.8	26.6	**18.3**	**4.7**
	24	504.5	553.4	247.9	318.4	484.0	486.5	408.9	468.0	311.6	534.1	412.9	653.4	**448.6**	**114.9**

One dose is 250µg terbutaline sulphate

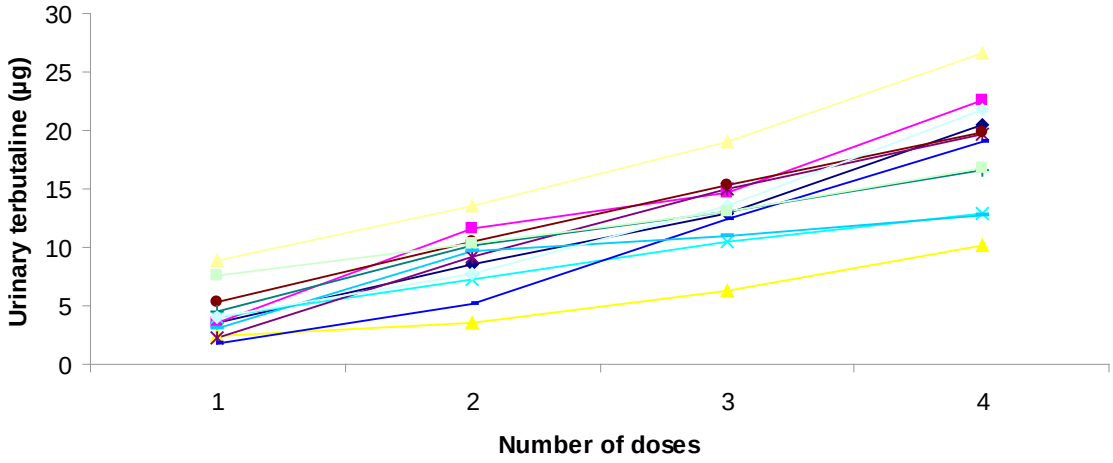


Figure 7.4 The individual amounts of urinary terbutaline excreted 30 minutes post MDI dosing (n=12).

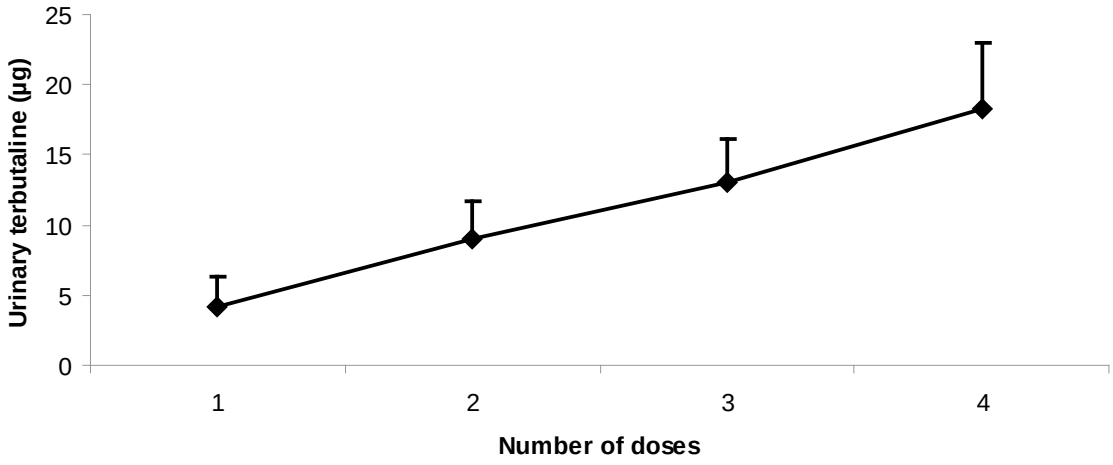


Figure 7.5 The mean (SD) [n=12] amounts of urinary terbutaline excreted 30 minutes post MDI dosing.

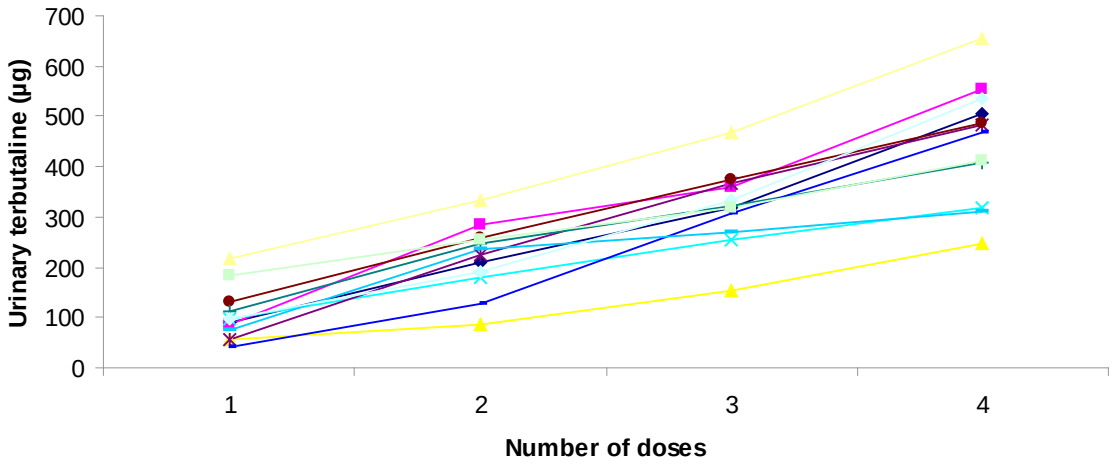


Figure 7.6 The individual amounts of urinary terbutaline excreted 24 hours post MDI dosing (n=12).

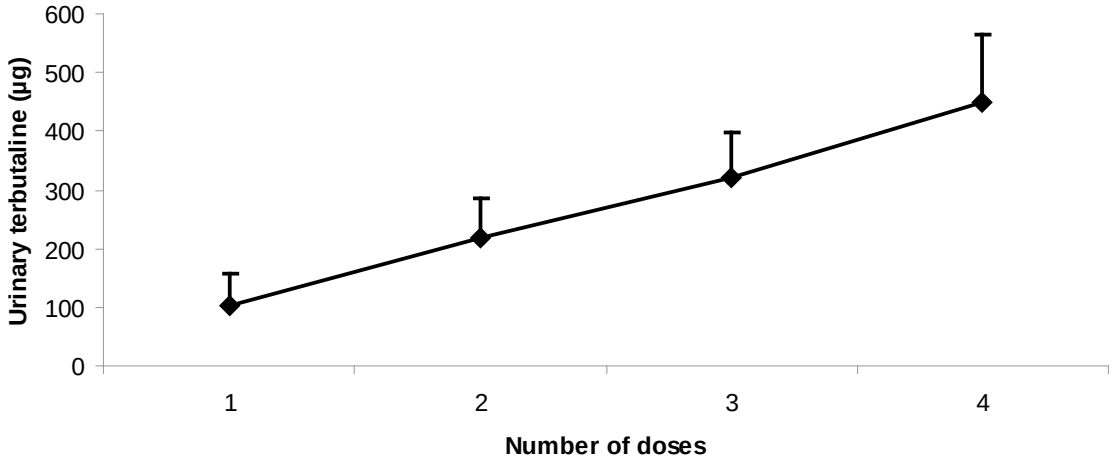


Figure 7.7 The mean (SD) [n=12] amounts of urinary terbutaline excreted 24 hours post MDI dosing.

7.4.3 Discussion

The dose–response relationships for the 30 minute and 24 hours terbutaline urinary excretion post dosing were linear. This is consistent with the results of a similar study for salbutamol by Tomlinson et al. (2003). In this study Tomlinson and colleges concluded that the 30 minute salbutamol urinary excretion post inhalation pharmacokinetic method was linear with the inhaled dose ($p<0.001$) and reproducible.

Regulatory Authorities favour the demonstration of clinical equivalence using the degree of protection to a bronchoprovocation agent together with in-vitro characterization of the emitted dose (Adams et al., 1994). Hence simultaneous measurements of 30 minute urinary salbutamol excretion and the dose of methacholine to reduce the FEV_1 by 20% following one and two doses would support the pharmacokinity method as a positive link to the clinical bioassay. The study of salbutamol from an Easibreathe in 12 asthmatics had shown the two methods to be equal (Tomlinson et al., 2005). The study demonstrated a correlation between the pharmacokinetic and pharmacodynamic method. Twelve asthmatics inhaled 0, 1 and 2 doses of 100 mg salbutamol from a breath-activated MDI on separate study days. The mean (SD) salbutamol 30 minutes post dose was 0, 2.25 (0.65) and 5.18 (1.68) mg, respectively, with a ratio between 1 : 2 doses of 2.30. The mean (SD) PD_{20} was 0.21 (0.24), 0.74 (0.70) and 1.69 (1.60) mg, respectively, with a ratio of 2.28 between 1 and 2 doses. This positive link to a clinical bioassay together with the reproducibility and dose–response properties highlight the potential of this 30 minute index of lung deposition in studies to compare different inhalation products and inhalation methods.

7.5 General conclusions

The amount excreted following an inhaled terbutaline sulphate dose taken together with an oral dose of activated charcoal represents the pulmonary absorbed fraction and because of the lack of difference between the inhaled and inhaled plus oral charcoal (IC) in the first 30 minutes

then the use of activated charcoal is not necessary when this method is used to compare between different products or techniques. In addition, the magnitude of the difference between the amount excreted in the first 30 minute following oral and inhaled dosing confirm that this 30 minutes urinary excretion of terbutaline post inhalation is an index of the relative bioavailability of terbutaline to the lungs following inhalation. The similarity between the 24 hours excretion post inhaled dosing (I) and the oral (O) dosing confirms that the 24 hours urinary excretion of terbutaline is an index that identifies the relative total systemic bioavailability of terbutaline post inhalation and can be used to compare the delivered dose to the body from two different inhalation products or methods. The method is linear and reproducible similar to that for urinary salbutamol method (Tomlinson et al., 2003).

Thus the urinary drug excretion technique, which has already been considered to be the basis for a novel clinical equivalence test for inhalation products of salbutamol (Hindle and Chrystyn, 1992) and extended to sodium cromoglycate (Aswania et al., 1997; Aswania and Chrystyn, 2002), nedocromil (Aswania et al., 1998), gentamicin (Al-Amoud et al., 2005; Al-Amoud et al., 2002), tobramycin. (Barber, 2002) and formoterol (Nadarassan et al., 2007), can also be used for other water-soluble inhaled drugs like terbutaline.

8.1 Introduction

The bioequivalence of inhaled products is complicated because the therapeutic effect is influenced by the topical deposition of drug in the lungs and safety is determined by systemic delivery. For inhaled corticosteroids, oropharyngeal impaction is also considered with respect to safety because of the potent side effect of the corticosteroids in this area. Regulatory Authorities favour the demonstration of clinical equivalence using the degree of protection to a bronchoprovocation agent together with in-vitro characterization of the emitted dose (Adams et al., 1994). This method measures the amount of inhaled bronchoprovocation agent that is required to reduce the FEV_1 by 20% following the inhalation of a placebo and active drug. Any method used in bioequivalence studies should be reproducible and have a dose response relationship. This has been reported for the bronchoprovocation method (Creticos et al., 2002). However, most clinical studies are carried out using measurements at the plateau portion of the dose response relationship (Chrystyn, 2000).

Hindle and Chrystyn (1992) have shown that the amount of salbutamol excreted in the urine during the first 30 minutes post inhalation represents the amount of drug deposited in the lungs. This urinary salbutamol pharmacokinetic method has been used to compare metered dose inhalers when attached to a large volume spacer (Chege and Chrystyn, 1994) and dry powder inhalers (Hindle et al., 1995; Hindle et al., 1994). Another study by Tomlinson et al. (2005) has shown that this method is more sensitive to demonstrate a difference between inhalation techniques than bronchoprovacation challenge test using methacholine (Tomlinson et al., 2005). The validation of this method for terbutaline is described in chapter 7.

To demonstrate the application of the urinary terbutaline pharmacokinetic method the aims of this chapter are

1. To evaluate the effect of inhalation technique on the lung and systemic bioavailability following inhalation from a dry powder inhaler after slow and fast inhalation flow.

2. To detect the effect of different spacers on the lung and the systemic bioavailability of inhaled terbutaline from the MDI.

3. To show the effect of different nebulisers on the lung and the systemic bioavailability of nebulised terbutaline.

8.2 Relative lung and systemic bioavailability of terbutaline inhaled from a dry powder inhaler using different inhalation flow

8.2.1 Methods

8.2.1.1 Equipment and inhalation devices

Inhaler device:	Bricanyl Turbuhaler labelled as a nominal dose of 500µg terbutaline sulphate per inhalation (AstraZeneca, UK)
In-Check Dial:	Clement Clarke International, UK
HPLC condition:	Previously described in section 3.3 in this thesis

8.2.1.2 Procedure

Ethical approval was obtained from the University of Bradford and all volunteers gave signed informed consent. Twelve non-smoking volunteers (six females), older than 18 years with an average FEV_1> 90% of predicted, agreed to inhale through a terbutaline sulphate Turbuhaler (Bricanyl, labelled 500µg terbutaline sulphate nominal dose, AstraZeneca, UK) using slow and fast inhalation flows. They were trained how to inhale using a slow inhalation flow (30 L min^{-1}) and also a fast inhalation flow (60 L min^{-1}) with aid of the In-Check Dial. They all received training on how to use a DPI. Doses were loaded for the subject before use according to the patient information leaflet. Subjects were trained to breathe out gently but not breathe out through the inhaler, prepare a dose for inhalation as recommended in the patient information leaflet, then place the mouthpiece between their lips and inhale through their mouth, according to the inhalation flow to be used, inhalation continued till their lungs were full (total lung capacity) and they then removed the Turbuhaler from their mouth and held

their breath for 10 seconds before breathing out slowly followed by normal breathing. Each subject inhaled two doses from two separate Bricanyl Turbuhalers using either a slow or a fast inhalation flow in random order of slow and fast inhalation manoeuvres.

The use of the two Turbuhalers was to avoid the inhalation of the residual part that would remain in the Turbuhaler from first dose. The residual part was later removed from the Turbuhaler by discharge to waste using a very high inhalation flow (over 90 L min^{-1}) for 16 seconds. This flow has been reported to clear the Turbuhaler from residue (Tarsin et al., 2004).

On each study day subjects were allowed to have a light breakfast and no caffeine or alcohol containing drinks for at least 12 hours before dosing. Immediately before each study dose each subject voided their urine and then provided a urine sample 30 minutes after the start of the first dose and cumulatively collected their urine for 24 hours. The volume of urine samples was measured and the urinary terbutaline assayed using the previously validated HPLC method described in section 3.3 of this thesis. The urine samples at slow and fast inhalation flows were compared using a two way analysis of variance (ANOVA) test using SPSS V15.0 (SPSS Inc., Chicago, USA).

8.2.2 Results

Twelve (six females) healthy non-smoking subjects with a mean (SD) age, weight and height of 29.2(4.8) years, 66.3(11.8) kg and 170.5(7.4) cm, respectively and FEV_1 96.1 (3.7) % of predicted completed this DPI flow effect study. Table 8.1 describe the demographic data of the subjects. The individual and mean (SD) urinary excretion of terbutaline post inhalation of two doses of 500µg terbutaline sulphate from the Turbuhaler DPI at slow and fast inhalation flow are shown in Tables 8.2 and 8.3 and Figure 8.1 and 8.2.

The mean (SD) terbutaline excreted after slow and fast inhalation flow during the 30 minutes post start of the inhalation was 5.1(4.3) and 18.3(12.4) µg, respectively as shown in Figure 8.1. These values are equivalent to 0.6(0.5) and 2.2(1.5) %, respectively when calculated as a

percentage of the nominal dose. The mean (SD) amount of terbutaline excreted in the urine over the first 24 hours post inhalation was 249.7(210.6) and 534.9(199.2) µg, respectively as shown in Figure 8.2. These values are equivalent to 30.4(25.6) and 65.1(24.3) %, respectively when calculated as a percentage of the nominal dose. Statistical analysis revealed that the *mean difference (95%; confidence interval*) between fast and slow inhalation flow for the terbutaline excreted in 0.0-0.5 hour interval was 13.2 (6.5, 19.9; $p<0.001$) µg and for the amounts of terbutaline excreted over the 24 hours period post inhalation was 285.1 (154.4, 415.8; $p<0.001$) µg.

Table 8.1 Demographic data of the patients that participated in the study

Patient code	Gender	Age	Height	Weight	FEV_1	Sequence of flow*
1	Female	25.0	161.0	50.0	99.0	A2
2	Male	25.0	168.0	68.0	95.0	A1
3	Male	32.0	170.0	80.0	99.0	A2
4	Female	32.0	165.0	54.0	98.0	A1
5	Male	34.0	180.0	74.0	90.0	A1
6	Male	27.0	173.0	72.0	99.0	A2
7	Male	37.0	180.0	80.0	98.0	A2
8	Female	36.0	175.0	57.0	97.0	A2
9	Female	24.0	172.0	62.0	92.0	A2
10	Female	24.0	160.0	54.0	99.0	A1
11	Female	25.0	162.0	60.0	89.0	A1
12	Male	29.0	180.0	85.0	98.0	A1
Mean		**29.2**	**170.5**	**66.3**	**96.1**	
SD		**4.8**	**7.4**	**11.8**	**3.7**	

*A1is fast flow first and A2 is slow flow first

Table 8.2 Mean (SD) and individual urinary excretion of terbutaline 0.5 hr. post inhalation of two 500µg terbutaline sulphate doses from the Turbuhaler using slow and fast inhalation flows [expressed in µg] (n=12).

Flow	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**Mean**	**SD**
Slow	1.3	4.8	2.4	0.9	6.4	3.3	3.2	5.0	0.8	10.8	7.3	15.0	**5.1**	**4.3**
Fast	4.4	17.1	25.4	21.4	8.5	3.2	20.0	15.2	7.1	48.3	22.2	26.4	**18.3**	**12.4**

Table 8.3 Mean (SD) and individual urinary excretion of terbutaline over the first 24 hr. post inhalation of two 500µg terbutaline sulphate doses from the Turbuhaler using slow and fast inhalation flows [expressed in µg] (n=12).

Time	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**Mean**	**SD**
Slow	66.0	233.6	117.6	42.2	313.3	160.4	156.1	244.4	39.0	529.9	356.2	737.7	**249.7**	**210.6**
Fast	215.4	698.0	658.7	625.0	419.8	156.0	634.6	598.7	349.3	738.4	610.5	713.8	**534.9**	**199.2**

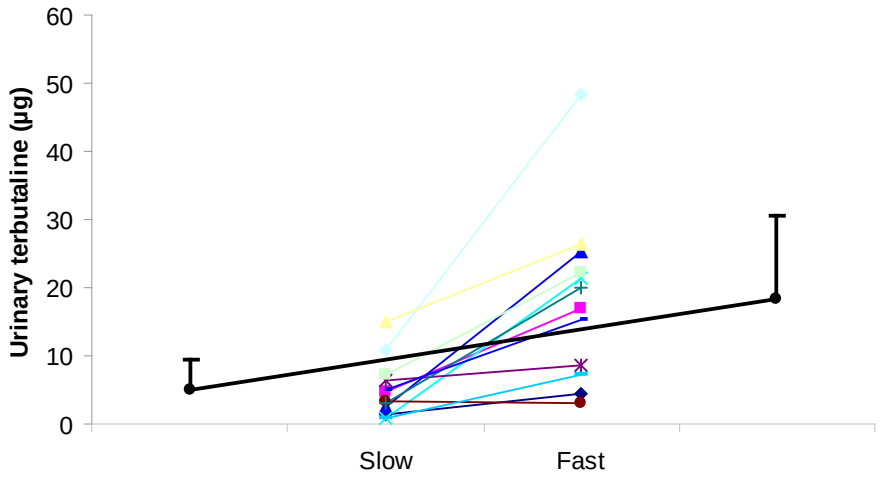


Figure 8.1 Mean (n=12) and the individual amounts of urinary terbutaline excreted 30 minutes post 500µg terbutaline sulphate dosing from the DPI after using a fast and slow inhalation flow.

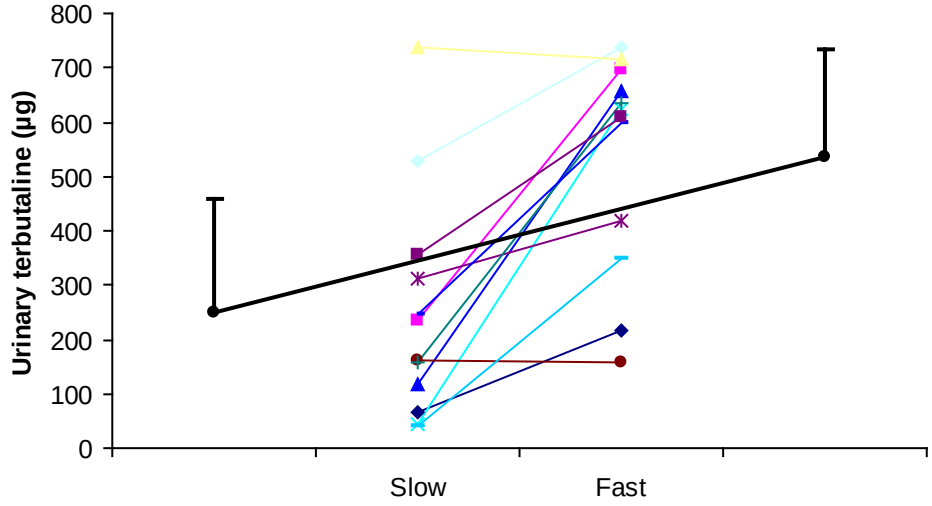


Figure 8.2 Mean (n=12) and the individual amounts of urinary terbutaline excreted over the first 24 hours post 500µg terbutaline sulphate dosing from the DPI after using a fast and slow inhalation flow.

8.2.3 Discussion

The amount excreted 30 minutes and 24 hours post dosing using inhalation flows 30 and 60 L min^{-1} confirm the application of the urinary terbutaline pharmacokinetic method for inhaled terbutaline.

The results indicates that the fast inhalation flow resulted in a significantly higher amount of urinary terbutaline excretion 30 minutes and 24 hours post dosing than slow inhalation ($P<0.001$). These results are consistent with the instructions on the use of the Turbuhaler, as explained in the device pamphlet, which is to inhale as fast and as deep as possible.

The results were also consistent with the most desirable inhalation flow for use with the Turbuhaler which has been reported to be 60 L min^{-1} (Borgstrom et al., 1996). Most importantly the results are consistent with the in-vitro results in chapter 4 which showed that the emitted dose, FPD and FPF are affected by the inhalation flow used. The data shown in chapter 4 showed that the increase in the inhalation flow increased the FPD, FPF and the total emitted dose. At low flow the decrease in lung deposition is due to the incomplete de-aggregation of the drug from the device.

A scintigraphy study by Newman et al (1991) has also showed similar results that support the inhalation at fast inhalation flow from a Turbuhaler. In this study the deposition of 500µg terbutaline sulphate from the Turbuhaler was measured in 10 asthmatic subjects at two inhalation flows (fast, mean 57 L min^{-1} and slow, mean 28 L min^{-1}). When using a fast inhalation flow, a mean (SD) 16.8 (2.6) % of the dose was deposited in the lungs compared with 9.1 (1.5) % of the dose for slow inhalation flow ($p<0.01$). At either flows, the majority of the dose was deposited in the oropharynx. This represents a higher relative bioavalaibility than found 30 minutes post fast and slow inhalation flows. This might be due to that the gamma scintigraphy methodology that includes drug deposited into the lungs which is cleared by mucociliary clearance.

Radiolabeled budesonide inhaled from a Turbuhaler by 10 healthy volunteers revealed that the mean (SD) total lung dose was 14.8% (3.3) of nominal dose when using a slow inhalation flow rate (36 L min^{-1}) and 27.7% (9.5) for a fast flow rate (58 L min^{-1}). Hence the inhalation of budesonide at the fast flow resulted in a significant increase in lung deposition compared with the slower flow which was matched by a decrease in deposition in the oropharynx and mouthpiece (Borgstrom et al., 1994).

A recent study by Meyer et al (2004) had compared the in-vivo and the in-vitro data representing the deposition of radiolabeled foradil (Aeroliser) in human lungs. The study involved in-vivo results of 10 healthy volunteers and the in-vitro results from the dose emission test and aerodynamic characteristics. The results were combined using a mathematical model of lung deposition (Meyer et al., 2004). Both in-vitro and in-vivo measurements showed a significant increase in the dose delivered as the flow increased (Meyer et al., 2004). A study by De Boer et al. (1996) showed that the higher inhalation flow result in more FPD. The study also showed that at low flows de-aggregation of drug and carrier was incomplete (de Boer et al., 1996).

8.3 Relative lung and systemic bioavailability of terbutaline inhaled from metered dose inhaler with different spacers using urinary drug excretion post inhalation

8.3.1 Materials and Methods

8.3.1.1 Equipment and inhalation devices

Inhaler and spacer devices

Bricanyl Metered Dose Inhaler:	Labelled as a nominal dose of 250µg terbutaline sulphate per shot (AstraZeneca, UK)
AeroChamber MAX spacer:	Valved Holding Chambers; Trudell Medical International Europe Ltd, UK

AeroChamber Plus spacer: Valved Holding Chambers; Trudell Medical International Europe Ltd, UK

Nebuhaler: Valved Holding Chambers; AstraZeneca, UK

HPLC condition: Previously described in sections 3.2 and 3.3 in this thesis

8.3.1.2 Procedure

Twelve non-smoking volunteers (six females), older than 18 years with an average FEV_1> 90% of predicted, gave their informed and written consent to take part in the study. Ethical approval has been obtained for the study. On four separate occasions, following a light breakfast and no caffeine or alcohol containing drinks for at least 12 hours, each subject inhaled two 250 µg terbutaline sulphate doses (500 µg in total) from a MDI (Bricanyl inhaler, AstraZeneca, UK)

(a) With no spacer [MDI]

(b) With the AeroChamber MAX spacer [AMAX] (Trudell Medical International Europe Ltd, UK)

(c) With the AeroChamber Plus spacer [APLUS] (Trudell Medical International Europe Ltd, UK)

(d) With the Nebuhaler spacer [NH] (AstraZeneca, UK).

The order of administration was randomized, and there was a seven day washout period between the study days. They all received training on how to use a metered dose inhaler (MDI) and the MDI was shaken and primed by firing two doses to waste before use (Barry and O'Callaghan, 2003). Subjects were trained to shake the MDI, remove the cap, exhale slowly as far as comfortable, put the MDI into their mouth and seal their lips round the mouthpiece. They were then instructed to start a slow inhalation through their mouth and activate the MDI immediately after the start of this slow inhalation. They continued to inhale slowly until total lung capacity. After the inhalation they held their breath for 10 seconds and

inhaled the second dose 30 seconds later. (Hindle et al., 1993). All subjects were trained how to use each spacer according to the manufacturer's recommended instruction. When using the spacer subjects exhaled to residual volume (as much as possible), the dose was discharged into the spacer and within 1 second a slow and deep inhalation was taken over a period of 5–10 seconds using the same inhalation procedure for the MDI alone. This was followed by a 10 seconds breath hold before the subject breathed out normally and inhaled the second dose 30 seconds later.

Subjects emptied their bladder prior to each study dose and urine samples were collected at 30 minutes post inhalation and cumulatively collected for 24 hours. The volume of each urine sample was measured, recorded and an aliquot was stored, at −20°C prior to analysis by a previously validated high performance liquid chromatography (HPLC) as explained in section 3.3 in this thesis. The amount of drug left in the spacer was determined following rinsing by HPLC analysis as described in section 3.2.

Statistical comparisons of the urinary excretion of terbutaline following inhalation from the MDI, AMAX, APLUS and nebuhaler in the urine samples collected at 30 minutes and 24 hours post inhalation and the amount left in the spacers were compared using a two way analysis of variance (ANOVA) test using SPSS V15.0 (SPSS Inc., Chicago, USA). The mean difference (95% confidence interval) was calculated for each inhaled method.

8.3.2 Results

Twelve (six females) healthy non-smoking subjects completed the study. Their mean (SD) age, weight and height was 29.2(4.8) years, 66.3(11.8) kg and 170.5(7.4) cm, respectively. The FEV_1 of all subjects was greater than 90% of predicted value with a mean (SD) of 96.1(3.7) % of predicted. Table 8.4 describe the demographic data of the subjects.

The mean (SD) and individual cumulative urinary excretion of terbutaline post inhalation of two doses of 250µg terbutaline sulphate via a MDI with different spacers are shown in Tables

8.5 and Figures 8.3 and 8.5. A summary of the mean (SD) amounts of urinary terbutaline excretion from the twelve subjects 30 minutes and 24 hours post inhalation of 500 µg terbutaline sulphate via MDI, AMAX, APLUS and NH are shown in Table 8.6 and Figures 8.4 and 8.6.

A summary of the statistical comparison between amounts of urinary terbutaline excretion for the twelve subjects 30 minutes and 24 hours post inhalation of 500 µg terbutaline sulphate via MDI, AMAX, APLUS and NH to highlight the differences are presented in Table 8.7 and 8.8, respectively. A summary of the statistical comparison between amounts of terbutaline sulphate retained in each spacer from the twelve subjects post inhalation of 500 µg terbutaline sulphate via AMAX, APLUS and NH is presented in Table 8.9.

Table 8.4 Demographic data of the patients that participated in the study

Patient code	Gender	Age	Height	Weight	FEV_1
1	Female	32.0	165.0	54.0	98.0
2	Female	24.0	172.0	62.0	92.0
3	Male	25.0	168.0	68.0	95.0
4	Male	32.0	170.0	80.0	99.0
5	Male	34.0	180.0	74.0	90.0
6	Female	36.0	175.0	57.0	97.0
7	Female	25.0	161.0	50.0	99.0
8	Female	24.0	160.0	54.0	99.0
9	Female	25.0	162.0	60.0	89.0
10	Male	27.0	173.0	72.0	99.0
11	Male	37.0	180.0	80.0	98.0
12	Male	29.0	180.0	85.0	98.0
Mean		**29.2**	**170.5**	**66.3**	**96.1**
SD		**4.8**	**7.4**	**11.8**	**3.7**

Table 8.5 Urinary excretion of terbutaline post inhalation of two doses of 250µg terbutaline sulphate and the amount of terbutaline sulphate deposited in each spacer expressed in µg, (n=12).

Method	Time	1	2	3	4	5	6	7	8	9	10	11	12	Mean	SD
MDI	**0.5**	8.5	11.6	3.5	7.3	9.2	10.5	10.1	5.1	9.7	7.8	10.4	13.6	**8.9**	**2.7**
	24	224.9	262.5	170.8	187.2	225.3	240.4	241.4	146.2	228.0	192.0	245.5	270.8	**219.6**	**37.8**
AMAX	**0.5**	32.6	30.1	18.2	12.8	15.7	16.2	14.7	39.0	37.8	28.6	21.5	33.5	**25.1**	**9.6**
	Spacer	105.4	108.6	102.4	110.5	108.7	103.6	114.7	153.4	150.7	105.0	104.0	153.9	**118.4**	**21.0**
	24	161.1	149.7	110.4	94.2	98.7	93.8	101.2	188.9	181.1	148.3	131.1	167.0	**135.5**	**35.1**
APLUS	**0.5**	12.4	21.8	10.1	10.0	11.6	12.3	12.6	30.0	32.7	21.6	16.8	31.1	**18.6**	**8.6**
	Spacer	135.2	189.3	135.3	163.6	159.8	139.3	163.1	66.2	67.2	181.9	176.0	65.5	**136.9**	**45.9**
	24	91.7	103.0	74.8	68.8	72.6	71.4	79.9	142.1	146.5	104.9	90.6	158.2	**100.4**	**31.8**
NH	**0.5**	11.0	14.8	6.6	12.8	13.3	11.8	14.7	18.3	21.9	16.2	18.0	24.0	**15.3**	**4.8**
	Spacer	106.1	93.9	108.6	108.6	93.9	108.0	118.2	116.0	118.2	92.1	99.4	120.1	**106.9**	**10.1**
	24	66.3	87.8	55.8	73.0	71.9	63.7	79.3	98.8	103.2	87.8	97.5	105.4	**82.5**	**16.6**

Table 8.6 Mean (SD) amount of terbutaline (in µg) excreted 30 minutes and 24 hours post-inhalation of two doses of 250µg terbutaline sulphate and the amount of terbutaline sulphate deposited in each spacer.

	Amount (S.D.)		
Method	**0.5 hours**	**Spacer**	**24 hours**
MDI	8.9 (2.7)	--	219.6 (37.8)
AMAX	25.1 (9.6)	118.4 (21.0)	135.5 (35.1)
APLUS	18.6 (8.6)	136.9 (45.9)	100.4 (31.8)
NH	15.3 (4.8)	106.9 (10.1)	82.5 (16.6)

Table 8.7 Mean difference (95% confidence interval) for the amount of terbutaline excreted post 30 minutes using MDI and MDI+Spacers.

Comparator	**MDI**	**AMAX**	**APLUS**
Nebuhaler	-6.3 (-10.4, -2.3)**	9.8 (5.7, 13.9)**	3.3 (-0.8, 7.4)
APLUS	-9.6 (-13.7, -5.6)**	6.5 (2.4, 10.6)**	---
AMAX	-16.1 (-20.2, -12.0)**	---	---

* $p<0.05$, ** <0.001 otherwise no significant difference.

Table 8.8 Mean difference (95% confidence interval) for the amount of terbutaline excreted post 24 hours using MDI and MDI+Spacers.

Comparator	**MDI**	**AMAX**	**APLUS**
Nebuhaler	137.1 (104.8, 169.3)**	52.8 (20.6, 85.1)**	17.9 (-14.4, 50.1)
APLUS	119.2 (87.0, 151.5)**	35.0 (2.8, 67.2)*	---
AMAX	84.2 (52.0, 116.5)**	---	---

* $p<0.05$, ** <0.001 otherwise no significant difference.

Table 8.9 Mean difference (95% confidence interval) for the amount terbutaline sulphate retained in each spacer.

Comparator	APLUS	AMAX
Nebuhaler	30.0 (0.2, 59.8)*	11.5 (-18.3, 41.3)
AMAX	18.5 (-11.4, 48.3)	---

* p<0.05, ** <0.001 otherwise no significant difference

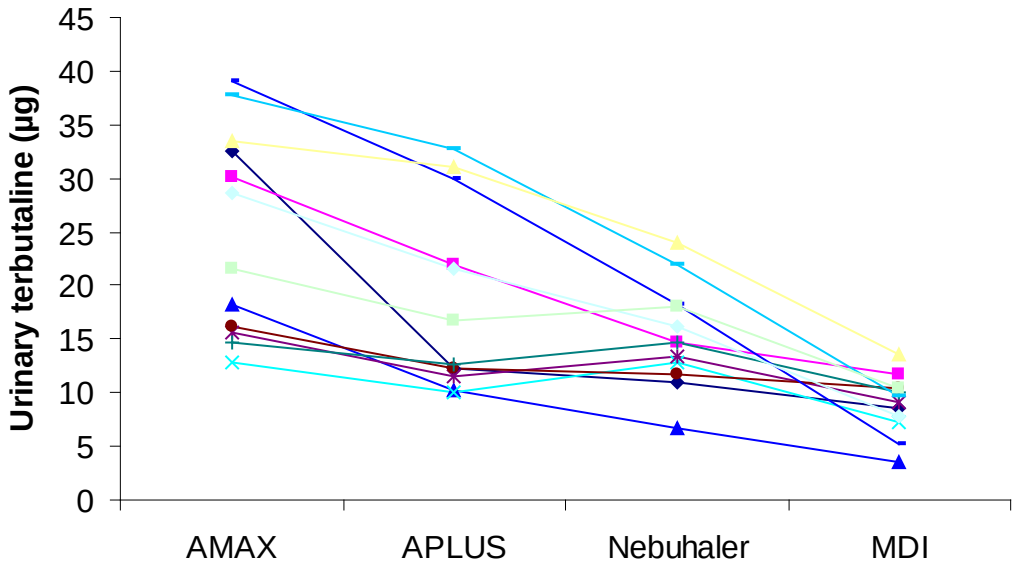


Figure 8.3 The individual (n=12) amounts of terbutaline excreted 30 minutes post-inhalation of 2 doses of 250µg terbutaline sulphate via AMAX, APLUS, Nebuhaler and MDI.

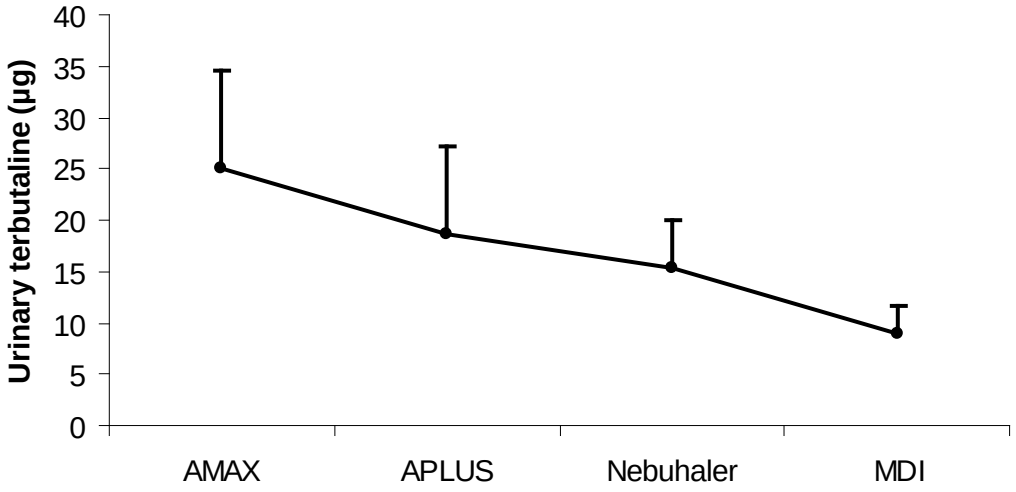


Figure 8.4 The mean (SD) [n=12] amounts of terbutaline excreted 30 minutes post-inhalation of 2 doses of 250µg terbutaline sulphate via AMAX, APLUS, Nebuhaler and MDI.

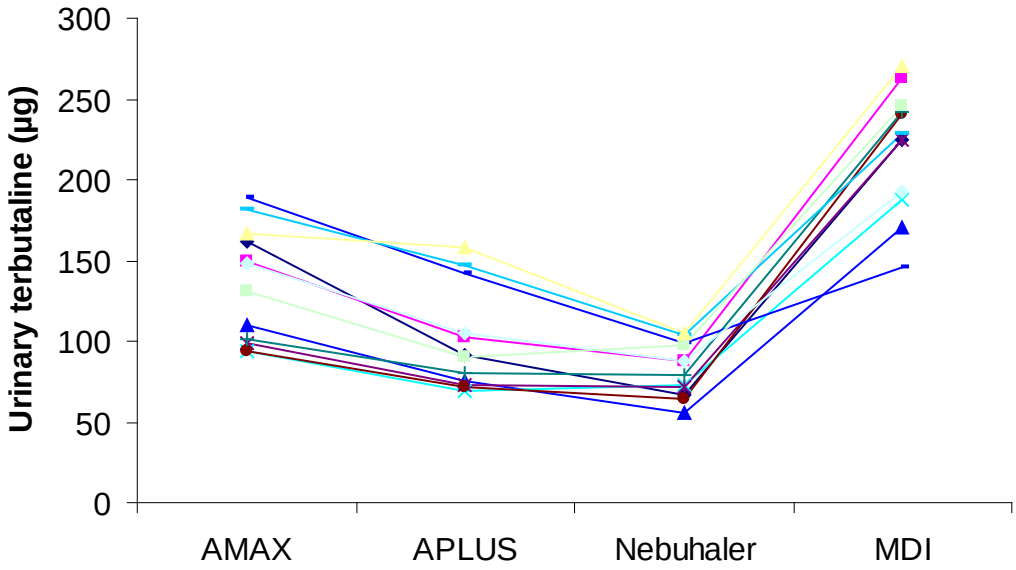


Figure 8.5 The individual (n=12) amounts of terbutaline excreted 24 hours post-inhalation of 2 doses of 250µg terbutaline sulphate via AMAX, APLUS, Nebuhaler and MDI.

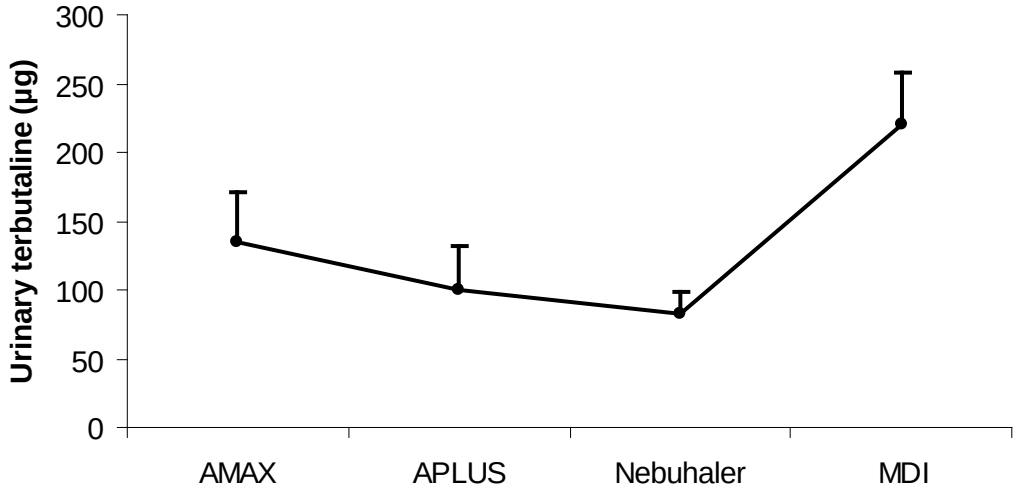


Figure 8.6 The mean (SD) [n=12] amounts of terbutaline excreted 24 hours post-inhalation of 2 doses of 250µg terbutaline sulphate via AMAX, APLUS, Nebuhaler and MDI.

8.3.3 Discussion

Add-on devices for MDI improve targeting of drug to the lungs and can correct poor hand-breath co-ordination (Newman and Newhouse, 1996).

The amounts of terbutaline excreted 30 minutes and 24 hours post dosing from the MDI and MDI+Spacer provide further evidence to the application of the urinary terbutaline pharmacokinetic method for inhaled terbutaline.

The results indicates that the use of the spacer resulted in significantly higher amounts of urinary terbutaline excretion after 30 minutes ($p<0.001$) and lower amounts of urinary terbutaline excretion in the first 24 hours ($p<0.001$) post dosing then the MDI alone for all individuals.

The results were consistent with that of a study by Hindle et al (1994). They found that in 10 volunteers the amount (SD) of urinary excreted salbutamol 30 minutes post inhalation of four

100µg salbutamol doses from the MDI alone (Ventolin, Allen and Hanburys Ltd, UK), MDI +Volumatic spacer (Allen and Hanburys Ltd, UK) and MDI + Nebuhaler (Astra Pharmaceutical) were 2.83 (0.78), 3.37 (0.69) and 4.34 (1.60)%, respectively. Also the the amount (SD) of urinary excreted salbutamol 24 hours post MDI alone , MDI +Volumatic spacer and MDI + Nebuhaler, expresses as percentage of nominal dose, were 55.6 (9.74)%, 26.6 (6.79)% and 27.0 (7.95)%, respectively. They showed that the spacer devices improve pulmonary bioavailability of salbutamol and reduce the systemic bioavailability (Hindle et al., 1994).

The results were also consistent with that of a study by Silkstone et al (2002). They compared the lung and systemic delivery of salbutamol following five 100 µg doses inhaled from a metered dose inhaler (MDI) and a MDI attached to a spacer (MDI+SP) using a urinary pharmacokinetic method. They found that in twelve (6 females) healthy subjects the mean (SD) 30 minutes urinary excretion amounts of salbutamol for MDI and MDI+SP was 12.6 (3.5) and 27.1 (6.0) µg, respectively. Also they found that the mean (SD) 24 hours urinary excretion of salbutamol was 287.0 (46.5) and 198.1 (34.7) µg, respectively. Following inhalation a mean of 202.9 (51.5) µg was left in the spacer. When normalised for the amounts available for inhalation, the mean amounts of salbutamol excreted in the urine during the first 30 minutes were 2.86 (0.78) and 9.15 (1.69) % following MDI and MDI+SP, respectively. Hence five 100 µg doses inhaled from a MDI attached to a spacer delivered more to the lungs and less to the systemic circulation than the same doses from a MDI used alone (Silkstone et al., 2002a).

Also the results were similar to the study by Aswania et al (1999). They have shown in 10 volunteers that the mean (SD) urinary excretion of sodium cromoglycate in the first 30 minutes post the inhalation of four 5mg doses from an Intal MDI (Fisons Ltd., UK) and an Intal MDI attached to the Fisonair large volume spacer (Fisons Ltd., UK) was 38.1 (27.5) and

222.3 (120.3) µg, respectively (Aswania, 1999). These were also similar to the results of another study by Aswania et al (2001) for the Cromogen MDI used alone and when attached to the Volumatic spacer. The mean (SD) urinary excretion of sodium cromoglycate in the first 30 minutes post-inhalation was 34.1 (20.2) and 211.7 (123.5) µg following MDI and MDI+Volumatic spacer, respectively. This shows that the MDI attached to a large volume spacer delivers more sodium cromoglycate to the lungs than MDI alone (Aswania and Chrystyn, 2001).

Although between 21 to 27 % of the nominal dose was retained in the spacers, there was upto a three-fold improvement in the relative amounts deposited in the lungs compared to the MDI alone. This large difference has been obtained in subjects who have been trained and demonstrated an excellent inhalation technique. The in-vitro analysis of the aerodynamic characterisation of the emitted dose for a Bricanyl MDI with and without a spacer in chapter 5 has shown an increase in the FPD and FPF. The FPD and FPF are representatives of the respirable amount that reach the lung which account for the amount excreted 30 minutes post dosing. However, the magnitude of difference of the in-vitro result is not as large as the in-vivo results obtained from this study.

In-vivo results here showed that, there was upto about 2.7-fold decrease in the relative amounts that reach the systemic circulation (24 hours results) compared to the MDI alone. The in-vitro analysis of the emitted dose for a Bricanyl MDI with and without a spacer in chapter 5 has shown a similar decrease.

Measurements of drug delivery from add-on devices by gamma scintigraphy have shown that, compared to an MDI, oropharyngeal deposition is always reduced, and that lung deposition is generally either increased or unchanged. The total body dose may be reduced by over 80% (Newman and Newhouse, 1996).

The results were also similar to a study by Richards et al (2001) to assess the deposition and pharmacokinetics of a novel formulation of flunisolide (Aerobid, Forest Laboratories) in hydrofluoroalkane (HFA) 134a delivered by a MDI. The design was a two-way crossover investigation in 12 healthy male subjects comparing HFA-134a flunisolide by MDI versus MDI + 50 ml spacer device. Four of these subjects also took part in a two-way crossover investigation comparing chlorofluorocarbon (CFC) flunisolide MDI versus MDI + AeroChamber holding chamber. The imaging technique of gamma scintigraphy was used to quantify total and regional lung deposition of flunisolide. Plasma levels of flunisolide and its major metabolite (6β-OH flunisolide) were also determined. The spacer and AeroChamber reduced the mean oropharyngeal deposition dramatically from 59.8 to 14.9% ($p < 0.01$) of dose for HFA product and 66.3 to 12.3% ($p < 0.01$) of dose for CFC product. That was due to deposition of part of the dose on the walls of the add-on devices themselves (Richards et al., 2001). The mean lung deposition was 22.6 and 40.4%, respectively ($p < 0.01$) of the dose for the HFA formulation used with MDI alone and with MDI + spacer. Mean lung deposition of the CFC formulation delivered via the AeroChamber (mean 23.4%) was higher than that for the CFC MDI alone (mean 17.0%), but this difference was not statistically significant. Plasma levels of flunisolide were higher for the MDI + spacer than for the MDI alone, reflecting higher lung deposition via the spacer. The plasma levels of the 6β-OH flunisolide metabolite were higher for the MDI alone as a consequence of higher oropharyngeal deposition which is ingested in the gastrointestinal tract.

A study by Hirst et al (2001) showed similar results. They assessed the deposition in the lungs and oropharynx of triamcinolone acetonide (TAA; Azmacort, Aventis Pharma, Collegeville, PA) delivered by a MDI formulated with HFA-134a by gamma scintigraphy. Also they measured the pharmacokinetic profile of TAA, to determine the extent to which the Azmacort spacer improves targeting of TAA to the lungs. The deposition of TAA, labelled with ^{99m}Tc,

was assessed by gamma scintigraphy in 10 patients with mild to moderate asthma (mean FEV_1 of 76% of predicted), who received, in randomized order, three delivered doses of 75 μg TAA via MDI + Azmacort spacer, and three delivered doses of 230 μg TAA via the MDI, but with no spacer. Mean lung deposition, expressed as mass of drug, was similar for each regimen (MDI alone 175 μg; MDI+Spacer 188 μg), but when expressed as a percentage of the delivered dose, lung deposition was higher for the MDI+Spacer (53.8%) versus the MDI alone (26.0%), indicating superior drug targeting for the MDI+Spacer. The spacer reduced oropharyngeal deposition. The pharmacokinetic data showed higher plasma levels of drug for the MDI alone, resulting from higher oropharyngeal deposition and delivered from the gastrointestinal tract (Hirst et al., 2001).

The results in this part of the thesis showed that the AMAX resulted in significantly higher urinary terbutaline excretion 30 minutes post dosing than either the APLUS or the Nebuhaler ($p<0.001$). Also the AMAX resulted in a significantly higher urinary terbutaline excreted post 24 hours post dosing than either the APLUS or the Nebuhaler ($p<0.05$ and $p<0.001$, respectively). This might be due to the antistatic property of AMAX.

The results are consistent with an in-vivo pharmacokinetic study by Anhoj et al (1999). They studied the effect of the electrostatic charge in plastic spacers on drug delivery to the lung of hydrofluoroalkane (HFA) salbutamol aerosol in children. Salbutamol HFA aerosol was delivered on different study days from a non-electrostatic 350 ml Babyhaler (coated with benzalkonium chloride) and a new 350 ml Babyhaler (rinsed in water). Plasma salbutamol was measured before and 5, 10, 15 and 20 minutes after inhalation of four 100 μg salbutamol doses. C_{max} was calculated as a reflection of lung dose.

They found in five children, aged 7–12 years that C_{max} for the non-electrostatic Babyhaler was 4.3 ng ml^{-1} and New Babyhaler was 1.9 ng ml^{-1}. The non-electrostatic Babyhaler delivered a significantly ($P<0.05$) higher lung dose than the New Babyhaler. Hence, the electrostatic

charge in plastic spacers reduces lung dose in children by more than two-fold (Anhoj et al., 1999).

The in-vitro analysis of the aerodynamic characterisation of the emitted dose for a Bricanyl MDI with and without a spacer in chapter 5 of this thesis has shown AMAX has a significantly higher total emitted dose when compared to the APLUS and the Nebuhaler ($p<0.01$) which account for the higher amounts excreted post 24 hours. The APLUS has a significantly high FPF and smaller MMAD when compared to the AMAX and Nebuhaler ($p<0.001$). However the AMAX has a relatively high FPD. The combination of the high total emitted dose together with the relatively high FPD of the AMAX could account for the significantly higher urinary terbutaline excreted 30 minutes post AMAX dosing. Since the urinary terbutaline excreted 30 minutes post dosing accounts for the relative amounts deposited in the lungs, it can be considered that the total emitted dose and the FPD together are more important for the lung deposition than the FPF and MMAD togather.

The mean amount of terbutaline excreted 30 minutes and 24 hours post APLUS was higher than that post Nebuhaler, but these differences were not statistically significant. However, the in-vitro results in chapter 5 showed that the FPF of the APLUS was significantly higher than that of the Nebuhaler ($p<0.001$) and the MMAD was significantly lower. The comparison between the in-vitro and the in-vivo results of the spacer could further raise doubts about the clinical meaning of FPF together with the MMAD when compared to the combination of the emitted dose and the FPD.

8.4 Relative lung and systemic bioavailability of terbutaline inhaled from two different nebulisers using urinary drug excretion post inhalation

8.4.1 Methods

8.4.1.1 Equipment and inhalation devices

Inhalation devices and HPLC system

Aeroneb Professional Nebuliser: Vibrational mesh Nebuliser System; Aerogen Inc, USA

Sidestream Jet nebuliser: Intersurgical Ltd, UK; attached to a Porta Neb compressor.

Bricanyl Respules: Labelled as a nominal dose of 5.0 mg in 2 ml (2.5 mg/ml) terbutaline sulphate (AstraZeneca, UK)

Porta Neb compressor: Respironics, UK

HPLC condition: Previously described in sections 3.2 and 3.3 in this thesis

8.4.1.2 Procedure

Ethical approval was obtained from the University of Bradford and all volunteers gave signed informed consent. Twelve non-smoking volunteers (six females), older than 18 years with an average (SD) FEV_1>90% of predicted, agreed to inhale nebulised aerosol of Bricanyl respiratory solution (Bricanyl Respules, labelled 5.0 mg/2ml terbutaline sulphate, AstraZeneca, UK) through two different nebulisers using normal tidal breathing. They were first trained on how to inhale through the nebulisers. Subjects were trained to place the mouthpiece between their lips and breathe in and out gently through their mouth. Each subject randomly inhaled one whole Bricanyl Respules dose from two different nebulisers (Aeroneb Pro and Sidestream jet nebuliser). The dose was loaded in the nebuliser for the subject before use according to the patient information leaflet.

On each of the two occasions, following a light breakfast and no caffeine or alcohol containing drinks for at least 12 hours, each subject inhaled their doses from the nebuliser. Immediately before each study dose subjects voided their urine and then provided a urine sample 30 minutes after the start of inhaling the dose and cumulatively collected their urine for 24 hours. The volume was measured and the urinary terbutaline was assayed using the previously validated HPLC method described in section 3.3. The nebulisers and connections were rinsed with water and terbutaline sulphate was assayed using the previously validated HPLC method

described in section 3.2. The urine samples were compared using a two way analysis of variance (ANOVA) test using SPSS V15.0 (SPSS Inc., Chicago, USA).

8.4.2 Results

Twelve (six females) with a mean (SD) age, weight and height of 29.7(4.7) years, 65.8(12.8) kg and 167.3(6.2) cm, respectively completed the study. The FEV_1 of all subjects was greater than 90% of predicted value with a mean (SD) of 95.4(4.0) % of predicted. Table 8.10 describe the demographic data of the subjects. The mean (SD) and individual cumulative urinary excretion of terbutaline post inhalation are shown in Tables 8.11 and Figure 8.7 and 8.8.

The mean (SD) terbutaline excreted after inhalation from the Sidestream jet nebuliser and Aeroneb Pro nebuliser during the 30 minutes post start of the inhalation was 11.6 (5.7) and 31.2 (7.2) µg, respectively as shown in Figure 8.7 and the mean (SD) 24 hours was 164.5 (44.0) and 440.4 (101.4) µg, respectively as shown in Figure 8.8. The mean (SD) amounts of terbutaline sulphate left in the nebulisation champers and the mouthpiece after inhalation from the Sidestream jet nebuliser and Aeroneb Pro nebuliser were 2797.4(181.0) and 1546.2(183.2) µg, respectively as shown in Tables 8.11.

A summary of the statistical comparison between Aeroneb Pro and the Sidestream jet nebuliser is shown in Table 8.12.

Table 8.10 Demographic data of the patients that participated in the study

Patient code	Gender	Age	Height	Weight	FEV_1	Sequence of nebulisers*
1	Female	32.0	165.0	54.0	98.0	A1
2	Female	24.0	172.0	62.0	92.0	A1
3	Male	25.0	168.0	68.0	95.0	A1
4	Male	32.0	170.0	80.0	99.0	A2
5	Male	34.0	180.0	74.0	90.0	A1
6	Female	36.0	175.0	57.0	97.0	A2
7	Female	25.0	161.0	50.0	99.0	A1
8	Female	24.0	160.0	54.0	99.0	A2
9	Female	25.0	162.0	60.0	89.0	A2
10	Male	32.0	169.0	70.0	99.0	A1
11	Male	36.0	163.0	65.0	90.0	A2
12	Male	31.0	163.0	95.0	98.0	A2
Mean		**29.7**	**167.3**	**65.8**	**95.4**	
SD		**4.7**	**6.2**	**12.8**	**4.0**	

A1 is Aeroneb Pro first and A2 is Sidestream first

Table 8.11 Mean (SD) and individual urinary excretion of terbutaline and the amount of terbutaline sulphate left in the nebulisers post inhalation of one dose of 5.0mg via two different nebuliser expressed in µg, (n=12).

Nebuliser	Time (Hours)	1	2	3	4	5	6	7	8	9	10	11	12	Mean	SD
Aeroneb	**0.5**	42.2	28.5	38.1	41.7	26.2	25.9	28.8	25.1	24.7	27.4	40.7	25.1	**31.2**	**7.2**
Pro	**24**	595.7	401.7	537.8	588.9	369.5	365.7	406.2	354.1	349.1	386.9	575.3	354.2	**440.4**	**101.4**
	Remaining	1755.4	1512.6	1771.6	1768.6	1360.0	1725.4	1519.4	1269.3	1356.7	1509.5	1642.2	1363.6	**1546.2**	**183.2**
Sidestream	**0.5**	12.6	10.9	13.9	17.0	1.5	16.7	8.8	3.4	21.6	13.1	8.0	12.4	**11.6**	**5.7**
jet	**24**	178.4	153.9	161.3	204.5	111.2	205.7	123.9	117.9	254.4	175.1	112.7	175.0	**164.5**	**44.0**
nebuliser	**Remaining**	2632.1	2732.5	2649.5	2684.4	3106.9	2638.0	2785.3	2922.6	3039.7	2641.7	2689.2	3047.1	**2797.4**	**181.0**

Table 8.12 Mean difference (95% confidence interval) of Aeroneb Pro *vs* the Sidestream jet nebuliser.

Comparator	Aeroneb Pro *vs* Sidestream jet nebuliser
Amount excreted 30 minutes post dose	19.5 (14.1, 25.0, p<0.001)
Amount excreted 24 hours post dose	105.6 (76.2, 135.0, p<0.001)
Amount remaining in the nebuliser chamber	-1251.2 (-1473.4, -1029.1, p<0.001)

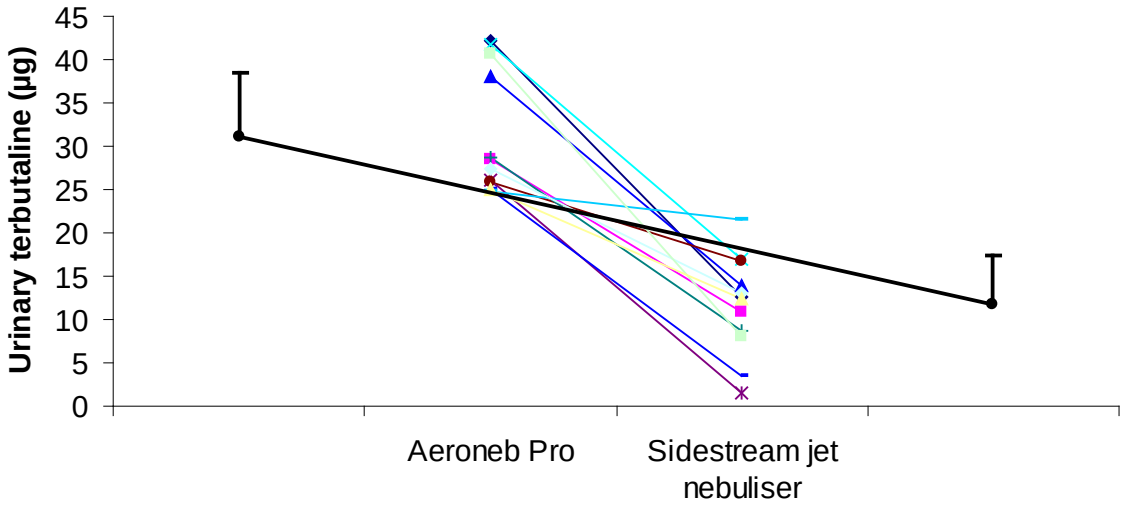


Figure 8.7 Mean (n=12) and the individual amounts of urinary terbutaline excreted 30 minutes post 5.0 mg nebulised terbutaline sulphate dosing.

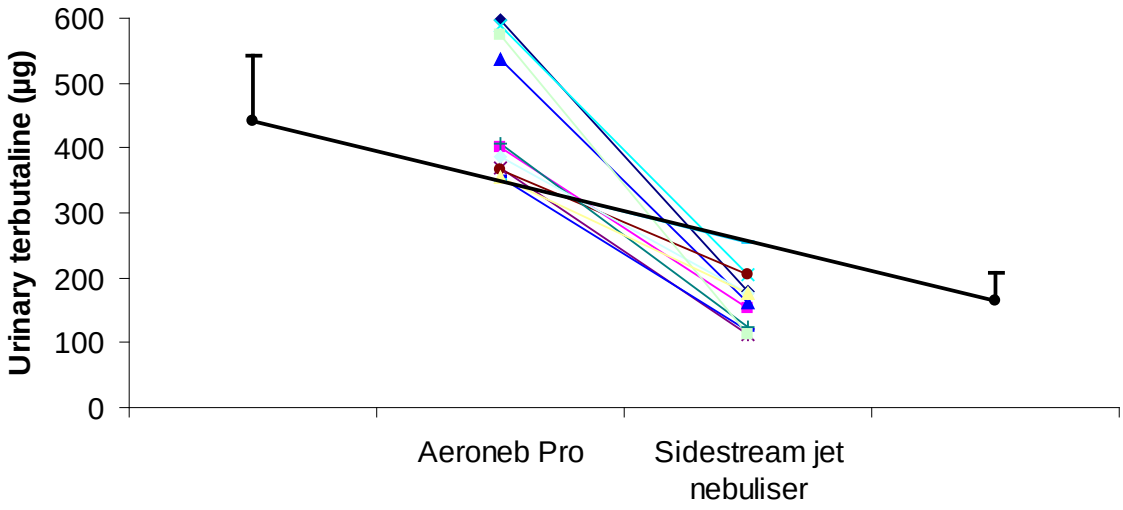


Figure 8.8 Mean (n=12) and the individual amounts of urinary terbutaline excreted in the first 24 hours post 5.0 mg nebulised terbutaline sulphate dosing.

8.4.3 Discussion

The amount left in the nebulisation chamber and the T-mouthpiece of the Sidestream jet nebuliser [55.95 (3.62) % of nominal dose] is significantly higher than that of the Aeroneb pro nebuliser [30.92 (3.66) % of nominal dose] ($p<0.001$). Also, the Aeroneb Pro had a significantly higher amount of systemically absorbed terbutaline than the Sidestream jet nebuliser, as found from the 24 hours urinary terbutaline ($p<0.001$) and the relative lung bioavailability ($p<0.001$) as found from the 30 minutes urinary terbutaline. These represents an approximate three-fold improvement in the relative amounts deposited in the lungs from the Aeroneb pro compared to the Sidestream jet nebuliser, and a 2.7-fold increase of the relative amount delivered to the body (systemic bioavailability). This difference has been obtained in subjects who have been trained and demonstrated an excellent inhalation technique. It has been shown previously using in-vitro analysis that the Aeroneb Pro has a three to five fold higher efficiency for delivering drug to the lungs than conventional jet or

ultrasonic nebulisers (Fink et al., 2001; Fink and Schmidt, 2002; Kristin et al., 2003). This is consistent with our in-vivo results.

The in-vivo results here are also consistent with a study by Fink et al (2003) who determined how Aeroneb Go nebuliser performed compared to a range of commercially available pneumatic jet nebulisers. They compared the inhaled mass and performance characteristics of three compressor-driven pneumatic jet nebulisers (Omron CompAir Elite, DeVilbiss Model 800 and Pari LC Plus) to a prototype nebuliser (Aerogen Aeroneb Go). Each nebuliser was filled with 2.5 mg (3 ml of 0.083%) salbutamol sulphate. Drug was collected on filters placed between the nebuliser and a breath simulator modeling an adult breathing pattern (tidal volume 500 ml, rate 15 cycle per minute). The amount of drug deposited on the filter (Inhaled Mass) was determined for each device (n=3). The Aeroneb Go had the lowest residual drug volumes (<0.3 ml) and delivered more inhaled mass than the other nebulisers tested. Hence, the Aerogen Aeroneb Go prototype nebuliser performed better than the pneumatic jet nebulisers tested (Fink et al., 2003).

Moreover the results are consistent with the in-vitro results in chapter 6 as the vibrational mesh nebulisers emit higher amounts of the nebulisation solution (emitted dose) and a higher respirable amount (the fine particle dose) than the Sidestream jet nebuliser ($p<0.001$). That may be in part due to the small residual volume of the Aeroneb Pro which is less than 0.3ml (Fink et al., 2001) and the Sidestream jet nebuliser have a big residual volume. However, the magnitude of the in-vitro results in chapter 6 is not as large as the in-vivo results obtained from this study.

The result are similar to that reported by Silkstone et al. (2002) to correlate the in-vivo salbutamol pharmacokinetic method and in-vitro CEN method results using eight nebuliser systems. They found a significant correlation ($p=0.02$) between the in-vitro respirable dose and the amount of salbutamol excreted in the urine 30 minutes post the start of nebulisation

[relative bioavailability of salbutamol to the lung]. Also, there was a significant ($p<0.001$) correlation between the in-vitro dose available for inhalation and the total amount of salbutamol cumulatively excreted over the 24 hours post the start of nebulisation [relative bioavailability of salbutamol to the body] (Silkstone et al., 2002b).

8.5 Conclusions

The results of this chapter demonstrate that the urinary pharmacokinetic method that has previously been reported for terbutaline in chapter 7 is a potential tool to compare different inhalation technique, methods and products. The results were consistent with the previously published literature and the in-vitro results in this thesis. Although this measurement (the 30 minutes post-inhalation urinary excretion) does not differentiate between depositions into different zones of the lungs, it is an index of total lung bioavailability and thus is useful for comparing different devices, techniques, methods etc. Also the 24 hours urinary terbutaline has showed that it can be used as an index of systemic bioavailability.

9.1 Introduction

Non-invasive positive pressure ventilation (NPPV) is being increasingly employed for the treatment of patients with acute and chronic respiratory failure (Carlucci et al., 2001). Successful application of NPPV with a nasal or face mask can often prevent the need for endotracheal intubation and improve mortality (Brochard et al., 1995; Plant and Elliott, 1998). NPPV may be employed as a first line mode of mechanical ventilation in about 50% of patients with hypercapnic respiratory failure (Carlucci et al., 2001). Such patients with acute or chronic hypercapnic respiratory failure who are receiving NPPV often require inhaled bronchodilators for the relief of their airway obstruction. This can be provided to them during NPPV by the use of a nebuliser or MDI with spacer in the circuit (Dhand and Tobin, 1997; Parkes and Bersten, 1997). The optimal techniques for aerosol delivery in patients receiving NPPV have been investigated using in-vitro models (Chatmongkolchart et al., 2002). Aerosol delivery can vary as much as five fold during NPPV, depending on the inspiratory and expiratory pressures employed and position of the nebuliser (Chatmongkolchart et al., 2002). Although the optimum settings required for maximum drug delivery during NPPV have not been well established, significant bronchodilator responses occur after salbutamol administration with a jet nebuliser or a MDI with spacer (Dolovich et al., 1977; Nava et al., 2001).

Traditionally, nebulisers have been used to deliver bronchodilators, antibiotics, and surfactant to mechanically ventilated patients, whereas MDIs have been used to deliver β_2-adrenergic and anticholinergic bronchodilators (Dhand and Tobin, 1997). A new generation of nebulisers that are based on vibrating mesh technology have been designed to deliver aerosol in a mechanical ventilation circuit. It have been demonstrated that a version of this new generation is approximately 3 times more efficient than a Sidestream jet nebuliser at delivering drug to the lungs of non-ventilated patients (Ismail and Chrystyn, 2004).

In-vivo deposition studies to determine aerosol deposition in the lower respiratory tract can be estimated by radionuclide studies (Fok et al., 1996; Harvey et al., 1997) but the radioactivity effects on the patient limit the use of these studies to estimate the aerosol deposition. Also the set-up procedure and specialized equipment limit the use of this methodology for patients that are mechanically ventilated. Furthermore the incorporation of a radionuclide alters the formulation of the product and thus its product license.

The 30 minute urinary salbutamol pharmacokinetic method has previously been used in patient with nebuliser studies (Ismail and Chrystyn, 2004) but this has involved switching patients onto terbutaline from salbutamol for their routine management and using salbutamol as the study drug. When investigating nebulised dosing it is the inhalation method rather than the bronchodilator solution that determines the lung and systemic delivery. Hence the urinary terbutaline pharmacokinetic method previously validated in chapter 7 can be used with patients without switching their salbutamol therapy. Terbutaline sulphate respiratory solution can be therefore used as the test. This involves administering a study dose of terbutaline sulphate in place of their prescribed salbutamol medication.

Ex-vivo methods have been described to identify the total emitted dose that a patient would have received (Fink and Simmons, 2004; O'Doherty et al., 1992; O'Riordan et al., 1994; O'Riordan et al., 1992). This is achieved by placing a filter between the patient's mouth and the mouthpiece of the inhalation method. The filter used has no resistance so does not affect the breathing pattern of the patient and does not allow any drug to pass through it. This method has been previously used to determine the amount of drug that mechanically ventilated patients would receive from a nebulised dose (Palmer et al., 1998; Smaldone, 2004; Smaldone and Palmer, 2000; Diot et al., 1997; Fink et al., 1999). The estimation of the amount of drug entrained on the filter, left in the nebuliser's chamber, the tubing and the mouthpiece provides a mass balance fate of the nebulised dose. However this method does not differentiate between

the amounts of drug that would be deposited into the lungs or swallowed. Nevertheless if linked with pharmacokinetic studies would provide valuable data on the lung deposition efficiency of an inhalation method.

The aim of this study was to compare the in-vivo (linked to ex-vivo) fate of nebulised terbutaline sulphate from the Aeroneb Pro nebuliser (a vibrating mesh nebuliser) to that of the routinely used Sidestream jet nebuliser in hospitalized patients requiring non-invasive mechanically ventilation.

9.2. Methods

Local hospital research ethics committee approval was obtained for this study. Patients prescribed non-invasive positive pressure mechanical ventilation mostly for 72 hours post admission were eligible for the study. Due to the necessary washout period of at least 36 hours to allow for the body to excrete the entire drug delivered from each study dose it was only possible for each subject to inhale two study doses and provide urine samples. This study was designed to compare the Sidestream and Aeroneb Pro nebulisers.

Patients were given their first study dose within the first 12 hours of admission. It was anticipated that to obtain consent within this period of time was not ethical or practical. The study dose that they received was part of their medication because the only difference was that the terbutaline sulphate replaced a prescribed salbutamol dose. The only extra procedure compared to routine management was that they were asked to provide a urine sample 30 minute post study dosing and to collect all their urine into a container over the next 24 hours. The volume of the 30 minute and pooled urine samples were measured and aliquots were retained for terbutaline analysis using the previously validated HPLC assay method described in section 3.3 of this thesis. All urine samples were labelled with a code and no other information. Analysis of samples was carried out at the University of Bradford.

The bi-level ventilator was set in spontaneous mode at an inspiratory pressure of 20 cmH_2O and expiratory pressure of 5 cmH_2O. The patient's lung internal pressure triggered the bi-level ventilator. The ventilator pressures are the typical levels mostly used for COPD patients when bi-level ventilation is initiated during acute exacerbations.

The attending physician made a decision whether or not a patient would be suitable for the study and that substitution of two of their prescribed salbutamol doses by the two terbutaline sulphate study doses was acceptable.

9.2.1 Equipment and inhalation devices

The Aeroneb Professional Nebuliser:	Vibrational mesh Nebuliser System; Aerogen,Inc,USA
Sidestream jet nebuliser:	Intersurgical Ltd, UK; attached to a Porta Neb compressor
Porta Neb compressor:	Respironics, UK
Ventilator:	Nippy2 (B&D Electromedical, UK)
NIV breathing circuit:	A 180 cm length of autoclavable corrugated tubing, diameter of 22 mm and a fixed leak exhalation valve (B&D Electromedical, UK)
Electrostatic filter:	An electrostatic filter enclosed in a filter holder (Filta Guard breathing filter, Intersurgical Ltd, UK)
Normal saline:	Sodium chloride 0.9% respiratory solution, Teva, UK
Inhalation solution:	Terbutaline sulphate Respules labelled as a nominal dose of 5mg terbutaline sulphate in 2 ml (2.5mg/ml, Breath Limited, UK)
HPLC conditions:	Previously described in sections 3.2 and 3.3 in this thesis

9.2.2 Consent Procedure

This study involved patients who were seriously ill and thus would not have the capacity to consent at enrolment. This study therefore used a two stage consent procedure. In the first instance patients were assessed to determine their capacity to consent on the day of admission (defined and assessed by the attending physician according to Mental Capacity Act 2005). The study investigators who obtained the consent underwent formal training in this prior to the start of the study. In those with capacity, consent was obtained from the patient. Where capacity did not exist, the patient was enrolled into the study only with the agreement from a close relative. If a patient recovered his capacity within 72 hours of admission, his consent was sought in retrospect. If a patient declined to give this consent after regaining capacity then the samples that had been collected were destroyed. Where capacity was not regained the patient's data remained in the study.

On day 2 the study procedure was described and the patient was invited to the study. Hence delayed written informed consent procedures had been applied. If a patient decided not to be part of the study then they were withdrawn together with all their data and their samples were disposed.

9.2.3 Patients

Inclusion criteria

- Patients with an acute exacerbation of COPD requiring non-invasive mechanical ventilation for respiratory acidosis [as per NICE Guidelines (2004)]
- Under the care of a consultant chest physician
- Receiving nebulised bronchodilators

Exclusion Criteria

- Previous enrolment in the study

- Known hypersensitivity to terbutaline or salbutamol.
- Systolic blood pressure of <100 mmHg.
- Moderate or severe renal impairment defined as a Creatinine Clearance or eGFR of <20 ml min^{-1}.

9.2.4 Procedure

Patients were randomised to the terbutaline study dose on days 1 and 3 nebulised using either the Aeroneb Pro or the Sidestream nebuliser. The patients received their terbutaline sulphate study doses in place of their afternoon salbutamol dose.

The study doses were as follows:

- 2mg (0.8ml) of terbutaline sulphate respiratory solution (Terbutaline sulphate Respules, Breath Limited, UK) were nebulised from the Aeroneb Professional Nebuliser System (Aerogen, USA).
- 5mg (2ml) of terbutaline sulphate respiratory solution (Terbutaline sulphate Respules, Breath Limited, UK) with 2ml normal saline (sodium chloride 0.9% respiratory solutions, Teva, UK) were nebulised from a Sidestream jet nebuliser (Intersurgical limited, UK).

Patients voided their urine 15 minutes before the study dose. The nebuliser was placed in the ventilatory circuit as shown in Figure 9.1. The patients provided a urine sample 30 minute after the start of the study dose. Their urine was then pooled for the next 24 hours. The volume of the 30 minute and 24 hours collection samples were measured and assayed for the terbutaline concentration using an HPLC method previously described in section 3.3 in this thesis. All parts of the non-invasive ventilation tubing and the nebuliser were rinsed with water. The rinsing solutions were also assayed to determine their terbutaline sulphate amount using an HPLC method previously described in section 3.2 of this thesis.

On day 2 subjects received both the study doses for the ex-vivo part of the study. On this occasion a filter (Filta Guard breathing filter, Intersurgical limited, UK) was placed between the inhalation method and the patient's mask, as shown in Figure 9.1, hence the patients would receive no drug. This procedure on day 2 was carried out between two of each patient's routine bronchodilator doses and was extra to the routine management of the patient. These procedures only took about 5-10 minutes each and thus any disruption or inconvenience was minimal.

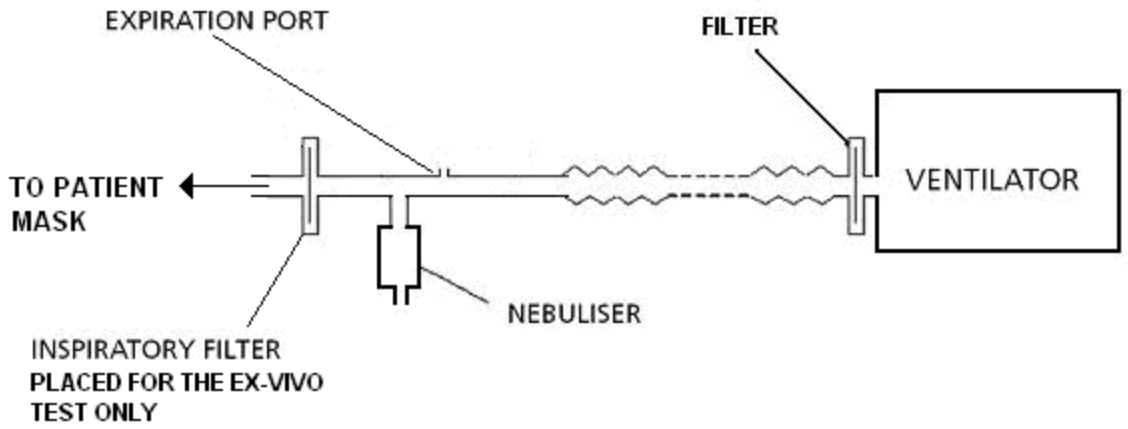


Figure 9.1 Schematic design of the nebuliser positions within the non-invasive circuit bi-level ventilator. The inspiratory filter was placed as shown in the circuit in the ex-vivo part of the study only.

Drug entrained on the filter was desorbed and the amount of terbutaline sulphate was determined. This represents the emitted dose the patient would have normally received. After each study dose the inhalation method was rinsed with water to collect the residual dose. The rinsing solutions were assayed for their terbutaline sulphate concentration using an HPLC method previously described in section 3.2 of this thesis.

The Sidestream jet nebulisers were one patient use according to standard ward practice and the ward washing procedures for the nebulisers applied. The Aeroneb Pro nebulisers were sterilised between use according to standard ward practice and the manufacturer's procedure for re-use.

9.2.5 Data analysis

The primary outcome measures were

- The relative bioavailability of terbutaline to the lungs following each inhalation method (30 minute urinary excretion).
- The relative bioavailability of terbutaline to the body following each inhalation method (24 hours urinary excretion).
- The fraction of the nominal dose that the patient would have received – (day 2).

The urine samples from the Aeroneb Pro and the Sidestream jet nebuliser were compared using a two way analysis of variance (ANOVA) test using SPSS V15.0 (SPSS Inc., Chicago, USA). Also a comparison between day 1 and day 3 treatments regardless of the inhalation method used (30 minute and 24 hours urinary excretion) were done using a two way analysis of variance (ANOVA) test using SPSS V15.0 (SPSS Inc., Chicago, USA). The ex-vivo results from the Aeroneb Pro and the Sidestream jet nebuliser were compared using a paired T-Test using SPSS V15.0 (SPSS Inc., Chicago, USA).

9.3 Results

Twelve (six females) NPPV patients with a mean (SD) age, weight and height of 74.8 (8.2) years, 61.0 (10.7) kg and 169.8 (12.4) cm, completed this study. FEV_1 is not measured in these patients due to the severity of their lungs and was anticipated to be <30 % predicted. Table 9.1 describe the demographic data of the patients. The mean (SD) and individual urinary excretion of terbutaline post inhalation of one dose of 5.0 mg terbutaline sulphate via Sidestream jet

nebuliser and 2 mg terbutaline sulphate via Aeroneb Pro are shown in Tables 9.2 and 9.3 and summarized in Figures 9.2-9.5. The mean (SD) and individual urinary excretion of terbutaline post inhalation of day 1 and day 3 (irrespective of inhalation method) are shown in Tables 9.4 and 9.5 and summarized in Figures 9.6 and 9.7. Tables 9.6 and 9.7 and Figures 9.8 and 9.9 show the mean (SD) and individual amount of terbutaline sulphate from the filter, T-piece and nebulisation chamber used in the ex-vivo part of the study post inhalation of one dose of 2 mg terbutaline sulphate via Aeroneb Pro and 5.0 mg terbutaline sulphate via Sidestream jet nebuliser expressed in µg, (n=12). The corrugated tubing and the filter next to the ventilator did not contain any terbutaline sulphate.

The mean (SD) terbutaline excreted in 30 minute period after the start inhalation of the study dose from the Sidestream jet nebuliser and Aeroneb Pro nebuliser were 10.4 (4.1) and 9.4 (3.7) µg, respectively as shown in Figure 9.2 and the mean (SD) 24 hours urinary excretion were 205.3 (58.0) and 192.3 (52.5) µg, respectively as shown in Figure 9.3. These values are equivalent to 0.25 (0.10), 0.57 (0.23), 5.00 (1.41) and 11.71 (3.19) %, respectively when calculated as a percentage of the nominal dose. The mean (SD) terbutaline excreted in day 1 and day 3 during the 30 minute post start of the inhalation irrespective of the nebulisation method was 9.6 (4.1) and 9.5 (3.8) µg, respectively as shown in Figure 9.6 and the mean (SD) 24 hours was 197.8 (65.0) and 198.9 (44.0) µg, respectively as shown in Figure 9.7. These values are equivalent to 0.40 (0.20), 0.40 (0.27), 8.41 (3.94) and 8.41 (4.61) %, respectively when calculated as a percentage of the nominal dose. The mean (SD) amounts of terbutaline sulphate entrained on the filter from the ex-vivo study arm after inhalation from the Sidestream jet nebuliser and Aeroneb Pro nebuliser were 1140.6 (186.7) and 771.7 (42.7) µg, respectively as shown in Tables 9.6 and 9.7. These values are equivalent to 22.8 (4.0) and 38.6 (2.3) %, respectively when calculated as a percentage of the nominal dose.

A summary of the statistical comparison between Aeroneb Pro and the Sidestream jet nebuliser is shown in Table 9.8 and between day 1 and day 3 (irrespective of inhalation method) in Table 9.9.

Table 9.1 Demographic data of the patients that participated in the study

Patient code	Gender	Age	Height	Weight	Sequence of nebulisers*
1	male	70.0	180.0	65.0	A1
2	female	83.0	150.0	40.0	A1
3	male	82.0	182.0	65.0	A3
4	female	63.0	162.0	61.0	A3
5	male	64.0	188.0	76.0	A3
6	female	81.0	160.0	61.0	A1
7	female	85.0	165.0	71.0	A1
8	female	83.0	156.0	43.0	A3
9	male	76.0	176.0	60.0	A1
10	female	64.0	160.0	55.0	A3
11	male	74.0	177.0	65.0	A1
12	male	72.0	182.0	70.0	A3
Mean		**74.8**	**169.8**	**61.0**	
SD		**8.2**	**12.4**	**10.7**	

*A1 Aeroneb Pro was used on day 1 and Sidestream on Day 3 and A3 Aeroneb Pro was used on day 3 and Sidestream on Day 1.

Table 9.2 Mean (SD) and individual urinary excretion of terbutaline and the amount of terbutaline sulphate left in the nebulisers post inhalation of one dose of 5.0 mg terbutaline sulphate via Sidestream jet nebuliser and 2.0 mg terbutaline sulphate via Aeroneb Pro expressed in µg, (n=12).

Nebuliser	Time (Hour)	1	2	3	4	5	6	7	8	9	10	11	12	Mean	SD
Aeroneb Pro	**0.5**	5.2	3.9	6.5	14.7	10.2	7.5	15.4	8.2	6.5	11.7	9.5	12.9	**9.4**	**3.7**
	24	275.8	198.4	208.4	276.9	164.8	174.3	194.1	186.7	143.7	237.4	102.9	143.8	**192.3**	**52.5**
	Remaining	950.0	1190.0	1250.2	1084.3	976.4	1134.8	1096.7	1204.9	995.7	1083.4	1108.3	976.4	**1087.6**	**98.0**
Sidestream jet nebuliser	**0.5**	5.4	4.6	7.9	16.8	11.8	8.7	16.9	8.7	6.7	12.8	10.9	13.7	**10.4**	**4.1**
	24	182.7	207.7	175.4	197.8	295.7	143.7	254.8	124.8	176.5	279.5	148.5	276.4	**205.3**	**58.0**
	Remaining	2489.3	2651.2	2479.3	2864.5	2795.3	2821.5	2719.9	2357.8	2265.8	2854.7	2497.6	2594.3	**2615.9**	**200.8**

Table 9.3 Mean (SD) and individual urinary excretion of terbutaline and the amount of terbutaline sulphate left in the nebulisers post inhalation of one dose of 5.0 mg terbutaline sulphate via Sidestream jet nebuliser and 2.0 mg terbutaline sulphate via Aeroneb Pro expressed in percentage of nominal dose, (n=12).

Nebuliser	Time (Hour)	1	2	3	4	5	6	7	8	9	10	11	12	Mean	SD
Aeroneb Pro	**0.5**	0.32	0.24	0.40	0.89	0.62	0.46	0.94	0.50	0.40	0.71	0.58	0.79	**0.57**	**0.23**
	24	16.79	12.08	12.69	16.86	10.03	10.61	11.82	11.37	8.75	14.45	6.26	8.76	**11.71**	**3.19**
	Remaining	47.5	59.5	62.5	54.2	48.8	56.7	54.8	60.2	49.8	54.2	55.4	48.8	**54.6**	**4.6**
Sidestream jet nebuliser	**0.5**	0.13	0.11	0.19	0.41	0.29	0.21	0.41	0.21	0.16	0.31	0.27	0.33	**0.25**	**0.10**
	24	4.45	5.06	4.27	4.82	7.20	3.50	6.21	3.04	4.30	6.81	3.62	6.73	**5.00**	**1.41**

	Remaining	49.8	53.0	49.6	57.3	55.9	56.4	54.4	47.2	45.3	57.1	50.0	51.9	**52.5**	**4.3**

Table 9.4 Mean (SD) and individual urinary excretion of terbutaline post inhalation of day 1 and day 3 (irrespective of inhalation method) expressed in µg, (n=12).

Nebuliser	**Day**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**Mean**	**SD**
30 minute	**Day 1**	5.2	3.9	7.9	16.8	11.8	7.5	15.4	8.7	6.5	12.8	9.5	13.7	**9.6**	**4.1**
	Day 3	5.4	4.6	6.5	14.7	10.2	8.7	16.9	8.2	6.7	11.7	10.9	12.9	**9.5**	**3.8**
24 hours	**Day 1**	275.8	198.4	175.4	197.8	295.7	174.3	194.1	124.8	143.7	279.5	102.9	276.4	**197.8**	**65.0**
	Day 3	182.7	207.7	208.4	276.9	164.8	143.7	254.8	186.7	176.5	237.4	148.5	143.8	**198.9**	**44.0**

Table 9.5 Mean (SD) and individual urinary excretion of terbutaline post inhalation of day 1 and day 3 (irrespective of inhalation method) expressed in percentage of nominal dose, (n=12).

Nebuliser	**Day**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**Mean**	**SD**
30 minute	**Day 1**	0.32	0.24	0.19	0.41	0.29	0.46	0.94	0.21	0.40	0.31	0.58	0.33	**0.40**	**0.20**
	Day 3	0.13	0.11	0.40	0.89	0.62	0.21	0.41	0.50	0.16	0.71	0.27	0.79	**0.40**	**0.27**
24 hours	**Day 1**	16.79	12.08	4.27	4.82	7.20	10.61	11.82	3.04	8.75	6.81	6.26	6.73	**8.41**	**3.94**
	Day 3	4.45	5.06	12.69	16.86	10.03	3.50	6.21	11.37	4.30	14.45	3.62	8.76	**8.41**	**4.61**

Table 9.6 Mean (SD) and individual amount of terbutaline sulphate recovered from the filter, T-piece and nebuliser's chamber from the day 2 ex-vivo study dosing of 2.0 mg terbutaline sulphate via Aeroneb Pro expressed in µg, (n=12).

Dose	Amount in µg Filter	T-piece	nebuliser	Amount deposited on the filter in % of nominal dose
1	800.0	210.0	848.3	40.0
2	743.4	252.0	933.7	37.2
3	741.3	248.8	848.9	37.1
4	751.6	206.2	909.1	37.6
5	815.1	250.0	717.0	40.8
6	809.4	231.2	752.0	40.5
7	850.4	170.2	731.1	42.5
8	724.0	164.0	885.0	36.2
9	741.9	179.0	616.8	37.1
10	714.2	289.2	993.4	35.7
11	764.8	181.2	968.6	38.2
12	804.8	189.7	927.1	40.2
Mean	**771.7**	**214.3**	**844.3**	**38.6**
STD	**42.7**	**39.6**	**115.9**	**2.3**
RSD	**5.5**	**18.5**	**13.7**	**5.9**

Table 9.7 Mean (SD) and individual amount of terbutaline sulphate recovered from the filter, T-piece and nebuliser's chamber from the day 2 ex-vivo study dosing of 5.0 mg terbutaline sulphate via Sidestream jet nebuliser expressed in µg, (n=12).

Dose	Amount in µg Filter	T-piece	nebuliser	Amount deposited on the filter in % of nominal dose
1	1032.6	295.7	2243.7	20.7
2	1248.5	399.4	2683.8	25.0
3	1545.0	167.5	2468.1	30.9
4	1289.1	176.5	1381.7	25.8
5	860.3	500.2	3356.9	17.2
6	1025.9	283.4	1091.9	20.5
7	887.5	490.2	3195.4	17.7
8	1128.9	258.7	1674.9	22.6
9	1111.2	576.8	3234.3	22.2
10	1097.6	303.8	2601.9	22.0
11	1226.7	382.7	2488.3	24.5
12	1233.6	335.6	3144.1	24.7
Mean	**1140.6**	**347.5**	**2463.7**	**22.8**
STD	**186.7**	**127.4**	**749.6**	**4.0**
RSD	**16.4**	**36.7**	**30.4**	**17.5**

Table 9.8 Mean difference (95% confidence interval) of Aeroneb Pro *vs* the Sidestream jet nebuliser.

Comparator	Aeroneb Pro *vs* Sidestream jet nebuliser
• **Amount excreted 30 minute post dose**	-1.1 (-1.4, -0.7)***
• **Amount excreted 24 hours post dose**	-13.0 (-60.8, 34.7)
• **Percent of the amount excreted 30 minute post dose**	0.32 (0.24, 0.39)***
• **Percent of the amount excreted 24 hours post dose**	6.7 (4.6, 8.9)***
• **Amount remaining in the nebuliser chamber and the connections in the in-vivo study**	-1528.342 (-1673.0, -1383.7)***
• **Amount resolved from the inhalation filter in the ex-vivo study**	-368.8 (-505.2, -232.5)***
• **Percent of the amount resolved from the inhalation filter in the ex-vivo study**	15.8 (12.4, 19.1)***

* p<0.05, ** <0.01 *** <0.001 otherwise no significant difference.

Table 9.9 Mean difference (95% confidence interval) of Day 1 *vs* Day 3 (irrespective of inhalation method).

Comparator	Day 1 *vs* Day 3
• **Amount excreted 30 minute post dose**	0.2 (-0.6, 1.0)
• **Amount excreted 24 hours post dose**	8.9(-39.3, 57.1)
• **Percent of the amount excreted 30 minute post dose**	-0.05 (0.27, 0.18)
• **Percent of the amount excreted 24 hours post dose**	-0.2 (-5.1, 4.8)

* p<0.05, ** <0.01 *** <0.001 otherwise no significant difference.

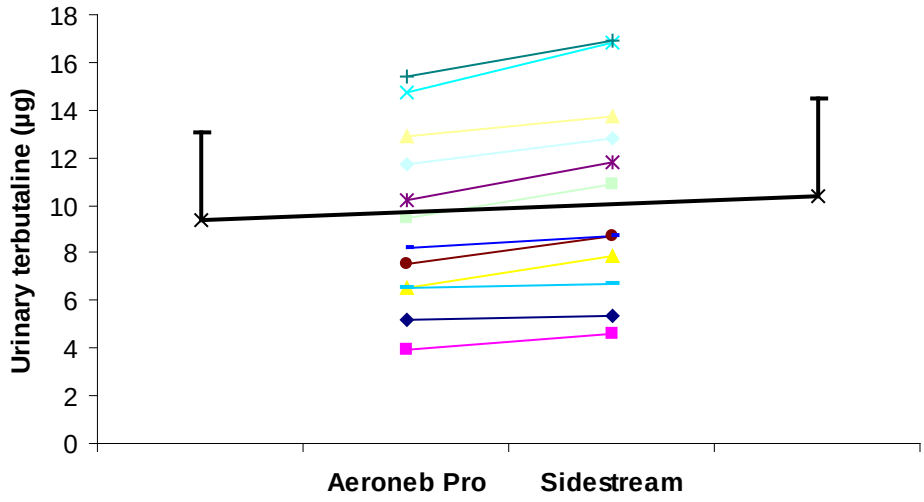


Figure 9.2 Mean (n=12) and the individual amounts in µg of urinary terbutaline excreted 30 minute post 5.0 mg terbutaline sulphate dosing via Sidestream jet nebuliser and 2.0 mg terbutaline sulphate via Aeroneb Pro.

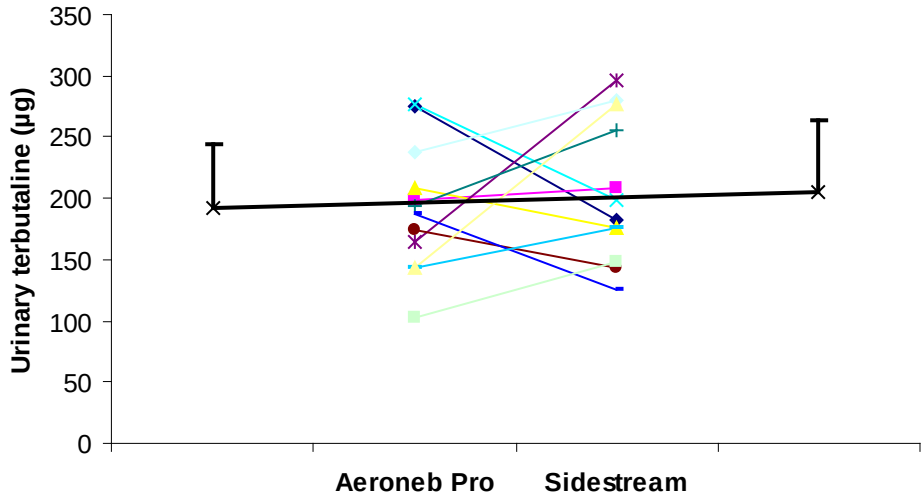


Figure 9.3 Mean (n=12) and the individual amounts in µg of urinary terbutaline excreted in the first 24 hours post 5.0 mg terbutaline sulphate dosing via Sidestream jet nebuliser and 2.0 mg terbutaline sulphate via Aeroneb Pro.

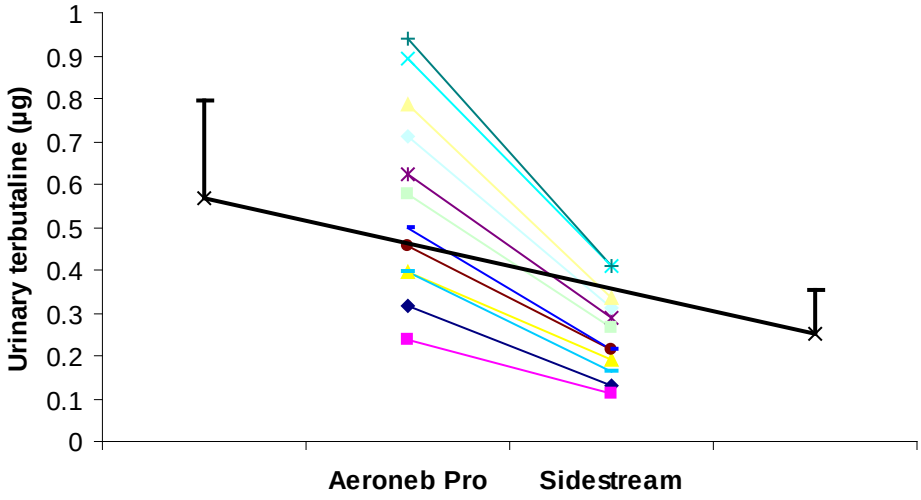


Figure 9.4 Mean (n=12) and the individual percentage of nominal dose of urinary terbutaline excreted 30 minute post 5.0 mg terbutaline sulphate dosing via Sidestream jet nebuliser and 2.0 mg terbutaline sulphate via Aeroneb Pro.

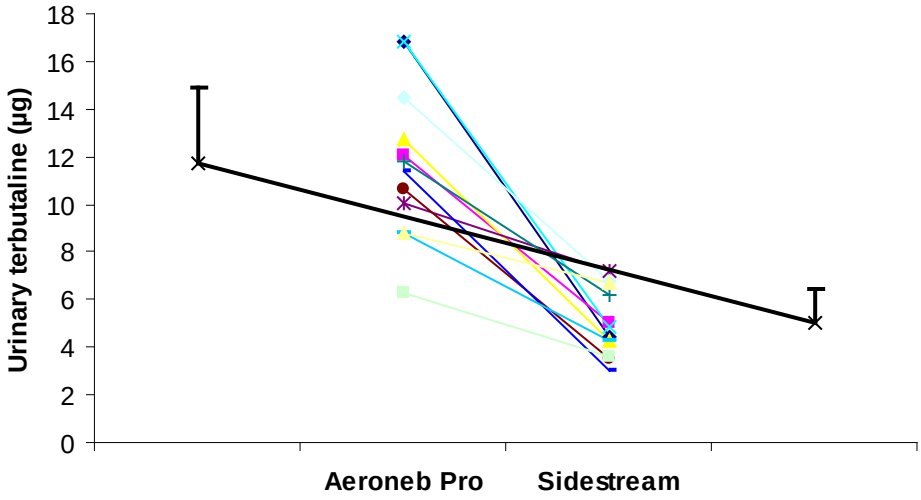


Figure 9.5 Mean (n=12) and the individual percentage of nominal dose of urinary terbutaline excreted in the first 24 hours post 5.0 mg terbutaline sulphate dosing via Sidestream jet nebuliser and 2.0 mg terbutaline sulphate via Aeroneb Pro.

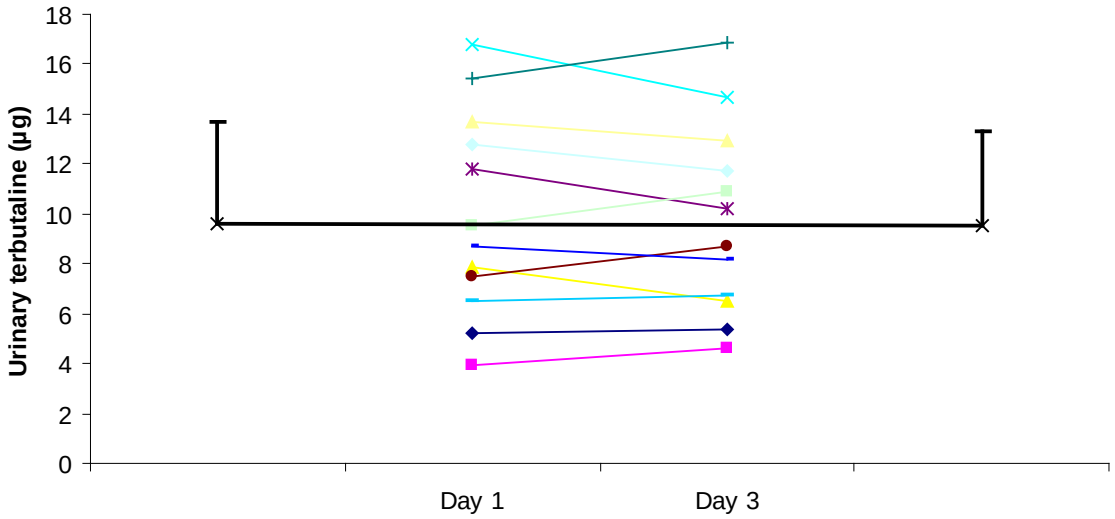


Figure 9.6 Mean (n=12) and the individual amounts in µg of urinary terbutaline excreted 30 minute post inhalation of day 1 and day 3 (irrespective of inhalation method) expressed in µg.

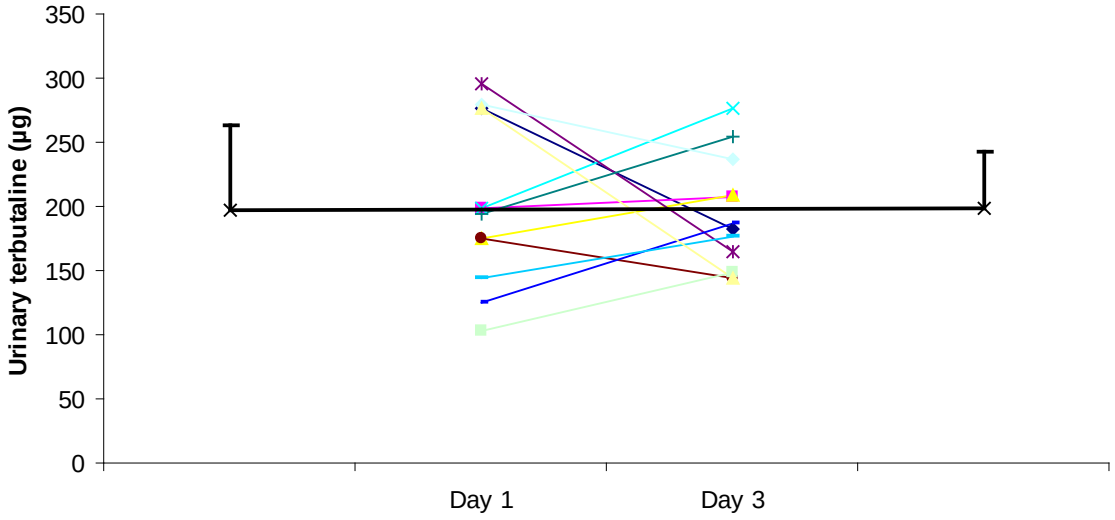


Figure 9.7 Mean (n=12) and the individual amounts in µg of urinary terbutaline excreted in the first 24 hours post inhalation of day 1 and day 3 (irrespective of inhalation method) expressed in µg.

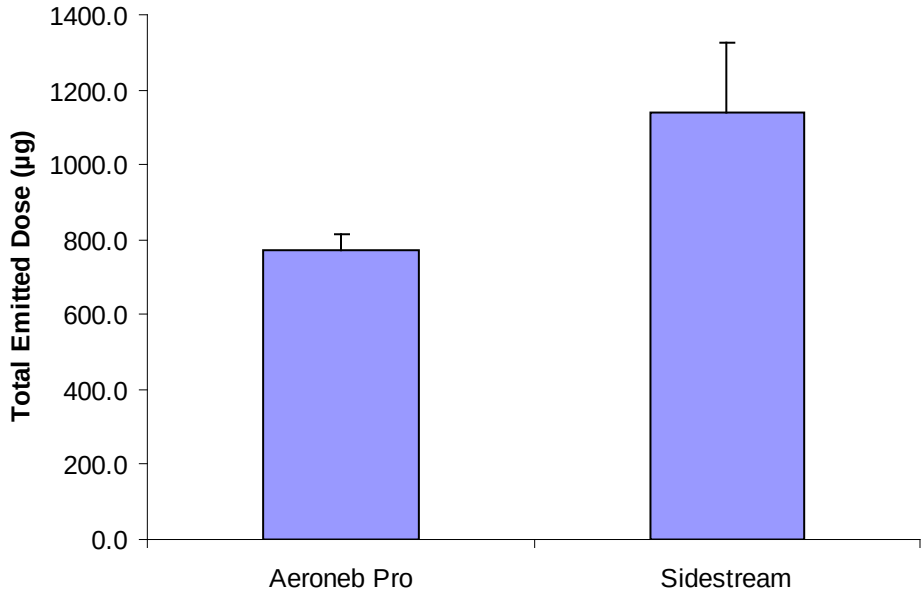


Figure 9.8 Mean (SD) amounts of terbutaline sulphate recovered from the filter from the day 2 ex-vivo study. This filter was placed between the mask and the nebuliser post inhalation of one dose of 5.0 mg terbutaline sulphate from the Sidestream jet nebuliser and one dose of 2.0 mg terbutaline sulphate from the Aeroneb Pro expressed in µg, (n=12).

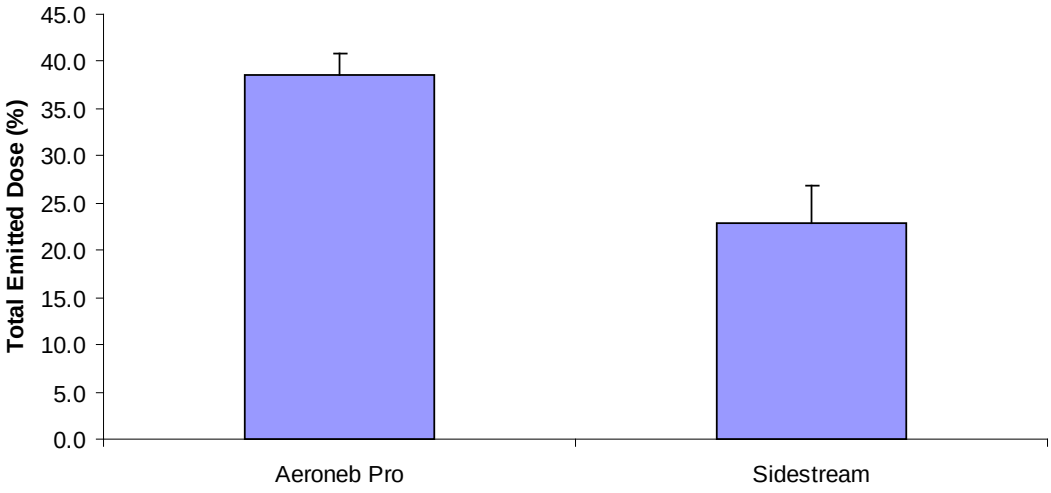


Figure 9.9 Mean (SD) amounts of terbutaline sulphate, expressed in % of nominal dose, sulphate recovered from the filter from the day 2 ex-vivo study. This filter was placed between the mask and the nebuliser post inhalation of one dose of 5.0 mg terbutaline sulphate from the Sidestream jet nebuliser and one dose of 2.0 mg terbutaline sulphate from the Aeroneb Pro, (n=12).

9.4 Discussion

Although it was appreciated that there would be a difference between each patient's condition on days 1 and 3 the statistical comparison between the amounts excreted on day one and day three for the 30 minute and 24 hours post dosing samples (irrespective of inhalation method) showed no significant difference. This was consistent with a study by Mazhar et al (2007) which has been recently completed measuring urinary drug excretion following study doses from a MDI+Spacer and a Sidestream nebuliser using patients with an acute exacerbation of asthma (n=11) and COPD (n=19) on day 2 and 4 following hospitalisation (Mazhar et al., 2008). The relative lung deposition between day 2 and 4 (irrespective of inhalation method) was similar. The latter suggests that lung deposition characteristics did not change significantly as the patient's FEV_1 improved and that if there were any changes in renal function the difference was not detected. None of these patients had moderate or severe renal impairment. The results is also consistent with another urinary pharmacokinetic studies post inhalation (Ismail and Chrystyn, 2004).

The corrugated tubing and the filter next to the ventilator did not contain any Terbutaline sulphate. This might be due to the effect of positive pressure which keeps most of the drug next to the patient.

The amount left in the nebulisation chamber and the connections of the Sidestream jet nebuliser [2615.9 (200.8) µg] were significantly higher than that left in the nebulisation chamber and connections of the Aeroneb pro nebuliser [1087.6 (98.0) µg] ($p<0.001$). The difference is about 2.5 times that of the Aeroneb pro nebuliser and this is due to the low residual volume of Aeroneb Pro compared to the Sidestream jet nebuliser.

As shown in table 9.8 the Sidestream jet nebuliser resulted in a significantly higher relative lung deposition than the Aeroneb Pro ($p<0.001$). That was consistent with the amount deposited on the inhalation filter in the ex-vivo results ($p<0.001$). Also there was no significant difference between the relative systemic bioavailability form the Aeroneb Pro

and the Sidestream jet nebuliser, however the doses were different. Hence, when the amounts were normalised to the percentage of the nominal dose; the relative lung and systemic bioavailability from the Aeroneb Pro was significantly greater ($p<0.001$) than that of the Sidestream jet nebuliser. The percentage of terbutaline sulphate deposited on the filter after Aeroneb Pro was also greater in the ex-vivo study ($p<0.001$). The results for the Aeroneb Pro may be in part due to the small residual volume of the Aeroneb Pro which is less than 0.3ml (Fink et al., 2001) while the Sidestream jet nebuliser had a very high residual volume.

The results are consistent with the in-vitro results in chapter 6 in that the vibrational mesh nebulisers emit higher emitted dose and a higher respirable amount (the fine particle dose) than the Sidestream jet nebuliser ($p<0.001$). Also the patient results are consistent with the in-vivo results from the healthy volunteers in chapter 8. There was upto about three-fold improvement in the relative amounts deposited in the lungs from the Aeroneb pro compared to the Sidestream jet nebuliser and a 2.7-fold increase of the relative amount delivered to the body (systemic bioavailability). This difference has been obtained in subjects who have been trained and demonstrated an excellent inhalation technique. The ratio here is lower than in chapter 8 and that might be due to the smaller fill volume (0.8 ml) of the Aeroneb Pro compared to 2 ml in chapter 8.

The amount of terbutaline excreted 30 minute post dosing from any of the two nebulisers used was close to that from two (250µg terbutaline sulphate) doses from MDI and much lower than that from two (250µg terbutaline sulphate) doses from MDI+Spacer to healthy volunteers as shown in section 8.3 of this thesis even though the nebulised dose was much higher. Although the results from section 8.3 are from health volunteer who were trained to use the device properly and with larger lung volumes, the lower lung deposition in this chapter show that the nebulisers are a poor method for aerosol delivery compared to properly used MDI with spacer.

The in-vivo and the ex-vivo results here are also consistent with a study by Fink et al (2001) who compared the ability of the Aeroneb Pro and two small volume jet nebulisers, MistyNeb (Allegiance) and Vix One (Westmed), to deliver 2.5 mg salbutamol sulphate in 3 ml to a simulated pediatric patient during mechanical ventilation. The Aeroneb Pro delivered significantly higher ($p<0.02$) amounts of salbutamol [582.0(89.0) µg] than the MistyNeb [201.0(87.0) µg] or the Vix One [197.0(50.0) µg] (Fink et al., 2001) with no modification of ventilator parameters. The authors also showed that operation of the Aeroneb Pro did not alter any of the monitored ventilator parameters. In contrast, the jet nebulisers increased the mean airway pressure during operation by >5 cm H_2O and required adjustment of ventilator flow settings during operation to maintain ventilator parameters with initiation and discontinuation of nebulization. They also found that when opening the medication reservoir to refill the Aeroneb Pro there was no change in any of the ventilator parameters. In contrast, both of the jet nebulisers required interruption of ventilation in order to refill.

A study by Dubus et al (2005) showed similar results to that shown in this chapter. The objective of their study was to assess lung delivery of aerosols in an animal model of neonatal ventilation using a conventional jet nebuliser and a novel vibrating mesh nebuliser. Aerosol deposition studies with 99mTc diethylenetriamine pentaacetate (99mTc-DTPA) were performed in four macaques (2.6 kg) that were ventilated with neonatal settings (peak inspiratory pressure 12-14 mbar (4.8-5.6 cm H_2O), positive end-expiratory pressure 2 mbar (0.8 cm H_2O), Inspiratory / Expiratory ratio 1/2, respiratory flow 40 L min^{-1}. They compared the MistyNeb jet-nebuliser (3-ml fill volume), with a vibrating mesh nebuliser operating continuously [Aeroneb Professional Nebuliser (APN-C); 0.5-ml fill volume] and another synchronized with inspiration [Aeroneb Professional Nebuliser Synchronized (APN-S); 0.5-ml fill volume]. The amount of radioactivity deposited into the lungs, the connections and remaining in the nebuliser was measured by a gamma counter.

Despite similar amounts of 99mTc-DTPA in the respiratory circuit with all nebulisers, both the APN-S and the APN-C delivered more drug to the lungs than the MistyNeb [14.0, 12.6, and 0.5% in terms of percentage of nebuliser fill volume, respectively; $p = 0.006$] (Dubus et al., 2005). Duration of delivery was shorter with the APN-C than with the two other nebulisers (2 versus 6 and 10 min for the APN-S and the MistyNeb, respectively; $p < 0.001$).

Even though the animal model has limited relevance to humans because of differences in anatomy, the absence of underlying lung disease and the lower lung deposition in these studies, the results of the above study as well as the results of the study in this chapter shows that Aeroneb Pro is more efficient than the Sidestream jet nebuliser.

The design of the vibrating mesh nebulisers is such that the fill volume required to produce the aerosol is much lower than with the jet nebuliser (0.5 versus 3 ml). The ability of the Aeroneb Pro nebulisers to deliver more aerosols to the lungs than the jet nebuliser may have positive implications in the treatment of critically mechanically ventilated patients.

9.5 Conclusions

The urinary terbutaline pharmacokinetic method was very sensitive in detecting the difference between the Aeroneb Pro and the Sidestream jet nebulisers.

The relative lung deposition from 5.0 mg terbutaline sulphate nebulised from the Sidestream “jet nebuliser” was 1.1 times that from 2.0 mg terbutaline sulphate nebulised from an Aeroneb Pro “vibrating mesh nebuliser”. However the dose was 2.5 times higher. Thus when comparing the percentage of the nominal dose as the relative lung deposition, the Aeroneb Pro results in more than a 2 fold increase than the Sidestream jet nebuliser. The small residual volume of the Aeroneb Pro (0.3 ml) allows the maximum use of the nebulised solution. The results were consistent with the previously published literatures and the ex-vitro results in this chapter.

Vibrating mesh nebulisers are more efficient to administer aerosols to the mechanically ventilated patient. This level of aerosol delivery may make aerosol delivery during mechanical ventilation more practical.

10.1 Summary and future work

Terbutaline sulphate is a potent short acting β_2-agonist which is recommended for the management of asthma and COPD by the BTS and NICE guideline, respectively, to be used as required to relieve symptoms. Terbutaline sulphate is available as the Bricanyl Turbuhaler in the form of a dry powder inhaler, a MDI and Respules for nebulisation (AstraZeneca).

The assessment of pulmonary drug absorption and deposition is becoming increasingly important in drug development. Several methods are available to investigate pulmonary drug absorption and deposition, e.g. simulated in-vitro experiments, in-vivo pharmacokinetic and pharmacodynamic and gamma scintigraphy. In combination, these methods can indicate the fate of an inhaled drug.

In-vitro methods are used as a quality assurance procedure to identify the quality of the inhaled product such as the total emitted dose, uniformity of dose, and the aerodynamic particle size distribution. Further, they are often extrapolated to give an estimation of in-vivo deposition.

Pharmacokinetic methods (using plasma or urine samples), can be used to identify the relative lung deposition of the drug (the effective lung dose) and total systemic delivery. Some studies have used charcoal, taken before and after an inhalation, to overcome the problem of oral absorption (Borgstrom and Nilsson, 1990), but the use of charcoal for patients with concomitant use of other drugs would not be ethical as it would block their therapeutic effect. It was found that the measurements of urine concentration of the drug and hence amount excreted in the absorption lag time of the orally swallowed portion (30 minutes) would account mainly for the drug absorbed from the lung after inhalation (Hindle and Chrystyn, 1992) and hence is a useful index of the lung deposition in cross-over studies.

This research work has focused on the identification and the development of this method, urinary terbutaline pharmacokinetic method along with identification of in-vitro parameters to characterise the aerodynamic properties of the inhalation methods used in the in-vivo studies. Hence the objectives were:

a) To validate HPLC methods for the determination of terbutaline in aqueous and urine samples.

b) To use in-vitro methods to highlight the potential of the Turbuhaler to deliver terbutaline sulphate to the lungs, to determine the effect of the spacers with the MDI on the dose emitted from the MDI and to compare the emitted dose nebulised from the Aeroneb Pro and Sidestream jet nebuliser.

c) To validate a urinary pharmacokinetic method for the evaluation of the relative lung bioavailability of terbutaline to the lungs and the body following inhalation.

d) To use the validated pharmacokinetic method to determine the effect of inhalation flow on the lung deposition from the Turbuhaler and the effect of spacers attached to the MDI. Also to compare the lung deposition following Aeroneb Pro and Sidestream jet nebuliser using volunteers and patients.

In Chapter 3 a novel HPLC assay for the estimation of terbutaline sulphate in aqueous samples was validated. The samples were separated using a 5µm Spherisorb, ODS1 (4.6 x 250mm, C-18, Waters Chromatography) column maintained at 30^{o}C. The mobile phase consisted of 5mM potassium dihydrogen orthophosphate buffer : acetonitrile (75:25). The buffer was adjusted to pH 2.5, with orthophosphoric acid. The mobile phase was filtered through a 45mm membrane filter (Millipore, Whatman Ltd, UK) and degassed under vacuum in an ultrasonic bath for 10 minutes prior to use. A fluorescence detector set with an excitation/emission of 267/313 nm was used. The calibration for terbutaline sulphate aqueous samples was linear using bamethane as the internal standard for terbutaline sulphate concentrations from 10µg/L to 800µg/L. The method had an accuracy of >95%

and intra and inter-day precision CV% of <2.8 and 4.5%, respectively at three (50, 300 and 700 µg/L corresponding to low, medium, and high) concentrations. The limit of detection (LOD) and lower limit of quantitation (LLOQ) for terbutaline sulphate was 10.9µg/L and 33.1µg/L, respectively.

A HPLC assay for the estimation of terbutaline in urine samples was also validated. Two solid phase extraction (SPE) methods, using Isolute HCX 130mg 10ml XL cartridge (Pre-hydrolysis) and Oasis HLB 30mg cartridge (Post hydrolysis), were validated to isolate terbutaline from a urine matrix followed by HPLC with fluorescence detector. This extraction procedure cleaned the final analytes from impurities such that their peaks were separated and easily detected. The urine assay was performed in accordance with FDA and ICH regulations of bio-analytical samples. The samples were separated on an ODS 5µm, (4.6 x 250mm, Zorbax, Phenomenex) C-18 HPLC column with a (4mm X 3 mm, Phenomenex); C-18 (ODS) guard column. Both were maintained at 30^{o}C. The mobile phase was acetonitrile : methanol : tetrahydrofuran : ethyl acetate : buffer 5:5:5:5:80% v/v. The buffer was 40mM phosphate buffer and 27.5mM sodium dodecyl sulphate with pH 6.75 adjusted using 10mM KOH. Fluorescence detection set with an excitation/emission of 267/313 nm was used. The extraction recovery of terbutaline from urine samples was >95%. The standard calibration for extracted terbutaline urine samples was with bamethane (r^2=0.9983) and salbutamol (r^2=0.9994), respectively as the internal standard over terbutaline concentrations from 10µg/L to 1200µg/L. The method had an accuracy of >90% and intra and inter-day precision CV% of <7.5 and 13.0%, respectively at three (100, 500 and 1000 µg/L corresponding to low, medium, and high) concentrations. The limit of detection (LOD) and lower limit of quantitation (LLOQ) for terbutaline was 24.2µg/L and 73.4µg/L, respectively.

Some patients with COPD can only inhale at low flows when using DPIs especially if the resistance of the inhaler is high. Most DPIs have flow dependent dose emission properties,

especially the Turbuhaler. It has been also shown that during its use the Turbuhaler has a minimum threshold for the inhalation flow for the emission of a dose with the potential for lung deposition. For the in-vitro determination of the aerodynamic characterization of the dose emitted from an inhaler the Pharmacopoeias recommend an inhalation volume of 4 L through the inhaler with the inhalation flow maintained constant at a flow that produces a pressure drop of 4 kPa across the inhaler. For the Turbuhaler this is equal to an inhalation flow of approximately 54 L min^{-1} which is not achievable by many patients. The main focus of chapter 4 was an in-vitro evaluation of the dose emitted from the Bricanyl Turbuhaler at different inhalation flows (ranging from 10 to 60 L min^{-1}). 4 L was used as the inhalation volume. In-vitro flow dependent dose emission was demonstrated for terbutaline sulphate emitted from the Turbuhaler. The effect of this was more pronounced for the fine particle dose. The higher inhalation flow resulted in a greater emitted dose and FPD than at a lower inhalation flow. The flow dependent dose emission results highlight the need for the Pharmacopoeias to use a variety of inhalation flows for in-vitro tests rather than one that produces a pressure drop of 4 kPa across the inhaler. Also these flows should be consistent with the range normally produced by the patients. The clinical relevance of the flow dependent and inconsistent dosage characteristics of terbutaline sulphate in the Turbuhaler requires further investigation.

It is claimed that spacers used with the MDI increase the FPD and hence decrease the gastrointestinal ingested portion as well as improving the lung deposition. They are recommended for use in children under 6 years and older patients that have a co-ordination problem. Hence in chapter 5 the in-vitro characteristics of the MDI and MDI plus four different spacers was studied in accordance with compendial methodology. The inhalation flow used was 28.3 L min^{-1} with an inhalation volume of 4 L. The MDI alone resulted in the highest emitted dose. The spacer removed most of the amount that would deposit in the mouth and throat hence the emitted dose from MDI+Spacer was less than the MDI alone.

The FPD from the AeroChamber Max was the highest but the AeroChamber Plus resulted in the smallest MMAD and the highest FPF.

The aerodynamic characteristic of the dose emitted from the AeroChamber Max (antistatic spacer) was better than the rest of the static spacers (Fisonair and Nebuhaler). The aerodynamic characteristic from the Fisonair without washing (antistatic spacer) was better than the Fisonair with washing (static spacer). Hence, the dose emitted and the FPD from plastic spacer devices will vary according to the electrostatic charge on the spacer walls.

A new type of nebuliser which use a vibrating mesh to produce aerosol was found to be more efficient than a jet nebuliser. In chapter 6 the in-vitro determination of nebulised terbutaline sulphate respiratory solution from two different nebulisers was studied using the CEN method and the NGI method. The Aeroneb Pro, as a vibrating mesh, produced a higher emitted dose and FPD than the Sidestream jet nebuliser but there was no significant difference in regard of FPF and MMAD. The higher emitted dose and FPD would suggest better lung deposition. Also using two different nebulizing systems it has been shown that the use of cooling and operation at an inhalation flow of 15 L min^{-1} limits evaporation effects when using the NGI. The aerodynamic characteristics of the nebulised dose from these two nebuliser systems using the CEN method were significantly different from when using NGI method. Hence further investigation for both methods is required together with the issue of the evaporation effects. Nevertheless the results consolidate the current growing consensus that a cooled NGI operated at 15 L min^{-1} should be the recommended methodology in the European pharmacopoeia.

In each of the in-vivo experiments twelve healthy non-smoking volunteers (6 females) completed the study. In chapter 7 validation of the salbutamol urinary pharmacokinetic method by Hindle and Chrystyn (1992) for the determination of urinary terbutaline is described. On a separate occasion each volunteer received the following doses:

(a) The oral administration of 500μg terbutaline sulphate in 20 ml water [O].

(b) The oral administration of 500μg terbutaline sulphate in 20 ml water with 20g activated charcoal (10g in 50 ml water before and after dosing) [OC].

(c) Two 250μg doses inhaled from a terbutaline sulphate metered dose inhaler (Bricanyl inhaler, AstraZeneca, UK) [I].

(d) Two 250μg doses inhaled from a terbutaline sulphate metered dose inhaler with 20g activated charcoal (10g in 50 ml water before and after dosing) [IC].

Each volunteer voided their urine pre-dosing and provide urine samples 0.5, 1, 2, 4, 6, 12, and 24 hours post study doses. No terbutaline was excreted post OC. The amount excreted following IC represents the pulmonary absorbed fraction. The use of activated charcoal was found to be unnecessary when this method is used to compare between different products or techniques. That was due to the lack of a difference between I and IC in the first 30 minutes because the majority of drug excreted in this time period is delivered to the body via the lungs. This is shown by the amount of terbutaline excreted 30 minutes post I and IC which were significantly higher than post O dosing ($p<0.001$). Also the cumulative amount of terbutaline excreted 24 hours post I and O was relatively similar. Hence the 24 hours excrtion can be used as an index of the relative systemic bioavailability.

Another group of volunteers inhaled 1, 2, 3 and 4 doses from the Bricanyl MDI (AstraZeneca, UK) on separate occasion to study the dose response relationship of the terbutaline urinary excretion method. The volunteers voided their urine pre-dosing and provide urine samples at 0.5 and cumulatively 24 hours post study dose. The method was found to be linear similar to that for the urinary salbutamol method (Tomlinson et al., 2003). To further validate the method a group of volunteers inhaled two doses on five separate occasions from the Bricanyl MDI (AstraZeneca, UK) in separate occasion to identify the intra and inter-subject variability of the terbutaline urinary excretion method. The volunteers voided their urine pre-dosing and provide urine samples at 0.5 hour post

study dose. The method was found to be reproducible similar to that for the urinary salbutamol method (Tomlinson et al., 2003).

Thus the urinary drug excretion technique, which has already been considered to be the basis for a novel clinical equivalence test for inhalation products of salbutamol (Hindle and Chrystyn, 1992) and extended to sodium cromoglycate (Aswania et al., 1997; Aswania and Chrystyn, 2002), nedocromil (Aswania et al., 1998), gentamicin (Al-Amoud et al., 2005; Al-Amoud et al., 2002), tobramycin. (Barber, 2002) and formoterol (Nadarassan et al., 2007), can also be used for other water-soluble inhaled drugs like terbutaline.

Chapter 8 provides some applications of the validated urinary terbutaline pharmacokinetic method. The urinary terbutaline pharmacokinetic method was used to detect the effect of different inhalation techniques when using the Turbuhaler. The volunteers in this study inhaled two doses of 500µg terbutaline sulphate from a Bricanyl Turbuhaler (AstraZeneca, UK) on separate occasion using slow and fast inhalation flows. They were trained how to inhale at a slow inhalation flow (30 L min^{-1}) and at a fast inhalation flow (60 L min^{-1}) with their inhalation flow checked by the In-Check Dial. The volunteers voided their urine pre-dosing and provided urine samples at 0.5 and cumulatively 24 hours post study dose. The amount of terbutaline excreted 30 minutes and 24 hours post dosing using a fast flow were significantly higher than that using a slow flow ($p<0.001$). The results are consistent with that from the in-vitro study in chapter 4 and some other in-vivo studies which recommend the patient to inhale as fast as possible when inhaling a dose from the Turbuhaler.

The urinary terbutaline pharmacokinetic method was then used to determine the effect of different spacers on the lung and the systemic bioavailability of inhaled terbutaline from the MDI. On separate occasions the volunteers in this study inhaled two doses of 250µg terbutaline sulphate from a Bricanyl MDI (AstraZeneca, UK) either alone or with different spacers. Again the volunteers voided their urine pre-dosing and provided urine samples at 0.5 and cumulatively 24 hours post study dose. It was found that the MDI alone resulted in

a significantly higher urinary excretion of terbutaline 24 hours post dose and lower urinary excretion of terbutaline 30 minutes post dose than when using the MDI attached to any of the spacers ($p<0.001$). This was consistent with the in-vitro results in chapter 5 and the published literature which highlights that spacer removes most of the large particles that would normally deposit in the mouth which are than swallowed followed by gastrointestinal absorption. The spacer also allows the aerosol to evaporate resulting in smaller particles and hence increase lung deposition. The AeroChamber Max resulted in a significantly higher urinary excretion of terbutaline 30 minutes and 24 hours post dose than any other spacers ($p<0.001$-0.05). When comparing these results to the in-vitro results in chapter 5 it was found that even though the AeroChamber Plus resulted in the best MMAD and FPF the AeroChamber Max with its high emitted dose and FPD provided the highest lung deposition.

The last study in this chapter was to check whether the urinary terbutaline pharmacokinetic method was able to determine the effect of different nebuliser on the lung and the systemic bioavailability of nebulised terbutaline. The volunteers inhaled, on separate occasions, 5mg/2ml terbutaline sulphate from Bricanyl Respules (AstraZeneca, UK) from Aeroneb Pro (vibrating mesh nebuliser) and Sidestream (Jet nebuliser). The volunteers voided their urine pre-dosing and provided urine samples at 0.5 and cumulatively 24 hours post study dose. Similar to the result of the in-vitro work in chapter 6 the Aeroneb Pro produced higher amounts excreted 30 minutes and 24 hours post dosing than the Sidestream jet nebuliser ($p<0.001$).

The three above mentioned studies in chapter 8 demonstrates that the urinary pharmacokinetic method previously reported for terbutaline in chapter 7 is a potential tool to compare different inhalation techniques, methods and products. This consolidate that the 30 minutes post-inhalation urinary excretion is a reliable index of total lung bioavailability

and the 24 hours urinary terbutaline measurement can be used as an index of the systemic bioavailability.

An extension of the applications of the urinary terbutaline pharmacokinetic method to patients is described in chapter 9. The study in this chapter was to investigate whether the urinary terbutaline pharmacokinetic method was able to determine the effect of different nebuliser on the lung and the systemic bioavailability of nebulised terbutaline in non-invasive positive pressure mechanically ventilated patient. The results were compared to an ex-vivo experiments performed on the same patients.

The patients inhaled, on separate occasions, 5mg in 2ml terbutaline sulphate from terbutaline sulphate Respules (Breath Limited, UK) from Sidestream (Jet nebuliser) and 2mg in 0.8ml terbutaline sulphate from terbutaline sulphate Respules (Breath Limited, UK) from Aeroneb Pro (vibrating mesh nebuliser). The volunteers voided their urine pre-dosing and provided urine samples at 0.5 and cumulatively 24 hours post study dose. The Aeroneb Pro nebuliser resulted was found to have better efficacy than the Sidestream nebuliser. That was shown from results of the urinary terbutaline pharmacokinetic method to patients and the ex-vivo method.

10.2 Future work

The in-vitro characterization of the nebulised dose using a cooled NGI operated at low flow of 15 L min^{-1} has consolidate the consensus that this methodology limits evaporation and should be the compendial method. Further work in this area is required. First there should be some investigation of using the NGI in a cooled environment possibly inside a fridge. Another aspect could look into using a relative humidity similar to that in the lungs and also using a temperature of 37 ^{o}C. The thermal and the metal effect from the metal NGI at these conditions would need to be investigated. In addition to this work in-vivo studies are essential. These could use the urinary pharmacokinetic method to compare

different systems with simultaneous clinical measurements. Such data could than be compared to that of the in-vitro methods to identify which was the most relevant.

11. References

Adams, N. P., Bestall, J. C., Lasserson, T. J.Jones, P. W. (2005) Fluticasone versus placebo for chronic asthma in adults and children. *Cochrane Database Syst Rev,* **4**.

Adams, W. P., Poochikian, G., Taylor, A. S., Patel, R. M., Burke, G. P.Williams, R. L. (1994) Regulatory aspects of modifications to innovator bronchodilator metered dose inhalers and development of generic substitutes. *J Aerosol Med,* **7**(2)**,** 119-34.

Al-Amoud, A. I., Clark, B. J., Assi, K. A.Chrystyn, H. (2005) Determination of the bioavailability of gentamicin to the lungs following inhalation from two jet nebulisers. *Br J Clin Pharmacol,* **59**(5)**,** 542-545.

Al-Amoud, A. I., Clark, B. J.Chrystyn, H. (2002) Determination of gentamicin in urine samples after inhalation by reversed-phase high-performance liquid chromatography using pre-column derivatisation with o-phthalaldehyde. *J Chromatogr B Analyt Technol Biomed Life Sci,* **769**(1)**,** 89-95.

Al-Showair, R. A. M., Pearson, S. B.Chrystyn, H. (2007) The potential of a 2Tone Trainer to help patients use their metered dose inhalers. *Chest,* chest.06-2765.

Allen, S. C. and Prior, A. (1986) What determines whether an elderly patient can use a metered dose inhaler correctly? *Br J Dis Chest,* **80**(1)**,** 45-9.

Allen, T. (1990) Sampling and sizing from the atmosphere. In: *Particle size measurement*. Vol. 1 New York: Chapman and Hall, pp. 78-80.

Altman, L. C., Hill, J. S., Hairfield, W. M.Mullarkey, M. F. (1981) Effects of corticosteroids on eosinophil chemotaxis and adherence. *J Clin Invest,* **67**(1)**,** 28-36.

Alvine, G. F., Rodgers, P., Fitzsimmons, K. M.Ahrens, R. C. (1992) Disposable jet nebulisers. How reliable are they? *Chest,* **101**(2)**,** 316-319.

Ambrosino, N., Foglio, K., Rubini, F., Clini, E., Nava, S.Vitacca, M. (1995) Non-invasive mechanical ventilation in acute respiratory failure due to chronic obstructive pulmonary disease: correlates for success. *Thorax,* **50**(7)**,** 755-7.

Analytical Profiles (1990) Terbutaline. In: *Analytical profiles of drug substances.* Vol. 19 London: Academy of Pharmaceutical Sciences. Pharmaceutical Analysis and Control Section. Academic press, INC., pp. 601-625.

Anderson, G. P. (1993) Formoterol: pharmacology, molecular basis of agonism, and mechanism of long duration of a highly potent and selective beta 2-adrenoceptor agonist bronchodilator. *Life Sci,* **52**(26)**,** 2145-60.

Andersson, P. (1976) Bronchospasmolytic and cardiovascular effects in anaesthetized cats of ibuterol and terbutaline given intravenously and after inhalation: drug and prodrug compared. *Acta Pharmacol Toxicol (Copenh),* **39**(2)**,** 225-31.

Anhoj, J., Bisgaard, H.Lipworth, B. J. (1999) Effect of electrostatic charge in plastic spacers on the lung delivery of HFA-salbutamol in children. *Br J Clin Pharmacol,* **47**(3)**,** 333-336.

Ashurst, I., Malton, A., Prime, D.Sumby, B. (2000) Latest advances in the development of dry powder inhalers. *PSTT,* **3**(7)**,** 246-256.

Aswania, O. and Chrystyn, H. (2001) Relative lung bioavailability of generic sodium cromoglycate inhalers used with and without a spacer device. *Pulm Pharmacol Ther,* **14**(2)**,** 129-33.

Aswania, O. and Chrystyn, H. (2002) Relative lung and systemic bioavailability of sodium cromoglycate inhaled products using urinary drug excretion post inhalation. *Biopharm Drug Dispos,* **23**(4)**,** 159-63.

Aswania, O. A. (1999) Relative deposition characteristics of inhaled Cromones. In: *Postgraduate Studies in Pharmaceutical Technology,* UK: School of Pharmacy, University of Bradford.

Aswania, O. A., Corlett, S. A.Chrystyn, H. (1997) Development and validation of an ion-pair liquid chromatographic method for the quantitation of sodium cromoglycate in urine following inhalation. *J Chromatogr B Biomed Sci Appl,* **690**(1-2)**,** 373-378.

Aswania, O. A., Corlett, S. A.Chrystyn, H. (1998) Validation of a high-performance liquid chromatography assay for urinary nedocromil sodium following oral and inhaled administration. *J Chromatogr B Biomed Sci Appl,* **718**(2)**,** 290-295.

Aswania, O. A., Corlett, S. A.Chrystyn, H. (1999) Relative bioavailability of sodium cromoglycate to the lung following inhalation, using urinary excretion. *Br J Clin Pharmacol,* **47**(6)**,** 613-8.

Barber, S. J. (2002) The relative bioavailability to the lungs of inhaled tobramycin using a urinary pharmacokinetic method : development and validation of a high performance liquid chromatographic method. Identification of a urinary pharmacokinetic method to evaluate the relative bioavailability to the lungs of tobramycin following inhalation. Bradford, 253.

Barnes, N. C., Qiu, Y.-S., Pavord, I. D., Parker, D., Davis, P. A., Zhu, J., Johnson, M., Thomson, N. C., Jeffery, P. K.on behalf of the, S. C. O. S. G. (2006) Antiinflammatory Effects of Salmeterol/Fluticasone Propionate in Chronic Obstructive Lung Disease. *Am J Respir Crit Care Med,* **173**(7)**,** 736-743.

Barnes, P. J. (2000) Chronic obstructive pulmonary disease. *N Engl J Med,* **343**(4)**,** 269-280.

Barnes, P. J. (2004a) The size of the problem of managing asthma. *Respir Med,* **98**(Supplement 2)**,** S4-S8.

Barnes, P. J. and Pride, N. B. (1983) Dose-response curves to inhaled beta-adrenoceptor agonists in normal and asthmatic subjects. *Br J Clin Pharmacol,* **15**(6)**,** 677-82.

Barnes, P. J. P. J. (2004b) Asthma guidelines: recommendations versus reality. *Respir Med,* **98**(Supplement 1)**,** S1-S7.

Barry, P. and O'Callaghan, C. (1996) Inhalational drug delivery from seven different spacer devices. *Thorax,* **51**(8)**,** 835-840.

Barry, P. W. and O'Callaghan, C. (1994) Multiple actuations of salbutamol MDI into a spacer device reduce the amount of drug recovered in the respirable range. *Eur Respir J,* **7**(9)**,** 1707-1709.

Barry, P. W. and O'Callaghan, C. (1995) The effect of delay, multiple actuations and spacer static charge on the in vitro delivery of budesonide from the Nebuhaler. *Br J Clin Pharmacol.,* **40**(1)**,** 76-8.

Barry, P. W. and O'Callaghan, C. (2003) The influence of inhaler selection on efficacy of asthma therapies. *Adv Drug Deliv Rev,* **55**(7)**,** 879-923.

Beasley, R., Nishima, S., Pearce, N.Crane, J. (1998) Beta-agonist therapy and asthma mortality in Japan. *Lancet,* **351**(9113)**,** 1406-7.

Berg, E. and Asking, L. (2004) Nebuliser droplet size distribution-refrigerated NGI at 15 L/min. *Resp Drug Deliv,* **IX**361-363.

Berg, E., Lamb, P., Ali, A., Dennis, J., Tservistas, M.Mitchell, J. (2008) Assessment of the need to coat

particle collection cups of the ngi to mitigate droplet bounce when evaluating nebuliser-produced droplets. *PharmEuropa Sci. Notes,* **1**21-26.

Berg, E., Lamb, P., Dennis, J., Tservistas, M.Mitchell, J. (2007a) European Pharmaceutical Aerosol Group (EPAG) Nebuliser Sub-Team: Assessment of the need to coat collection cup surfaces of the Next generation Impactor (NGI) to mitigate droplet bounce. *J Aerosol Med,* **20**(2)**,** 189-190.

Berg, E., Svensson, J. O.Asking, L. (2007b) Determination of Nebuliser Droplet Size Distribution: A Method Based on Impactor Refrigeration. *J Aerosol Med,* **20**(2)**,** 97-104.

Berico, M., Luciani, A.Formignani, M. (1997) Atmospheric aerosol in an urban area--measurements of TSP and PM10 standards and pulmonary deposition assessments. *Atmos Environ,* **31**(21)**,** 3659-3665.

Berridge, M., Lee, Z., Leisure, G., Moraldi, F.Heald, D. (1998) Nasal delivery and kinetics of fluticasone propionate using positron tomography (PET). *Pharm Sci,* **1(Suppl 4)**S208-S209.

Bisgaard, H. (1995) A metal aerosol holding chamber devised for young children with asthma. *Eur Respir J,* **8**(5)**,** 856-860.

Bisgaard, H. (1997a) Delivery of inhaled medication to children. *J Asthma,* **34**(6)**,** 443-67.

Bisgaard, H. (1997b) What dose fraction represents the respirable dose? *Respir Med,* **91**20-21.

Bisgaard, H. (1998) Targeting drugs to the respiratory tract. *Res Immunol* **149**(3)**,** 229-231.

Bisgaard, H., Anhoj, J., Klug, B.Berg, E. (1995) A non-electrostatic spacer for aerosol delivery. *Arch Dis Child,* **73**(3)**,** 226-30.

Boe, J., Dennis, J., O'Driscoll, B., Members of Task Force:, Bauer, T., Carone, M., Dautzenberg, B., Diot, P., Heslop, K.Lannefors, L. (2001) European Respiratory Society Guidelines on the use of nebulisers: Guidelines prepared by a European Respiratory Society Task Force on the use of nebulisers. *Eur Respir J,* **18**(1)**,** 228-242.

Bondesson, E., Bengtsson, T., Borgstrom, L., Nilsson, L.-E., Norrgren, K., Trofast, E.Wollmer, P. (2003) Planar gamma scintigraphy--points to consider when quantifying pulmonary dry powder aerosol deposition. *Int J Pharm,* **251**(1-2)**,** 33-47.

Borgstrom, L., Bengtsson, T., Derom, E.Pauwels, R. (2000) Variability in lung deposition of inhaled drug, within and between asthmatic patients, with a pMDI and a dry powder inhaler, Turbuhaler. *Int J Pharm,* **193**(2)**,** 227-30.

Borgstrom, L., Bondesson, E., Moren, F., Trofast, E.Newman, S. P. (1994) Lung deposition of budesonide inhaled via Turbuhaler: a comparison with terbutaline sulphate in normal subjects. *Eur Respir J,* **7**(1)**,** 69-73.

Borgstrom, L., Derom, E., Stahl, E., Wahlin-Boll, E.Pauwels, R. (1996) The inhalation device influences lung deposition and bronchodilating effect of terbutaline. *Am J Respir Crit Care Med,* **153**(5)**,** 1636-1640.

Borgstrom, L. and Newman, S. (1993) Total and regional lung deposition of terbutaline sulphate inhaled via a pressurised MDI or via Turbuhaler(R). *Int J Pharm,* **97**(1-3)**,** 47-53.

Borgstrom, L., Newman, S., Weisz, A.Moren, F. (1992) Pulmonary deposition of inhaled terbutaline: comparison of scanning gamma camera and urinary excretion methods. *J Pharm Sci,* **81**(8)**,** 753-755.

Borgstrom, L. and Nilsson, M. (1990) A method for determination of the absolute pulmonary bioavailability of inhaled drugs: terbutaline. *Pharm Res,* **7**(10)**,** 1068-70.

Boulton, D. W. and Fawcett, J. P. (1996) Interaction of [beta]2-adrenoceptor agonists with native cyclodextrins: Application to the development of chiral assays for terbutaline. *Pharm Acta Helv,* **71**(4)**,** 293-296.

British Pharmacopoeia (2005a) Preparations for inhalation. Aerodynamic assessment of fine particles-fine particle dose and particle size distribution (Ph. Eur. method 2.9.18). In: *British Pharmacopoeia.* Vol. 4 (Ed, Stationery Office) London, pp. A277-290.

British Pharmacopoeia (2005b) Terbutaline. In: *British pharmacopoeia.* Vol. 2 London: Stationery Office, pp. 1917-1918.

British Thoracic Society (2007) British guideline on the management of asthma. *Thorax,* 58 Suppl. I:1-98.

Britton, J., Hanley, S. P., Garrett, H. V., Hadfield, J. W.Tattersfield, A. E. (1988) Dose related effects of salbutamol and ipratropium bromide on airway calibre and reactivity in subjects with asthma. *Thorax,* **43**(4)**,** 300-305.

Brochard, L., Lofaso, F., Simonneau, G., Conti, G., Mancebo, J., Wysocki, M., Gasparetto, A., Rauss, A.Benito, S. (1995) Noninvasive ventilation for acute exacerbations of chronic obstructive pulmonary disease. *N Engl J Med,* **333**(13)**,** 817-22.

Brodde, O.-E., Buscher, R., Leineweber, K.Bruck, H. (2002) Beta-adrenoceptor polymorphisms: does altered in vitro function predict in vivo effects? *Int Congr Ser,* **1244**69-76.

Broeders, M. E. A. C., Molema, J., Hop, W. C. J.Folgering, H. T. M. (2003) Inhalation Profiles in Asthmatics and COPD Patients: Reproducibility and Effect of Instruction. *J Aerosol Med,* **16**(2)**,** 131-141.

Brown, P. H., Ning, A. C., Greening, A. P., McLean, A.Crompton, G. K. (1995) Peak inspiratory flow through Turbuhaler in acute asthma. *Eur Respir J,* **8**(11)**,** 1940-1941.

Bulletti, C., Mansour, S., Rossi, S., Pelli, V., de Moustier, B., Massonneau, M., de Ziegler, D., Giacomucci, E., Albonetti, A., Alfieri, S., Negrini, V., Bolelli, G.Fraceschetti, F. (1997) First uterine pass of radioactive terbutaline in human uterus. *Fertil Steril,* **68**(Supplement 1)**,** S75.

Burge, P. S., Calverley, P. M. A., Jones, P. W., Spencer, S., Anderson, J. A.Maslen, T. K. (2000) Randomised, double blind, placebo controlled study of fluticasone propionate in patients with moderate to severe chronic obstructive pulmonary disease: the ISOLDE trial. *BMJ,* **320**(7245)**,** 1297.

Calverley, P., Pauwels, R.Vestbo, J. (2003) Combining salmeterol and fluticasone in the treatment of chronic obstructive pulmonary disease. *Lancet,* **361**449–456.

Carlucci, A., Lepage, E., Brochard, L., Richard, J. C.Wysocki, M. (2001) Noninvasive versus conventional mechanical ventilation. An epidemiologic survey. *Am J Respir Crit Care Med,* **163**(4)**,** 874-80.

Casterline, C. L., Evans 3rd, R.Ward Jr, G. W. (1976) The effect of atropine and albuterol aerosols on the human bronchial response to histamine. *J Allergy Clin Immunol,* **58**(5)**,** 607-13.

Castro-Rodriguez, J. A. and Rodrigo, G. J. (2004) ß-agonists through metered-dose inhaler with valved holding chamber versus nebuliser for acute exacerbation of wheezing or asthma in children under 5 years of age: A systematic review with meta-analysis. *J Pediatr,* **145**(2)**,** 172-177.

Cates, C. (2003) Spacers and nebulisers for the delivery of beta-agonists in non-life-threatening acute asthma. *Respir Med,* **97**(7)**,** 762-769.

Cates, C. J., Bara, A., Crilly, J. A.Rowe, B. H. (2003) Holding chambers versus nebulisers for beta-agonist treatment of acute asthma. *Cochrane Database Syst Rev,* (2)**,** 1-53.

Cazzola, M. and Dahl, R. (2004) Inhaled Combination Therapy With Long-Acting {beta}2-Agonists and Corticosteroids in Stable COPD. *Chest,* **126**(1)**,** 220-237.

Cazzola, M., Marco, F. D., Santus, P., Boveri, B., Verga, M., Matera, M. G.Centanni, S. (2004) The pharmacodynamic effects of single inhaled doses of formoterol, tiotropium and their combination in patients with COPD. *Pulm Pharmacol Ther,* **17**(1)**,** 35-39.

Cazzola, M., Noschese, P., Salzillo, A., De Giglio, C., D'Amato, G.Gabriella Matera, M. (2005) Bronchodilator response to formoterol after regular tiotropium or to tiotropium after regular formoterol in COPD patients. *Respir Med,* **99**(5)**,** 524-528.

Cegla, U. H. U. H. (2004) Pressure and inspiratory flow characteristics of dry powder inhalers. *Respir Med,* **98**(Supplement 1)**,** S22-S28.

Celli, B. R., MacNee, W., Agusti, A., Anzueto, A., Berg, B., Buist, A. S., Calverley, P. M. A., Chavannes, N., Dillard, T., Fahy, B., Fein, A., Heffner, J., Lareau, S., Meek, P., Martinez, F., McNicholas, W., Muris, J., Austegard, E., Pauwels, R., Rennard, S., Rossi, A., Siafakas, N., Tiep, B., Vestbo, J., Wouters, E.ZuWallack, R. (2004) Standards for the diagnosis and treatment of patients with COPD: a summary of the ATS/ERS position paper. *Eur Respir J,* **23**(6)**,** 932-946.

Chatmongkolchart, S., Schettino, G. P., Dillman, C., Kacmarek, R. M.Hess, D. R. (2002) In vitro evaluation of aerosol bronchodilator delivery during noninvasive positive pressure ventilation: Effect of ventilator settings and nebuliser position. *Crit Care Med,* **30**(11)**,** 2515-2519.

Chege, J. K. and Chrystyn, H. (1994) Volumatic usage: some generic salbutamol metered dose inhalers can be used. *Thorax,* **49**(11)**,** 1162-3.

Chege, J. K. and Chrystyn, H. (1996) Evaluation of in-vivo lung deposition following inhalation from a Rotahaler and cyclohaler using urinary salbutamol excretion. *Pharm Sci,* **2**569-570.

Chege, J. K. and Chrystyn, H. (2000) The relative bioavailability of salbutamol to the lung using urinary excretion following inhalation from a novel dry powder inhaler: the effect of inhalation rate and formulation. *Respir Med,* **94**(1)**,** 51-56.

Chege, J. K., Parry-Billings, M.Chrystyn, H. (1996a) A comparison of the relative bioavailability of salbutamol to the lung and body following treatment via two

versions of a novel dry powder inhaler an a metered dose inhaler In: *Resp Drug Deliv V*, Vol. 1 California, 318-319.

Chege, J. K., Parry-Billings, M.Chrystyn, H. (1996b) Relative bioavailability of salbutamol to the lung following treatment via two versions of a novel dry powder inhaler and a metered dose inhaler. *Eur J Pharm Sci,* **4**(Supplement 1)**,** S142.

Chou, K. J., Cunningham, S. J.Crain, E. F. (1995) Metered-dose inhalers with spacers vs nebulisers for pediatric asthma. *Arch Pediatr Adolesc Med,* **149**(2)**,** 201-205.

Chrystyn, H. (1994) Standards for bioequivalence of inhaled products. *Clin Pharmacokinet,* **26**(1)**,** 1-6.

Chrystyn, H. (1999) The Diskus Inhaler. *Clin Drug Investig,* **18**(4)**,** 287-296.

Chrystyn, H. (2000) Methods to determine lung distribution of inhaled drugs - could gamma scintigraphy be the gold standard? *Br J Clin Pharmacol.,* **49**(6)**,** 525-528.

Chrystyn, H. (2001) Methods to identify drug deposition in the lungs following inhalation. *Br J Clin Pharmacol.,* **51**(4)**,** 289-299.

Chrystyn, H. (2003) Is inhalation rate important for a dry powder inhaler? using the In-Check Dial to identify these rates. *Respir Med,* **97**(2)**,** 181-187.

Chrystyn, H. (2005) Do patients show the same level of adherence with all dry powder inhalers? *Int J Clin Pract,* **59**(Suppl 149)**,** 19-25.

Chrystyn, H. (2006) Closer to an'ideal inhaler' with the easyhaler®: An innovative dry powder inhaler. *Clin Drug Investig,* **26**(4)**,** 175-183.

Chrystyn, H. (2007) The DiskusTM: a review of its position among dry powder inhaler devices. *Int J Clin Pract,* **61**(6)**,** 1022-1036.

Chuffart, A. A., Sennhauser, F. H.Wildhaber, J. H. (2001) Factors affecting the efficiency of aerosol therapy with pressurised metered-dose inhalers through plastic spacers. *Swiss Med Wkly,* **131**14-8.

Cipolla, D. C., Clark, A. R., Chan, H. K., Gonda, I.Shire, S. J. (1994) Assessment of aerosol delivery systems for recombinant human deoxyribonuclease. *STP. Pharm Sci,* **4**(1)**,** 50-62.

Clark, A. R. and Hollingworth, A. M. (1993) The relationship between powder inhaler resistance and peak inspiratory conditions in healthy volunteers--implications for in vitro testing. *J Aerosol Med,* **6**(2)**,** 99-110.

Clay, M. and Clarke, S. (1987) Wastage of drug from nebulisers: a review. *JR Soc Med,* **80**(1)**,** 38-39.

Clay, M. M., Pavia, D., Newman, S. P.Clarke, S. W. (1983a) Factors influencing the size distribution of aerosols from jet nebulisers. *Thorax,* **38**(10)**,** 755-759.

Clay, M. M., Pavia, D., Newman, S. P., Lennard-Jones, T.Clarke, S. W. (1983b) Assessment of jet nebulisers for lung aerosol therapy. *Lancet,* **2**(8350)**,** 592-4.

Cockcroft, D. W., Killian, D. N., Mellon, J. J.Hargreave, F. E. (1977) Protective effect of drugs on histamine-induced asthma. *Thorax,* **32**(4)**,** 429-437.

Comis, A., Valletta, E., Sette, L., Andreoli, A.Boner, A. (1993) Comparison of nedocromil sodium and sodium cromoglycate administered by pressurized aerosol, with and without a spacer device in exercise-induced asthma in children. *Eur Respir J,* **6**(4)**,** 523-526.

Comité-Européen-Normalisation. (2001) Respiratory therapy equipment-Part 1:Nebulizing system and their componants. prEN 13544-1. CEN Brssels, Belgium. 33-38.

Copley (2007) *Copley Scientific Catalogue* [cited 20:05pm, 16/12/2006]. Available from http://www.copleyscientific.co.uk/new/inhaler%20brochure%202006%20(hi%20res).pdf.

Crane, J., Pearce, N., Flatt, A., Burgess, C., Jackson, R., Kwong, T., Ball, M.Beasley, R. (1989) Prescribed fenoterol and death from asthma in New Zealand, 1981-83: case-control study. *Lancet,* **1**(8644)**,** 917-22.

Creticos, P. S., Adams, W. P., Petty, B. G., Lewis, L. D., Singh, G. J. P., Khattignavong, A. P., Molzon, J. A., Martinez, M. N., Lietman, P. S.Williams, R. L. (2002) A methacholine challenge dose-response study for development of a pharmacodynamic bioequivalence methodology for albuterol metered-dose inhalers. *J Allergy Clin Immunol,* **110**(5)**,** 713-720.

Croes, K., McCarthy, P. T.Flanagan, R. J. (1995) HPLC of basic drugs and quaternary ammonium compounds on microparticulate strong cation-exchange materials using methanolic or aqueous methanol eluents containing an ionic modifier. *J Chromatogr A,* **693**(2)**,** 289-306.

Crompton, G. K. (1982) Problems patients have using pressurized aerosol inhalers. *Eur J Respir Dis Suppl,* **119**101-4.

Crompton, G. K. (2004) How to achieve good compliance with inhaled asthma therapy. *Respir Med,* **98**(Supplement 2)**,** S35-S40.

Dahl, R., Lundback, B., Malo, J. L., Mazza, J. A., Nieminen, M. M., Saarelainen, P.Barnacle, H. (1993) A dose-ranging study of fluticasone propionate in adult patients with moderate asthma. International Study Group. *Chest,* **104**(5)**,** 1352-1358.

Daniel, K. N., Chow, P.Lam, Y. (2003) *Administration of Aerosolized Medications* [cited 12:40pm, 12 October 2005]. Available from http://www.fmshk.com.hk/publications/hkps/200301.htm.

Daraghmeh, N., Al-Omari, M. M., Sara, Z., Badwan, A. A.Jaber, A. M. Y. (2002) Determination of terbutaline sulfate and its degradation products in pharmaceutical formulations using LC. *J Pharm Biomed Anal,* **29**(5)**,** 927-937.

Davies, D. S. (1975) Pharmacokinetics of inhaled substances. *Postgrad Med J,* **51**(7 SUPPL)**,** 69-75.

de Boer, A. H., Bolhuis, G. K., Gjaltema, D.Hagedoorn, P. (1997) Inhalation characteristics and their effects on in vitro drug delivery from dry powder inhalers: Part 3: the effect of flow increase rate (FIR) on the in vitro drug release from the Pulmicort 200 Turbuhaler. *Int J Pharm,* **153**(1)**,** 67-77.

de Boer, A. H., Gjaltema, D.Hagedoorn, P. (1996) Inhalation characteristics and their effects on in vitro drug delivery from dry powder inhalers Part 2: Effect of peak flow rate (PIFR) and inspiration time on the in vitro drug release from three different types of commercial dry powder inhalers. *Int J Pharm,* **138**(1)**,** 45-56.

de Boer, T. and Ensing, K. (1998) Determination of the enantiomeric purity of (-) terbutaline by capillary electrophoresis using cyclodextrins as chiral selectors in a polyethylene glycol gel. *J Pharm Biomed Anal,* **17**(6-7)**,** 1047-1056.

de Koning, J. P., van der Mark, T. W., Coenegracht, P. M. J., Tromp, T. F. J.Frijlink, H. W. (2002) Effect of an external resistance to airflow on the inspiratory flow curve. *Int J Pharm,* **234**(1-2)**,** 257-266.

de Moustier, B., Boulard, C., Levin, H., Brion, N.Mier, L. (1997) Transvaginal Administration of Terbutaline Using a New Controlled and Prolonged Release Delivery Gel, COL 2301. *Fertil Steril,* **68**(Supplement 1)**,** S76.

Dennis, J., Berg, E., Sandell, D., Ali, A., Lamb, P., Tservistas, M., Karlsson, M.Mitchell, J. (2008) Cooling the NGI – An approach to size a nebulised aerosol more accurately. *PharmEuropa Sci. Notes,* **1**27-30.

Dennis, J. and Pieron, C. (2004) Quality Control and Standards in Nebuliser Performance and Use. In: *Practical Handbook of Nebilizer Technology*. (Eds, J Boe, R., et al.) London, UK Martin Dunitz.

Dennis, S. M., Sharp, S. J.Vickers, M. R. (2000) Regular inhaled salbutamol and asthma control: the TRUST randomised trial. Therapy Working Group of the National Asthma Task Force and the MRC General Practice Research Framework. *Lancet,* **355**1675-1679.

Derom, E., Borgstrom, L., Van Schoor, J., Lofroos, A. B.Pauwels, R. (2001) Lung deposition and protective effect of terbutaline delivered from pressurized metered-dose inhalers and the Turbuhaler in asthmatic individuals. *Am J Respir Crit Care Med,* **164**(8 Pt 1)**,** 1398-402.

Desiderio, C. and Fanali, S. (1995) Use of negatively charged sulfobutyl ether-[beta]-cyclodextrin for enantiomeric separation by capillary electrophoresis. *J Chromatogr A,* **716**(1-2)**,** 183-196.

Dhand, R. (2000) Special problems in aerosol delivery: artificial airways. *Respir Care,* **45**(6)**,** 636-45.

Dhand, R. (2002) Nebulisers that use a vibrating mesh or plate with multiple apertures to generate aerosol. *Respir Care,* **47**(12)**,** 1406-16.

Dhand, R. and Fink, J. B. (1999) Dry powder inhalers. *Respir Care,* **44**(8)**,** 940-951.

Dhand, R. and Tobin, M. J. (1996) Bronchodilator delivery with metered-dose inhalers in mechanically-ventilated patients. *Eur Respir J,* **9**(3)**,** 585-95.

Dhand, R. and Tobin, M. J. (1997) Inhaled Bronchodilator Therapy in Mechanically Ventilated Patients. *Am J Respir Crit Care Med,* **156**(1)**,** 3-10.

Diot, P., Palmer, L. B., Smaldone, A., Decelie-Germana, J., Grimson, R.Smaldone, G. C. (1997) RhDNase I Aerosol Deposition and Related Factors in Cystic Fibrosis. *Am J Respir Crit Care Med,* **156**(5)**,** 1662-1668.

Dolovich, M. B., Killian, D., Wolff, R. K., Obminski, G.Newhouse, M. T. (1977) Pulmonary aerosol deposition in chronic bronchitis: intermittent positive pressure breathing versus quiet breathing. *Am Rev Respir Dis,* **115**(3)**,** 397-402.

Duarte, A. G., Momii, K.Bidani, A. (2000) Bronchodilator therapy with metered-dose inhaler and spacer versus nebuliser in mechanically ventilated patients: comparison of magnitude and duration of response. *Respir Care,* **45**(7)**,** 817-23.

Dubus, J. C., Vecellio, L., De Monte, M., Fink, J. B., Grimbert, D., Montharu, J., Valat, C., Behan, N.Diot, P. (2005) Aerosol Deposition in Neonatal Ventilation. *Pediatr Res,* **58**(1)**,** 10.

Eaton, T., Lewis, C., Young, P., Kennedy, Y., Garrett, J. E.Kolbe, J. (2004) Long-term oxygen therapy improves health-related quality of life. *Respir Med,* **98**(4)**,** 285-293.

Eiser, N. M., Phillips, C.Wooler, P. A. (2001) Does the mode of inhalation affect the bronchodilator response in patients with severe COPD? *Respir Med,* **95**(6)**,** 476-483.

Elliott, J., Bergauer, N., Jacques, D.Stanziano, G. (1999) An assessment of pregnancy prolongation in 104 triplet gestations treated with oral then subcutaneous terbutaline. *Obstetrics & Gynecology,* **93**(4, Supplement 1)**,** S75.

Elliott, J., Istwan, N.Stanziano, G. (2002) Adverse events related to continuous subcutaneous terbutaline therapy. *Obstetrics & Gynecology,* **99**(4, Supplement 1)**,** S63-S64.

Elliott, M. W., Branthwaite, M. A., Steven, M. H.Phillips, G. D. (1990) Non-invasive mechanical ventilation for acute respiratory failure. *BMJ,* **300**(6721)**,** 358-60.

European Pharmacopeia (2001) Preparations for inhalation: aerodynamic assessment of fine particles. In: *European Pharmacopoeia*. (Ed, ed., t.), pp. 209-217.

Ferron, G. A., Kerrebijn, K. F.Weber, J. (1976) Properties of aerosols produced with three nebulisers. *Am Rev Respir Dis,* **114**(5)**,** 899-908.

Fink , J., Barraza, P.Bisgaard, J. (2001) Aerosol delivery during mechanical ventilation with high frequency oscillation: an in vitro evaluation. In: *American college of chest physicians annual meeting (ACCP).*

Fink, J., Schmidt, D.Power, J. (2001a) Comparison of a nebuliser using a novel aerosol generator with a standard ultrasonic nebuliser designed for use during mechanical ventilation. *Am J Respir Crit Care Med.,* **163**,A127.

Fink, J., Uster, P., Schmidt, D.Simon, M. (2001b) In vitro characteristics of the aeroneb portable nebuliser system. *J Aerosol Med,* **14**(3)**,** 1-11.

Fink, J. B. (2000) Metered-dose inhalers, dry powder inhalers, and transitions. *Respir Care,* **45**(6)**,** 623-35.

Fink, J. B., Dhand, R., Grychowski, J., Fahey, P. J.Tobin, M. J. (1999) Reconciling In Vitro and In Vivo Measurements of Aerosol Delivery from a Metered-Dose Inhaler during Mechanical Ventilation and Defining Efficiency-enhancing Factors. *Am J Respir Crit Care Med,* **159**(1)**,** 63-68.

Fink, J. B. and Schmidt, D. (2002) In vitro comparison of nebulisers for aerosol delivery during mechanical ventilation In: *American thoracic society international conference (ATS).*

Fink, J. B. and Simmons, R. (2004) Impact of Nebuliser Position in the Ventilator Circuit on Drug Delivery During Mechanical Ventilation Using the Aeroneb Professional Nebuliser. In: *June, Western Pacific Association of Critical Care Medicine Congress (WPACCM).*

Fink, J. B., Simon, M., MacLaughlan, R.N., B. (2003) In vitro comparison of a novel nebuliser with ultrasonic and pneumatic nebulisers. *J Allergy Clin Immunol,* **111**(1)**,** S143.

Finlay, W. H. and Stapleton, K. W. (1999) Undersizing of droplets from a vented nebuliser caused by aerosol heating during transit through an anderson impactor. *J Aerosol Sci,* **30**(1)**,** 105-109.

Fletcher, C. and Peto, R. (1977) The natural history of chronic airflow obstruction. *Br Med J,* **1**(6077)**,** 1645-8.

Fok, T. F., Lam, K., Ng, P. C., Leung, T. F., So, H. K., Cheung, K. L.Wong, W. (1998) Delivery of salbutamol to nonventilated preterm infants by metered-dose inhaler, jet nebuliser, and ultrasonic nebuliser. *Eur Respir J,* **12**(1)**,** 159-164.

Fok, T. F., Monkman, S., Dolovich, M., Gray, S., Coates, G., Paes, B., Rashid, F., Newhouse, M.Kirpalani, H. (1996) Efficiency of aerosol medication delivery from a metered dose inhaler versus jet nebuliser in infants with bronchopulmonary dysplasia. *Pediatr Pulmonol,* **21**(5)**,** 301-309.

Gleeson, J. G. and Price, J. F. (1988) Nebuhaler technique. *Br J Dis Chest,* **82**(2)**,** 172-4.

Global Initiative for Chronic Obstructive Lung Disease (GOLD) (2007) *Global Strategy for Diagnosis, Management, and Prevention of COPD* [cited March 2008]. Available from http://www.goldcopd.com.

Gorecka, D., Gorzelak, K., Sliwinski, P., Tobiasz, M.Zielinski, J. (1997) Effect of long-term oxygen therapy on survival in patients with chronic obstructive pulmonary disease with moderate hypoxaemia. *Thorax,* **52**(8)**,** 674-679.

Grainger, J., Woodman, K., Pearce, N., Crane, J., Burgess, C., Keane, A.Beasley, R. (1991) Prescribed fenoterol and death from asthma in New Zealand, 1981-7: a further case-control study. *Thorax,* **46**(2)**,** 105-11.

Gustafsson, P., Taylor, A., Zanen, P.Chrystyn, H. (2005) Can patients use all dry powder inhalers equally well? *Int J Clin Pract,* **59**(s149)**,** 13-18.

Hancox, R. J., Cowan, J. O., Flannery, E. M., Herbison, G. P., Mclachlan, C. R.Taylor, D. R. (2000) Bronchodilator tolerance and rebound bronchoconstriction during regular inhaled [beta] -agonist treatment. *Respir Med,* **94**(8)**,** 767-771.

Hardy, J. G., Jasuja, A. K., Frier, M.Perkins, A. C. (1996) A small volume spacer for use with a breath-operated pressurised metered dose inhaler. *Int J Pharm,* **142**(1)**,** 129-133.

Harvey, C. J., Thomas, S. H., Nunan, T. O., O'Doherty, M. J., Page, C. J.Treacher, D. F. (1997) Comparison of jet and ultrasonic nebuliser pulmonary aerosol deposition during mechanical ventilation. *Eur Respir J,* **10**(4)**,** 905-9.

Hawksworth, G. M., James, L.Chrystyn, H. (2000) Characterization of the inspiratory manoeuvre when asthmatics inhale through a Turbohaler pre- and post-counselling in a community pharmacy. *Respir Med,* **94**(5)**,** 501-504.

Heald, D., Berrdge, M., Lee, Z.Leisure, G. (1997) In vivo lung deposition of triamcinolone acetonide (Azmacort) with and without the integrated spacer. In Respiratory drug delivery, eds. Dalby RN, Byron PR, Farr SY. *Interpharm Press Inc.*, 345-347.

Henze, M. K., Opfermann, G., Spahn-Langguth, H.Schanzer, W. (2001) Screening of [beta]-2 agonists and confirmation of fenoterol, orciprenaline, reproterol and terbutaline with gas chromatography-mass spectrometry as tetrahydroisoquinoline derivatives. *J Chromatogr B Biomed Sci Appl,* **751**(1)**,** 93-105.

Herring, V. L. and Johnson, J. A. (2000) Simple method for determination of terbutaline plasma concentration by high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl,* **741**(2)**,** 307-312.

Hess, D., Fisher, D., Williams, P., Pooler, S.Kacmarek, R. M. (1996) Medication Nebuliser Performance: Effects Of Diluent Volume, Nebuliser Flow, and Nebuliser Brand. *Chest,* **110**(2)**,** 498-505.

Heyder, J., Gebhart, J., Rudolf, G., Schiller, C. F.Stahlofen, W. (1986) Deposition of particles in the human respiratory tract in the size range 0, 005-15 µm. *J Aerosol Sci,* **17**(5)**,** 811-825.

Hickey, A. J. (1992) Pharmaceutical inhalation aerosol technology. New York: Marcel Dekker, Inc.

Hill, L. S. and Slater, A. L. (1998) A comparison of the performance of two modern multidose dry powder asthma inhalers. *Respir Med,* **92**(1)**,** 105-110.

Hillery, A. M., Lloyd, A. W.Swarbrick, J. (2001) Pulmonary drug delivery. In: *Drug delivery and targeting for pharmacists and pharmaceutical scientists*. London: Taylor & Francis, pp. 269-300.

Hindle, M. and Chrystyn, H. (1992) Determination of the relative bioavailability of salbutamol to the lung following inhalation. *Br J Clin Pharmacol.,* **34**(4)**,** 311-315.

Hindle, M., Chrystyn, H.Newton, D. A. (1994) Relative bioavailability of salbutamol to the lung following inhalation using metered dose inhalation methods and spacer devices. *Thorax,* **49**(6)**,** 549-53.

Hindle, M., Newton, D. A.Chrystyn, H. (1993) Investigations of an optimal inhaler technique with the use of urinary salbutamol excretion as a measure of relative bioavailability to the lung. *Thorax,* **48**(6)**,** 607-10.

Hindle, M., Newton, D. A.Chrystyn, H. (1995) Dry powder inhalers are bioequivalent to metered-dose inhalers. A study using a new urinary albuterol (salbutamol) assay technique. *Chest,* **107**(3)**,** 629-33.

Hirst, P. H., Pitcairn, G. R., Richards, J. C., Rohatagi, S., Gillen, M. S.Newman, S. P. (2001) Deposition and Pharmacokinetics of an HFA Formulation of Triamcinolone Acetonide Delivered by Pressurized Metered Dose Inhaler. *J Aerosol Med,* **14**(2)**,** 155-165.

Hogg, P. J. C. (2004) Pathophysiology of airflow limitation in chronic obstructive pulmonary disease. *Lancet,* **364**(9435)**,** 709-721.

Hunninghake, G. W. and Crystal, R. G. (1983) Cigarette smoking and lung destruction. Accumulation of neutrophils in the lungs of cigarette smokers. *Am Rev Respir Dis,* **128**(5)**,** 833-8.

Huynh, N.-H., Karlsson, A.Pettersson, C. (1995) Enantiomeric separation of basic drugs using N-benzyloxycarbonylglyclyl--proline as counter ion in methanol. *J Chromatogr A,* **705**(2)**,** 275-287.

Ismail, N. E. and Chrystyn, H. (2004) The relative bioavailability of salbutamol to the lungs from a U22 MicroAir nebuliser. *J Pharm Pharmacol,* **56 (supplement)**S-39.

Isohama, Y., Kanemaru, M., Kai, H., Takahama, K.Miyata, T. (2001) Interaction between [beta]-adrenergic signaling and protein kinase C increases cytoplasmic Ca2+ in alveolar type II cells. *Life Sci,* **68**(21)**,** 2361-2371.

Jauernig, J., Hug, M.Knoch, M. (2002) Contribution to the aerodynamic particle size assessment of nebulised drugs using the next generation impactor (NGI). *Drug Delivery to the Lungs XIII. Aerosol Society, London, UK,* 44–47.

Jauernig, J., Ohl, S., Knoch, M.Keller, M. (2004) Effects of the test set-up formulation, and nebuliser type on aerodynamic droplet characteristics. *Resp Drug Deliv,* **IX**609-612.

Jauernig, J., Ohl, S., Luber, M.Keller, M. (2003) Differences in results obtained with the next generation impactor (NGI) for Pulmicort® suspension and according to the CEN standard EN13544-1 for a NaFSolution. *Resp Drug Deliv,* **XIV**41-44.

Jensen, E. J., Dahl, R.Steffensen, F. (2000) Bronchial reactivity to cigarette smoke; relation to lung function, respiratory symptoms, serum-immunoglobulin E and blood eosinophil and leukocyte counts. *Respir Med,* **94**(2)**,** 119-127.

Johnson, M. (2001) Beta2-adrenoceptors: mechanisms of action of beta2-agonists. *Paediatr Respir Rev,* **2**(1)**,** 57-62.

Johnson, M. A., Newman, S. P., Bloom, R., Talaee, N.Clarke, S. W. (1989) Delivery of albuterol and ipratropium bromide from two nebuliser systems in chronic stable asthma. Efficacy and pulmonary deposition. *Chest,* **96**(1)**,** 6-10.

Jones, B. and Kenward, M. G. (1989) *Design and Analysis of Cross-over Trials.*

Kamiya, A., Sakagami, M., Hindle, M.Byron, P. R. (2004) Aerodynamic sizing of metered dose inhalers: An evaluation of the andersen and next generation pharmaceutical impactors and their USP methods. *J Pharm Sci,* **93**(7)**,** 1828-1837.

Kavelaars, A., van de Pol, M., Zijlstra, J.Heijnen, C. J. (1997) [beta]2-Adrenergic activation enhances interleukin-8 production by human monocytes. *J Neuroimmunol,* **77**(2)**,** 211-216.

Keatings, V. M. and Barnes, P. J. (1997) Granulocyte activation markers in induced sputum: comparison between chronic obstructive pulmonary disease, asthma, and normal subjects. *Am J Respir Crit Care Med,* **155**449-453

Keatings, V. M., Collins, P. D., Scott, D. M.Barnes, P. J. (1996) Differences in interleukin-8 and tumor necrosis factor-alpha in induced sputum from patients with chronic obstructive pulmonary disease or asthma. *Am J Respir Crit Care Med,* **153**530-534

Kenyon, C. J., Thorsson, L., Borgstrom, L.Newman, S. P. (1998) The effects of static charge in spacer devices on glucocorticosteroid aerosol deposition in asthmatic patients. *Eur Respir J,* **11**(3)**,** 606-10.

Kesten, S. and Chapman, K. R. (1993) Physician perceptions and management of COPD. *Chest,* **104**(1)**,** 254-258.

Kim, K. H., Kim, H. J., Kim, J.-H., Lee, J. H.Lee, S. C. (2001a) Determination of the optical purity of (R)-terbutaline by 1H-NMR and RP-LC using chiral derivatizing agent, (S)-(-)-[alpha]-methylbenzyl isocyanate. *J Pharm Biomed Anal,* **25**(5-6)**,** 947-956.

Kim, K. H., Kim, H. J., Kim, J.-H.Shin, S. D. (2001b) Determination of terbutaline enantiomers in human urine by coupled achiral-chiral high-performance liquid chromatography with fluorescence detection. *J Chromatogr B Biomed Sci Appl,* **751**(1)**,** 69-77.

Kohler, D. (2004) The Novolizer(R): overcoming inherent problems of dry powder inhalers. *Respir Med,* **98**(Supplement 1)**,** S17-S21.

Kondili, E. and Georgopoulos, D. (2002) Aerosol medications. *Respir Care Clin N Am,* **8**(2)**,** 309-34.

Korn, S. H., Wouters, E. F. M., Wesseling, G., Arends, J.-W.Thunnissen, F. B. J. M. (1998) Interaction between glucocorticoids and [beta]2-agonists: [alpha] and [beta] glucocorticoid-receptor mrna expression in human bronchial epithelial cells. *Biochem Pharmacol,* **56**(12)**,** 1561-1569.

Kraemer, R. (1995) Babyhaler a new paediatric aerosol device. *J Aerosol Med,* **8**(2)**,** S19-26.

Kresch, M. J., Lima, D. M.Lu, H. (1996) Developmental regulation of phospholipid secretion by fetal type II pneumocytes. *Biochimica et Biophysica Acta (BBA) - Lipids and Lipid Metabolism,* **1299**(1)**,** 39-46.

Kristin, S., Tom, B., Tim, C.Thomas, H. (2003) Evaluation of three small volume nebulisers for use with infant ventilator circuits In: *American association for respiratory care congress(AARC).*

Lee, S. and Jung, S. (2003) Enantioseparation using cyclosophoraoses as a novel chiral additive in capillary electrophoresis. *Carbohydr Res,* **338**(10)**,** 1143-1146.

Lightowler, J. V., Wedzicha, J. A., Elliott, M. W.Ram, F. S. F. (2003) Non-invasive positive pressure ventilation to treat respiratory failure resulting from exacerbations of chronic obstructive pulmonary disease: Cochrane systematic review and meta-analysis. *BMJ,* **326**(7382)**,** 185.

Lipworth, B., ChB, S. T., Devlin, M., ChB, T. A., Baker, R.Hendrick, D. (1998) Effects of Treatment with Formoterol on Bronchoprotection against Methacholine. *Am J Med,* **104**(5)**,** 431-438.

Lipworth, B. and Clark, D. (1997a) Effects of airway calibre on lung delivery of nebulised salbutamol. *Thorax,* **52**(12)**,** 1036-1039.

Lipworth, B. J. and Clark, D. J. (1997b) Comparative lung delivery of salbutamol given via Turbuhaler and Diskus dry powder inhaler devices. *Eur J Clin Pharmacol,* **53**(1)**,** 47-49.

Lu, W. and Cole, R. B. (1998) Determination of chiral pharmaceutical compounds, terbutaline, ketamine and propranolol, by on-line capillary electrophoresis-electrospray ionization mass spectrometry. *J Chromatogr B Biomed Sci Appl,* **714**(1)**,** 69-75.

Lv, Y., Zhang, Z., Hu, Y., He, D.He, S. (2003) A novel chemiluminescence method for determination of terbutaline sulfate based on potassium ferricyanide oxidation sensitized by rhodamine 6G. *J Pharm Biomed Anal,* **32**(3)**,** 555-561.

Manthous, C. A., Hall, J. B., Chatila, W.Schmidt, G. A. (1995) Treatment of bronchospasm by metered-dose inhaler albuterol in mechanically ventilated patients. *Chest,* **107**(1)**,** 210-3.

Marik, P., Hogan, J.Krikorian, J. (1999) A comparison of bronchodilator therapy delivered by nebulization and metered-dose inhaler in mechanically ventilated patients. *Chest,* **115**(6)**,** 1653-7.

Marple, V., Olson, B., Santhanakrishnan, K., Roberts, D., Mitchell, J.Hudson-Curtis, B. (2004a) Next Generation Pharmaceutical Impactor: A New Impactor for Pharmaceutical Inhaler Testing. Part III. Extension of Archival Calibration to 15 L/min. *J Aerosol Med,* **17**(4)**,** 335-343.

Marple, V., Roberts, D.Jolyon, M. (2004b) A proposal for the use of the Next generation impactor (NGI) at flow rates between 15 and 30 L/min. *Resp Drug Deliv,* **IX**701-704.

Martindale (2002) Bronchodilators and Anti-asthmata drugs. In: *Martindale : the complete drug reference*. (Ed, Sweetman, C. S.) London: Pharmaceutical Society of Great Britain. Pharmaceutical Press, pp. 757-786.

Massey, D. G., Miyauchi, D.Fournier-Massey, G. (1982) Nebuliser function. *Bull Eur Physiopathol Respir,* **18**(4)**,** 665-71.

Mazhar, S., Ismail, N., Newton, D.Chrystyn, H. (2008) Relative lung deposition of salbutamol following inhalation from a spacer and a Sidestream jet nebuliser following an acute exacerbation. . *Brit J Clin Pharmacol,* **65**(3)**,** 334-337.

McCallion, O. N. M. and Patel, M. J. (1996) Viscosity effects on nebulisation of aqueous solutions. *Int J Pharm,* **130**(2)**,** 245-249.

McCallion, O. N. M., Taylor, K. M. G., Bridges, P. A., Thomas, M.Taylor, A. J. (1996) Jet nebulisers for pulmonary drug delivery. *Int J Pharm,* **130**(1)**,** 1-11.

Mead, J., Turner, J. M., Macklem, P. T.Little, J. B. (1967) Significance of the relationship between lung recoil and maximum expiratory flow. *J Appl Physiol,* **22**(1)**,** 95-108.

Meakin, B. J., Cainey, J. M.Woodcock, P. M. (1995a) Drug delivery characteristics of Bricanyl TurbohalerTM dry powder inhalers. *Int J Pharm,* **119**(1)**,** 91-102**(1)**.

Meakin, B. J., Cainey, J. M.Woodcock, P. M. (1995b) Simulated 'in-use' and 'mis-use' aspects of the delivery of terbutaline sulphate from Bricanyl TurbohalerTM dry powder inhalers. *Int J Pharm,* **119**(1)**,** 103-108**(2)**.

Melchor, R., Biddiscombe, M. F., Mak, V. H., Short, M. D.Spiro, S. G. (1993) Lung deposition patterns of directly labelled salbutamol in normal subjects and in patients with reversible airflow obstruction. *Thorax,* **48**(5)**,** 506-511.

Mercer, T. T. (1973) Production and characterization of aerosols. *Arch Intern Med,* **131**(1)**,** 39-50.

Mercer, T. T. (1981) Production of therapeutic aerosols; principles and techniques. *Chest,* **80**(6 Suppl)**,** 813-8.

Meyer, T., Brand, P., Ehlich, H., Kobrich, R., Meyer, G., Riedinger, F., Sommerer, K., Weuthen, T.Scheuch, G. (2004) Deposition of Foradil P in Human Lungs: Comparison of In Vitro and In Vivo Data. *J Aerosol Med,* **17**(1)**,** 43-49.

Miller, N. C., Maniaci, M. J., Dwivedi, S.Ward, G. H. (2000) Aerodynamic sizing with simulated inhalation profiles: total dose capture and measurement. *Resp Drug Deliv VII,* **1**191-196.

Mitchell, J. P. and Nagel, M. W. (2003) Cascade impactors for the size characterization of aerosols from medical inhalers: Their uses and limitation. *J Aerosol Med,* **16**341-376.

Mobley, C. and Hochhaus, G. (2001) Methods used to assess pulmonary deposition and absorption of drugs. *Drug Discov Today,* **6**(7)**,** 367-375.

Molina, M. J. and Rowland, F. S. (1974) Stratospheric sink for chlorofluoromethanes: chlorine atomc-atalysed destruction of ozone. *Nature,* **249**(5460)**,** 810-812.

Montreal (2000) *The montreal protocol on substances that deplete the ozone layer.* [cited 13/9/2005 12:30 pm]. Available from http://www.unep.org/ozone/pdfs/Montreal-Protocol2000.pdf.

Mullen, M., Mullen, B.Carey, M. (1993) The association between beta-agonist use and death from asthma. A meta-analytic integration of case-control studies. *Jama,* **270**(15)**,** 1842-5.

Nadarassan, D. K., Chrystyn, H., Clark, B. J.Assi, K. H. (2007a) Dose emission of Formoterol fumarate from a Turbuhaler using in-vitro and in-vivo methods In:

Postgraduate Studies in Pharmaceutical Technology, UK: School of Pharmacy, University of Bradford.

Nadarassan, D. K., Clark, B. J., Assi, K.Chrystyn, H. (2007b) Use of a mixing valve allows in-vitro characterisation of the emitted dose from a dry powder inhaler (dpi) using a low inhalation flow. *J Aerosol Med*, **20**(2)**,** 188.

National Institute for Health and Clinical Excellence (NICE) (2004) *Chronic obstructive pulmonary disease: national clinical guideline for management of chronic obstructive pulmonary disease in adults in primary and secondary care.* Available from http://www.nice.org.uk/guidance/.

Nava, S., Karakurt, S., Rampulla, C., Braschi, A.Fanfulla, F. (2001) Salbutamol delivery during non-invasive mechanical ventilation in patients with chronic obstructive pulmonary disease: a randomized, controlled study. *Intensive Care Med,* **27**(10)**,** 1627-1635.

Netter FH (1979) CIBA Collection of Medical Illustrations Vol. 7, pp. 60.

Newman, S. (2004) Spacer devices for metered dose inhalers. *Clin Pharmacokinet,* **43**(6)**,** 349-60.

Newman, S., Pavia, D., Moren, F., Sheahan, N.Clarke, S. (1981) Deposition of pressurised aerosols in the human respiratory tract. *Thorax,* **36**(1)**,** 52-55.

Newman, S. P. (1991) Aerosol physiology, deposition and metered dose inhalers. *Allergy Proc,* **12**(1)**,** 41-45.

Newman, S. P., Brown, J., Steed, K. P., Reader, S. J.Kladders, H. (1998) Lung deposition of fenoterol and flunisolide delivered using a novel device for inhaled medicines: comparison of RESPIMAT with conventional metered-dose inhalers with and without spacer devices. *Chest,* **113**(4)**,** 957-63.

Newman, S. P., Clark, A. R., Talaee, N.Clarke, S. W. (1989) Pressurised aerosol deposition in the human lung with and without an "open" spacer device. *Thorax,* **44**(9)**,** 706-710.

Newman, S. P. and Clarke, S. W. (1993) Bronchodilator delivery from Gentlehaler, a new low-velocity pressurized aerosol inhaler. *Chest,* **103**(5)**,** 1442-1446.

Newman, S. P. and Kenyon, C. J. (1994) Asthma products bioequivalence. *Pharm J,* **253**42.

Newman, S. P., Moren, F., Trofast, E., Talaee, N.Clarke, S. W. (1991a) Terbutaline sulphate Turbuhaler: effect of inhaled flow rate on drug deposition and efficacy. *Int J Pharm,* **74**(2-3)**,** 209-213.

Newman, S. P. and Newhouse, M. T. (1996) Effect of add-on devices for aerosol drug delivery: deposition studies and clinical aspects. *J Aerosol Med,* **9**(1)**,** 55-70.

Newman, S. P., Pavia, D., Garland, N.Clarke, S. W. (1982) Effects of various inhalation modes on the deposition of radioactive pressurized aerosols. *Eur J Respir Dis Suppl,* **119(suppl)**57-65.

Newman, S. P., Talaee, N.Clarke, S. W. (1991b) Salbutamol aerosol delivery in man with the Rondo Spacer. *Acta Ther,* **17**49-50.

Newman, S. P., Weisz, A. W., Talaee, N.Clarke, S. W. (1991c) Improvement of drug delivery with a breath actuated pressurised aerosol for patients with poor inhaler technique. *Thorax,* **46**(10)**,** 712-716.

Newman, S. P. and Wilding, I. R. (1999) Imaging techniques for assessing drug delivery in man. *Pharm Sci Technolo Today,* **2**(5)**,** 181-189.

Newnham, D. M., McDevitt, D. G.Lipworth, B. J. (1993) Comparison of the extrapulmonary beta2-adrenoceptor responses and pharmacokinetics of salbutamol given by standard metered dose-inhaler and modified actuator device. *Br J Clin Pharmacol,* **36**(5)**,** 445-50.

Nials, A. T., Ball, D. I., Butchers, P. R., Coleman, R. A., Humbles, A. A., Johnson, M.Vardey, C. J. (1994) Formoterol on airway smooth muscle and human lung mast cells: a comparison with salbutamol and salmeterol. *Eur J Pharmacol,* **251**(2-3)**,** 127-35.

Nicpon-Marieb, E. (2004) Respiratory System. In: *Human anatomy & physiology*. San Francisco; London: Pearson/Benjamin Cummings, pp. 828-880.

Niven, R. W. and Brain, J. D. (1994) Some functional aspects of air-jet nebulisers. *Int J Pharm,* **104**(1)**,** 73-85.

Nyberg, L. (1984) Pharmacokinetic parameters of terbutaline in healthy man. An overview. *Eur J Respir Dis Suppl,* **134**149-60.

O'Byrne, P., Barnes, P. J., Rodriguez-Gomez, G., Runnerstrom, E., Sandstrom, T.Svenson, K. (2001) Low dose inhaled budesonide and formoterol in mild persistent asthma: the OPTIMA randomized trial. *Am J Respir Crit Care Med,* **164**1392-1397.

O'Callaghan, C. and Barry, P. W. (2000) How to choose delivery devices for asthma. *Arch Dis Child,* **82**(3)**,** 185-7.

O'Callaghan, C., Lynch, J., Cant, M.Robertson, C. (1993) Improvement in sodium cromoglycate delivery from a spacer device by use of an antistatic lining, immediate inhalation, and avoiding multiple actuations of drug. *Thorax,* **48**(6)**,** 603-606.

O'Doherty, M. J., Treacher, D. F., Nunan, T. O., Thomas, S. H.Page, C. J. (1992) Delivery of a nebulised aerosol to a lung model during mechanical ventilation. Effect of ventilator settings and nebuliser type, position, and volume of fill. *Am Rev Respir Dis,* **146**(2)**,** 383-8.

O'Riordan, T. G., Palmer, L. B.Smaldone, G. C. (1994) Aerosol deposition in mechanically ventilated patients. Optimizing nebuliser delivery. *Am J Respir Crit Care Med,* **149**(1)**,** 214-9.

O'Riordan, T. G., Smaldone, G. C., Greco, M. J.Perry, R. J. (1992) Nebuliser function during mechanical ventilation. *Am Rev Respir Dis,* **145**(5)**,** 1117-22.

O'Sullivan, E. A. and Curzon, M. E. J. (1998) Drug treatments for asthma may cause erosive tooth damage. *Bmj,* **317**(7161)**,** 820.

O'Callaghan, C. and Barry, P. W. (1997) The science of nebulised drug delivery. *Thorax,* **52**(2)**,** S31-S44.

Palander, A., Mattila, T., Karhu, M.Muttonen, E. (2000) In vitro comparison of three salbutamol-containing multidose dry powder inhalers: Buventol Easyhaler®, Inspiryl Turbuhaler® and Ventoline Diskus®. *Clin Drug Investig,* **20**(1)**,** 25-33.

Palmer, L. B., Smaldone, G. C., Simon, S. R., O'Riordan, T. G.Cuccia, A. (1998) Aerosolized antibiotics in mechanically ventilated patients: delivery and response. *Crit Care Med,* **26**(1)**,** 31-9.

Parkes, S. N. and Bersten, A. D. (1997) Aerosol kinetics and bronchodilator efficacy during continuous positive airway pressure delivered by face mask. *Thorax,* **52**(2)**,** 171-175.

Patel, P., Mukai, D.Wilson, A. F. (1990) Dose-response effects of two sizes of monodisperse isoproterenol in mild asthma. *Am Rev Respir Dis,* **141**(2)**,** 357-60.

Pauwels, R., Newman, S.Borgstrom, L. (1997) Airway deposition and airway effects of antiasthma drugs delivered from metered-dose inhalers. *Eur Respir J,* **10**(9)**,** 2127-2138.

Pauwels, R. A., Buist, A. S., Calverley, P. M. A., Jenkins, C. R.Hurd, S. S. (2001) Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Pulmonary Disease . NHLBI/WHO Global Initiative for Chronic Obstructive Lung Disease (GOLD) Workshop Summary. *Am J Respir Crit Care Med,* **163**(5)**,** 1256-1276.

Pauwels, R. A., Pedersen, S. and Busse, W. W. (2003) Early intervention with budesonide in mild persistent asthma: a randomised, double-blind trial. *Lancet,* **361**(9363)**,** 1071-1076.

Pearce, N., Beasley, R., Crane, J., Burgess, C.Jackson, R. (1995) End of the New Zealand asthma mortality epidemic. *Lancet,* **345**(8941)**,** 41-4.

Pearce, N., Crane, J., Burgess, C., Jackson, R.Beasley, R. (1991) Beta agonists and asthma mortality: deja vu. *Clin Exp Allergy,* **21**(4)**,** 401-10.

Pearce, N., Grainger, J., Atkinson, M., Crane, J., Burgess, C., Culling, C., Windom, H.Beasley, R. (1990) Case-control study of prescribed fenoterol and death from asthma in New Zealand, 1977-81. *Thorax,* **45**(3)**,** 170-5.

Pedersen, S., Hansen, O. R.Fuglsang, G. (1990) Influence of inspiratory flow rate upon the effect of a Turbuhaler. *Arch Dis Child,* **65**(3)**,** 308-310.

Pedersen, S. and Mortensen, S. (1990) Use of different inhalation devices in children. *Lung,* **168**653-7.

Petty, T. L. (1995) Enjoying Life with Chronic Obstructive Pulmonary Disease. NJ: Cedar Grove Laennec Publishing.

Pierart, F., Wildhaber, J., Vrancken, I., Devadason, S.Le Souef, P. (1999) Washing plastic spacers in household detergent reduces electrostatic charge and greatly improves delivery. *Eur Respir J,* **13**(3)**,** 673-678.

Pieron, C., Gee-Turner, A.Dennis, J. (2007a) Equivalence between NGI Vs Marple 298x impactors in sizing nebulised aerosol. *J Aerosol Med,* **20**(2)**,** 188.

Pieron, C., Tasko, P.Dennis, J. (2007b) Cascade impactor equivalence in sizing nebulised aerosol: NGI Vs Marple 298. *J Aerosol Med,* **20**(2)**,** 187.

Pieron, C., Tasko, P.Dennis, J. (2007c) Long-term use of Marple 298X impactor yields robust and repeatable results. *J Aerosol Med,* **20**(2)**,** 187.

Plant, P. K. and Elliott, M. W. (1998) Non-invasive ventilation in acute exacerbations of COPD. *QJM,* **91**(10)**,** 657-660.

Polettini, A., Montagna, M., Hogendoorn, E. A., Dijkman, E., van Zoonen, P.van Ginkel, L. A. (1995) Applicability of coupled-column liquid chromatography to the analysis of [beta]-agonists in urine by direct sample injection I. Development of a single-residue reversed-phase liquid chromatography-UV method for clenbuterol

and selection of chromatographic conditions suitable for multi-residue analysis. *J Chromatogr A*, **695**(1)**,** 19-31.

Poole, P. J. and Black, P. N. (2001) Oral mucolytic drugs for exacerbations of chronic obstructive pulmonary disease: systematic review. *BMJ*, **322**(7297)**,** 1271.

Prime, D., Woodhouse, R. N., Grant, A. C.Slater, A. L. (1999) A critical comparison of the dose delivery characteristics of four alternative inhalation devices delivering salbutamol: pressurized metered dose inhaler, Diskus inhaler, Diskhaler inhaler, and Turbuhaler inhaler. *J Aerosol Med*, **12**(2)**,** 75-84.

Raphael, G. D., Lanier, R. Q., Baker, J., Edwards, L., Rickard, K.Lincourt, W. R. (1999) A comparison of multiple doses of fluticasone propionate and beclomethasone dipropionate in subjects with persistent asthma. *J Allergy Clin Immunol*, **103(**5 Pt 1)**,** 796-803.

Repine, J. E., Bast, A.Lankhorst, I. (1997) Oxidative stress in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*, **156**341-357

Retamales, I., Elliott, W. M., Meshi, B., Coxson, H. O., Pare, P. D., Sciurba, F. C., Rogers, R. M., Hayashi, S.Hogg, J. C. (2001) Amplification of Inflammation in Emphysema and Its Association with Latent Adenoviral Infection. *Am J Respir Crit Care Med*, **164**(3)**,** 469-473.

Richards, J., Hirst, P., Pitcairn, G., Mahashabde, S., Abramowitz, W., Nolting, A.Newman, S. P. (2001) Deposition and Pharmacokinetics of Flunisolide Delivered from Pressurized Inhalers Containing Non-CFC and CFC Propellants. *J Aerosol Med*, **14**(2)**,** 197-208.

Richter, K. (2004) Successful use of DPI systems in asthmatic patients--key parameters. *Respir Med*, **98**(Supplement 2)**,** S22-S27.

Rodriguez-Carballeira, M., Heredia, J. L., Rue, M., Quintana, S.Gomez, L. (2001) The Bronchodilator Test with Increasing Doses of Terbutaline in Chronic Obstructive Pulmonary Disease Patients. *Pulmonary Pharmacology & Therapeutics*, **14**(1)**,** 61-65.

Rogers, D. F. and Ganderton, D. (1995) Determining equivalence of inhaled medications. Consensus statement from a workshop of the British Association for Lung Research, held at Royal Brompton National Heart & Lung Institute, London 24 June 1994. *Respir Med*, **89**(4)**,** 253-61.

Roig, M., Berges, R., Ventura, R., Fitch, K. D., Morton, A. R.Segura, J. (2002) Quantification of terbutaline in urine by enzyme-linked immunosorbent assay and capillary electrophoresis after oral and inhaled administrations. *J Chromatogr B Analyt Technol Biomed Life Sci*, **768**(2)**,** 315-324.

Ross, D. L. and Schultz, R. K. (1996) Effect of inhalation flow rate on the dosing characteristics of dry powder inhaler (DPI) and metered dose inhaler (MDI) products. *J Aerosol Med*, **9**(2)**,** 215-26.

Ruffin, R. E., Kenworthy, M. C.Newhouse, M. T. (1978) Response of asthmatic patients to fenoterol inhalation: a method of quantifying the airway bronchodilator dose. *Clin Pharmacol Ther*, **23**(3)**,** 338-45.

Scottish Intercollegiate Guidelines (SIGN) (2002a) *Evidence table 4.4c: inhaled corticosteroid vs leukotriene receptor antagonists.* [cited 21/04/2008]. Available from http://www.sign.ac.uk/guidelines/published/support/guideline63/index.html.

Scottish Intercollegiate Guidelines (SIGN) (2002b) *Pharmacological management of asthma, Evidence table 4.1: inhaled short acting beta 2 agonists. Edinburgh:* [cited 21/04/2008]. Available from http://www.sign.ac.uk/guidelines/published/support/guideline63/index.html.

Scottish Intercollegiate Guidelines (SIGN) (2002c) *Pharmacological management of asthma. Evidence table 4.11b: add-on drugs for inhaled steroids: Long acting or oral B2 agonists* [cited 21/04/2008]. Available from http://www.sign.ac.uk/guidelines/published/support/guideline63/index.html.

Scottish Intercollegiate Guidelines (SIGN) (2002d) *Pharmacological management of asthma. Evidence table 4.11d: add-on drugs for inhaled steroids: theophylline, beclomethasone diproponate, budesonide.* [cited 21/04/2008]. Available from http://www.sign.ac.uk/guidelines/published/support/guideline63/index.html.

Sears, M. R. and Lotvall, J. (2005) Past, present and future--[beta]2-adrenoceptor agonists in asthma management. *Respir Med,* **99**(2)**,** 152-170.

Seeley, R. R., Stephens, Trent D., Tate, Philip. (2000) Respiratory system. In: *Anatomy and physiology*. Boston, Mass.; London : McGraw-Hill: McGraw-Hill, pp. 737-776.

Seppälä, O. P., Herrala, J., Hedman, J., Alanko, K., Liipo, K., Terho, E., Pietinalho, A., Nyholm, J. E.Nieminen, M. M. (1998) The bronchoprotective efficacy of salbutamol inhaled from a new metered-dose powder inhaler compared with a conventional pressurized metered-dose inhaler connected to a spacer. *Respir Med,* **92**(3)**,** 578-583.

Shrewsbury, S. B., Mohsen, N. M., Newman, S. P., Pitcairn, G.Armer, T. A. (2006) Novel Pressurised Metered Dose Inhaler Aerosol Delivery of Fluticasone Propionate via Tempo(R) in Humans. *J Allergy Clin Immunol,* **117**(2, Supplement 1)**,** S91.

Silkstone, V., Corlett, S.Chrystyn, H. (2002a) Relative lung and total systemic bioavailability following inhalation from a metered dose inhaler compared with a metered dose inhaler attached to a large volume plastic spacer and a jet nebuliser. *Eur J Clin Pharmacol,* **57**(11)**,** 781-786.

Silkstone, V., Dennis, J., Pieron, C.Chrystyn, H. (2002b) An Investigation of In Vitro/In Vivo Correlations for Salbutamol Nebulised by Eight Systems. *J Aerosol Med,* **15**(3)**,** 251-259.

Silkstone, V. L., Chrystyn, H., Tomlinson, H. S.Corlett, S. A. (2000) Relative bioavailability of salbutamol to the lung following inhalation when administration is prolonged. *Br J Clin Pharmacol,* **50**(3)**,** 281-4.

Silkstone, V. L., Corlett, S. A., Chrystyn, H.Tomlinson, H. S. (2002c) Determination of the relative bioavailability of salbutamol to the lungs and systemic circulation following nebulization. *Br J Clin Pharmacol,* **54**(2)**,** 115-9.

Singh, T. K., Abonyo, B., Narasaraju, T. A.Liu, L. (2004) Reorganization of cytoskeleton during surfactant secretion in lung type II cells: a role of annexin II. *Cell Signal,* **16**(1)**,** 63-70.

Smaldone, G. C. (2004) Aerosolized antibiotics in mechanically ventilated patients. *Respir Care,* **49**(6)**,** 635-9.

Smaldone, G. C. and Palmer, L. B. (2000) Aerosolized antibiotics: current and future. *Respir Care,* **45**(6)**,** 667-75.

Smigaj, D., Drago, N., Sultzman, C., Amini, S., Caritas, S., Kalhan, S.Catalano, P. (1996) The effect of oral terbutaline on maternal glucose metabolism and energy expenditure in pregnancy. *J Soc Gynecol Investig* **3**(2, Supplement 1)**,** 295A.

Smith, E. C., Denyer, J.Kendrick, A. H. (1995) Comparison of twenty three nebuliser/compressor combinations for domiciliary use. *Eur Respir J,* **8**(7)**,** 1214-1221.

Smith, S. R. and Kendall, M. J. (1986) Potentiation of the adverse effects of intravenous terbutaline by oral theophylline. *Br J Clin Pharmacol,* **21**(4)**,** 451-3.

Snell, J. and Ganderton, D. (1999) Assessing lung deposition of inhaled medications: Consensus statement from a workshop of the British Association of Lung Research. *Respir Med,* **93**123-133.

Sophie Thayer, J. (1996) In utero exposure to terbutaline: Effects on infant behavior and maternal self-esteem. *Infant Behav Dev,* **19**(Supplement 1)**,** 778.

Spitzer, W. O., Suissa, S., Ernst, P., Horwitz, R. I., Habbick, B., Cockcroft, D., Boivin, J. F., McNutt, M., Buist, A. S.Rebuck, A. S. (1992) The use of beta-agonists and the risk of death and near death from asthma. *N Engl J Med,* **326**(8)**,** 501-6.

Srichana, T., Martin, G. P.Marriott, C. (1998) Dry powder inhalers: The influence of device resistance and powder formulation on drug and lactose deposition in vitro. *Eur J Pharm Sci,* **7**(1)**,** 73-80.

Stanescu, D., Sanna, A., Veritier, C., Kostianev, S., Calcagni, P. G., Fabbri, L. M.Maestrelli, P. (1996) Airways obstruction, chronic expectoration and rapid decline of FEV1 in smokers are associated with increased levels of sputum neutrophils. *Thorax,* **51**267-271

Staun, P., Lennmarken, C., Eriksson, L. I.Wiren, J. E. (1990) The influence of 10 mg and 20 mg of bambuterol on the duration of succinylcholine-induced neuromuscular blockade. *Acta Anaesthesiol Scand,* **34**(6)**,** 498-500.

Szafranski, W., Cukier, A., Ramirez, A., Menga4, G., Sansores, R., Nahabedian, S., Peterson, S.Olsson, H. (2003) Efficacy and safety of budesonide/formoterol in the management of chronic obstructive pulmonary disease. *Eur Respir J,* **21**74-81.

Szeman, J., Roos, N.Csabai, K. (1997) Ruggedness of enantiomeric separation by capillary electrophoresis and high-performance liquid chromatography with methylated cyclodextrins as chiral selectors. *J Chromatogr A,* **763**(1-2)**,** 139-147.

Tal, A., Golan, H., Grauer, N., Aviram, M., Albin, D.Quastel, M. R. (1996) Deposition pattern of radiolabeled salbutamol inhaled from a metered-dose inhaler by means of a spacer with mask in young children with airway obstruction. *J Pediatr,* **128**(4)**,** 479-84.

Tarsin, W., Assi, K. H.Chrystyn, H. (2004) In-vitro intra- and inter-inhaler flow rate-dependent dosage emission from a combination of budesonide and eformoterol in a dry powder inhaler. *J Aerosol Med,* **17**(1)**,** 25-32.

Tarsin, W., Corrado, O. J., Brownlee, K., Kanthapillai, P., Pearson, S., Chetcuti, P.Chrystyn, H. (2001) Inter-individual variability of peak inhalation rate through different inhalers by asthmatic children and adults and those with COPD. *Eur Respir J,* **Vol 18, Suppl. 33,**P900 (133S).

Tarsin, W. Y., Pearson, S. B., Assi, K. H.Chrystyn, H. (2006) Emitted dose estimates from Seretide®, Diskus®, and Symbicort®, Turbuhaler® following inhalation by severe asthmatics. *Int J Pharm,* **316**(1-2)**,** 131-137.

Tattersfield, A. E. (1987) Effect of beta-agonists and anticholinergic drugs on bronchial reactivity. *Am Rev Respir Dis,* **136**(4 Pt 2)**,** S64-8.

Taylor, K. M. G. and McCallion, O. N. M. (1997) Ultrasonic nebulisers for pulmonary drug delivery. *Int J Pharm,* **153**(1)**,** 93-104.

Taylor, K. M. G., Venthoye, G.Chawla, A. (1992) Pentamidine isethionate delivery from jet nebulisers. *Int J Pharm,* **85**(1-3)**,** 203-208.

Terzano, C. (2001) Pressurized Metered Dose Inhalers and Add-on Devices. *Pulmonary Pharmacology & Therapeutics,* **14**(5)**,** 351-366.

Terzano, C. and Mannino, F. (1996) Probability of particle and salbutamol deposition in the respiratory tract: comparison between MDI and Autohaler. *Monaldi Arch Chest Dis,* **51**(3)**,** 236-42.

Terzano, C. and Mannino, F. (1999) Aerosol characterization of three corticosteroid metered dose inhalers with volumatic holding chambers and metered dose inhalers alone at two inspiratory flow rates. *J Aerosol Med,* **12**(4)**,** 249-54.

Tetley, T. D. (1993) New perspectives on basic mechanisms in lung disease. 6. Proteinase imbalance: its role in lung disease. *Thorax,* **48**560-565

Theophilus, A., Moore, A., Prime, D., Rossomanno, S., Whitcher, B.Chrystyn, H. (2006) Co-deposition of salmeterol and fluticasone propionate by a combination inhaler. *Int J Pharm,* **313**(1-2)**,** 14-22.

Thirstrup, S., Nielsen-Kudsk, F.Dahl, R. (1997) In vitro studies on the interactions of [beta]2-adrenoceptor agonists, methylxanthines, Ca2+-channel blockers, K+-channel openers and other airway smooth muscle relaxants in isolated guinea-pig trachea. *Eur J Pharmacol,* **326**(2-3)**,** 191-200.

Tomlinson, H. S., Corlett, S. A., Allen, M. B.Chrystyn, H. (2005) Assessment of different methods of inhalation from salbutamol metered dose inhalers by urinary drug excretion and methacholine challenge. *Br J Clin Pharmacol,* **60**(6)**,** 605-610.

Tomlinson, H. S., Corlett, S. A.Chrystyn, H. (2003) Dose-response relationship and reproducibility of urinary salbutamol excretion during the first 30 min after an inhalation. *Br J Clin Pharmacol,* **56**(2)**,** 225-7.

Tortora, G. J. G. J., Grabowski, Sandra Reynolds. (2003) Respiratory system. In: *Principles of anatomy and physiology.*: New York ; Chichester : Wiley, pp. 806-846.

United States Pharmacopeia (2002) Terbutaline. In: *The United States pharmacopeia 25 [and] The national formulary 20.*: The board of trustees, pp. 1658-1660.

United States Pharmacopeia (2005) Aerosols, nasal sprays, metered dose inhalers and dry powder inhalers. In: *The United States pharmacopeia 28 [and] The national formulary 23.*: The board of trustees, pp. 2359-2377.

Van der Palen, J. (2003) Peak inspiratory flow through Diskus and Turbuhaler, measured by means of a peak inspiratory flow meter (In-Check DIAL®). *Respir Med,* **97**(3)**,** 285-289.

Van der Vlis, E., Mazereeuw, M., Tjaden, U. R., Irth, H.van der Greef, J. (1995) Combined liquid-liquid electroextraction-isotachophoresis for loadability enhancement in capillary zone electrophoresis-mass spectrometry. *J Chromatogr A,* **712**(1)**,** 227-234.

van Noord, J. A., Aumann, J. L., Janssens, E., Smeets, J. J., Verhaert, J., Disse, B., Mueller, A.Cornelissen, P. J. G. (2005) Comparison of tiotropium once daily, formoterol twice daily and both combined once daily in patients with COPD. *Eur Respir J,* **26**(2)**,** 214-222.

Van Oort, M. (1995) In vitro testing of dry powder inhalers. *Aerosol Sci Technol,* **22**(4)**,** 364-373.

Van Vyncht, G., Preece, S., Gaspar, P., Maghuin-Rogister, G.DePauw, E. (1996) Gas and liquid chromatography coupled to tandem mass spectrometry for the multiresidue analysis of [beta]-agonists in biological matrices. *J Chromatogr A,* **750**(1-2)**,** 43-49.

Vaswani, S. K. and Creticos, P. S. (1998) Metered dose inhaler: past, present, and future. *Ann Allergy Asthma Immunol,* **80**(1)**,** 11-9; quiz 19-20.

Vestbo, J., Sorensen, T., Lange, P., Brix, A., Torre, P.Viskum, K. (1999) Long-term effect of inhaled budesonide in mild and moderate chronic obstructive pulmonary disease: a randomised controlled trial. *Lancet,* **353**(9167)**,** 1819-1823.

Vilsvik, J., Ankerst, J., Palmqvist, M., Persson, G., Schaanning, J., Schwabe, G.Johansson, A. (2001) Protection against cold air and exercise-induced bronchoconstriction while on regular treatment with Oxis(R). *Respir Med,* **95**(6)**,** 484-490.

Vogelberg, C., Kremer, H.-J., Ellers-Lenz, B., Engel, M., Maus, J., Conrad, F.Hermann, R. (2004) Clinical evaluation of the peak inspiratory flow generated by asthmatic children through the Novolizer(R). *Respir Med,* **98**(10)**,** 924-931.

Waldeck, B. (2002) [beta]-Adrenoceptor agonists and asthma--100 years of development. *Eur J Pharmacol,* **445**(1-2)**,** 1-12.

Waldrep, J. C., Keyhani, K., Black, M.Knight, V. (1994) Operating characteristics of 18 different continuous-flow jet nebulisers with beclomethasone dipropionate liposome aerosol. *Chest,* **105**(1)**,** 106-110.

Walters, E. H., Walters, J., Gibson, P.Jones, P. W. (2003) Inhaled short acting $beta_2$ agonist use in asthma: regular versus as needed treatment. *Cochrane Database Syst Rev,* **ISSN 1464-780X**(2)**,** CD001285.

Weda, M., Zanen, P., Boer, A. H. d., Barends, D. M.Frijlink, H. W. (2004) An investigation into the predictive value of cascade impactor results for side effects of inhaled salbutamol. *Int J Pharm,* **287**(1-2)**,** 79-87.

Wildhaber, J., Devadason, S., Hayden, M., James, R., Dufty, A., Fox, R., Summers, Q.LeSouef, P. (1996) Electrostatic charge on a plastic spacer device influences the delivery of salbutamol. *Eur Respir J,* **9**(9)**,** 1943-1946.

Wildhaber, J., Janssens, H., Piérart, F., Dore, N., Devadason, S.LeSouef, P. (2000) High-percentage lung delivery in children from detergent-treated spacers. *Pediatr Pulmonol,* **29**(5)**,** 389-393.

Williams, A. E. and Chrystyn, H. (2007) Survey of pharmacists' attitudes towards interchangeable use of dry powder inhalers. *Pharm World Sci*.

Witek, T. J., Jr. (2000) The fate of inhaled drugs: the pharmacokinetics and pharmacodynamics of drugs administered by aerosol. *Respir Care,* **45**(7)**,** 826-30.

Wong, A. G., O'Shaughnessy, A. D., Walker, C. M.Sears, M. R. (1997) Effects of long-acting and short-acting beta-agonists on methacholine dose-response curves in asthmatics. *Eur Respir J,* **10**(2)**,** 330-336.

Yanai, M., Sekizawa, K., Ohrui, T., Sasaki, H.Takishima, T. (1992) Site of airway obstruction in pulmonary disease: direct measurement of intrabronchial pressure. *J Appl Physiol,* **72(**3**),** 1016-1023.

Zanen, P., Go, L. T.Lammers, J. W. (1996) Optimal particle size for beta 2 agonist and anticholinergic aerosols in patients with severe airflow obstruction. *Thorax,* **51**(10)**,** 977-980.

Zanen, P., Go, L. T.Lammers, J. W. J. (1994) The optimal particle size for ß-adrenergic aerosols in mild asthmatics. *Int J Pharm,* **107**(3)**,** 211-217.

Zeng, X. M., Martin, G. P.Marriott, C. (1995) The controlled delivery of drugs to the lung. *Int J Pharm,* **124**(2)**,** 149-164.

Zhou, Y., Ahuja, A., Irvin, C. M., Kracko, D. A., McDonald, J. D.Chen, Y. S. (2005) Medical Nebuliser Performance: Effects of Cascade Impactor Temperature. *Respir Care,* **50**(8)**,** 1077.

12. Appendix

Date: Thu, 30 Jun 2005 12:49:00 +0100
From: "Prof. R.J. Naylor" <r.j.naylor@Bradford.ac.uk>
To: M.E.Abdelmobdy@Bradford.ac.uk
Subject: Ethics approval
2 unnamed text/html 1.76 KB

To: M.E.Abdelmobdy and Colleagues
School of Pharmacy
University of Bradford

From: Professor R.J.Naylor, Chairman of the Subcommittee on Ethics and Procedures involving Human Subjects.

Dear Mr. Abdelmobdy,

Re Project: Determination of the relative bioavailability of terbutaline to the lung and body following inhalation using urinary pharmacokinetic methods.

Thank you for the above submission to the 'Subcommittee on Ethics and Procedures involving Human Subjects' on behalf of yourself and other colleagues. The completed application and additional protocol have been reviewed.

As Chairman of the Subcommittee I formally approve the submission.

Yours sincerely,

Professor R.J.Naylor 30th June 2005

The Leeds Teaching Hospitals NHS

NHS Trust

PRIVATE & CONFIDENTIAL

Mohamed Abdelrahim
School of Pharmacy
Institute of Pharmaceutical Innovation
University of Bradford
Bradford
BD7 1DP

Central Recruitment
1st Floor, Trust HQ
Beckett Street
St James's University Hospital
Leeds
LS9 7TF

Direct Line 0113 2065980
Fax: 0113 2066556
www.leedsth.nhs.uk

Date: 05 June 2008

Dear Mr Abdelrahim

HONORARY CONTRACT IN THE POST OF HONORARY PhD STUDENT

1. I am instructed by Leeds Teaching Hospitals NHS Trust ("the Trust") to offer you an honorary contract conferring honorary status in the post of PhD Student commencing on 1st February 2008.

   The purpose of the contract is to undertake a research study - 'Determination of the relative lung bioavailability and systemic bioavailability of different inhalation methods in non invasive ventilation.'

   This Contract is for a fixed period and will terminate on 31st December 2008. During the continuance of the fixed term this Honorary Contract may be terminated by the Trust at any time upon giving one week's notice in writing to you.

2. The title and status does not create an employment relationship with the Trust and attracts no remuneration from the Trust. You are required to observe the policies and procedures of the Trust in so far as they apply to this appointment and to observe all policies and procedures in respect of clinical and research activities. In addition you will be expected to comply with the Trusts general conditions of employment in as far as they apply to you e.g. working hours.

3. You must notify Dr Paul Plant of your presence within the Trust and the likely duration of each visit.

4. Under the terms of this Contract you are permitted access to the Trust premises and equipment within the Trust's Ward 9 at St James's Hospital for the purpose of carrying out the functions associated with the position of PhD Student.

5. Whilst undertaking NHS duties you are normally covered by the NHS Hospital and community Health Services indemnity against claims for medical negligence. However in certain circumstances (especially in services for which you receive a separate fee) you may not be covered by the indemnity. You are therefore advised to maintain membership of your defence organisation.

6. You must observe the same standards of care and propriety in dealing with patients, staff, visitors, equipment and premises as is expected of any other contract holder and must act appropriately and responsibly at all times and in accordance with in other terms set out in this document.

7. (Not applicable).It is a condition of this Contract that you are required to be registered with a professional body, **(General Medical Council).** This contract will automatically terminate if you fail to maintain such registration. (Please provide evidence of this before you commence)

8. You are required to ensure the security and confidentiality of all information regarding patients or staff at all times and not release any such information to anyone other than an approved person in the course of their duties. Honorary contract holders handling information stored in any format must ensure, in particular, that they meet the requirements of the Data Protection Act in only using such information for a registered purpose and not disclosing it to any unauthorised person.

9. With regard to claims for losses or personal injury sustained whilst undertaking your duties you will be treated the same as Trust employees. The Trust has a risk management strategy, which includes Health & Safety, Security, Fire Safety and Control of Infection. In certain circumstances the Trust has indemnity schemes to share the risk.

   You must report any accident or injury, however trivial, arising out of or in the course of your activities in the Trust and make appropriate records and statements as required.

   The LTH NHS Trust accepts no responsibility for damage to or loss of personal property, with the exception of small valuables handed to their officials for safe custody. You are, therefore, recommended to take out an insurance policy to cover your personal property.

10. You will be required to ensure that you are familiar with, and adhere to, the policies and procedures of the Trust which are available on the Trust's intranet HR site, or from your line manager within the Trust. Whilst working with the Trust your actions and their consequences will be the responsibility of the Trust other than where you act in a wilfully negligent manner in disregard of the Policies and Procedures of the Trust.

11. The Trust manages all research in accordance with the requirements of the Department of Health Research Governance Framework. As an Honorary Contract holder you must comply with all reporting requirements, systems, duties and action put in place by the Trust to deliver research governance.

12. Any intellectual property rights created during and/or in the work undertaken on Trust premises is and remains the property of the Trust and should not be used by you without the express permission of the Medical Director.

13. The Trust accepts high standards of work and behaviour from holders of Honorary Contracts and appointees and failure to maintain such standard may lead termination of the Contract.

14. You agree to co-operate fully with the Trust and/or its legal advisor in the investigation of any patient complaints/incidents including but not limited to any allegation of negligence or misconduct on your part. You also agree to provide the Trust upon request with a full written statement concerning the said patient complaint/incident.

15. For the duration of this contract you must not, by act or omission, compromise or prejudice the business; commercial or other legitimate interests of the Trust.

16. Should you be absent due to sickness or any other reason you should inform your Trust contact at the first available opportunity.

17. a) Should you have any grievance relating to your work within the Trust please discuss them with the manager of your substantive post in the first instance.

    b) The arrangements for settling differences between you and the LTH NHS Trust will be in accordance with the agreed procedures laid down by your substantive employer.

    c) Any disciplinary action that needs to be taken will be done so following the Procedures of your substantive employer.

18. You will be expected to carry your Trust and University ID card at all times whilst undertaking Trust business.

19. Should your honorary contract take you into an area that requires staff to undergo a Criminal Records Bureau check then you will be expected to undertake the relevant checks before commencing work in the Trust.

20. If you agree to accept this honorary contract on the terms specified above, please sign the form of acceptance at the foot of this page and one copy to me at the above address. A second signed copy of this letter is also attached, which you should also sign and retain for your future reference.

21. The Trust reserves to withdraw this honorary contract at any time but will not do so without good reason. If you leave your post with your substantive employer your honorary contract will automatically be terminated – you should at this time return all Trust property e.g. ID badges to the Trust. It is the responsibility of your employer to inform the Trust when an individual holding an honorary contract leaves their employment.

22. During the period of your honorary contract your contact point will be Dr Paul Plant.

23. On starting work for the Trust you are required to attend the Induction Course Day and a place has been reserved for you on 6th August at 10:00am until 4.00pm in Suite D16, Josephs Well, (behind Clarendon Wing, Leeds General Infirmary). A programme of events will be given to you on the morning of the induction. It is essential that you attend a Trust Induction. In exceptional circumstances, if you are unable to attend on this day, please contact your line manager to arrange an alternative date.

Yours sincerely

Jennifer Tate
Recruitment Assistant

DO NOT DETACH

I hereby accept the honorary contract mentioned in the letter to me, of which the above is a copy, on the terms and subject to the conditions referred to in that letter.

Signed: ..... Date: 11/06/08

The Leeds Teaching Hospitals NHS
NHS Trust

Research & Development Directorate
A/B Corridor, Old Site

The General Infirmary at Leeds
Great George Street
Leeds
West Yorkshire
LS1 3EX

Tel: 0113 392 2878
Fax: 0113 392 6397

www.leedsteachinghospitals.com

04/05/2007

Mr Mohamed E.A. Abdelrahim
PH.D. Student
Institute of Pharmaceutical Innovation
University of Bradford
Bradford
BD7 1DP

Dear Mr Mohamed E.A. Abdelrahim

Re: LTHT R&D Approval of EX07/8175: Determination of the relative lung bioavailability and systemic bioavailability of different inhalation methods in non invasive ventilation

I write with reference to the above research study. I can now confirm that this study has R&D approval and the study may proceed at The Leeds Teaching Hospitals NHS Trust (LTHT). This organisational level approval is given based on the information provided in the documents listed below.

As principal investigator you have responsibility for the design, management and reporting of the study. In undertaking this research you must comply with the requirements of the *Research Governance Framework for Health and Social Care* which is mandatory for all NHS employees. This document may be accessed on the Department of Health website at http://www.dh.gov.uk/research

R&D approval is therefore given on the understanding that you comply with the requirements of the *Framework* as listed in the attached sheet "Conditions of Approval".

If you have any queries about this approval please do not hesitate to contact the R&D Department on telephone 0113 392 2878.

Indemnity Arrangements

The Leeds Teaching Hospitals NHS Trust participates in the NHS risk pooling scheme administered by the NHS Litigation Authority 'Clinical Negligence Scheme for NHS Trusts' for: (i) medical professional and/or medical malpractice liability; and (ii) general liability. NHS Indemnity for negligent harm is extended to researchers

with an employment contract (substantive or honorary) with the Trust. The Trust only accepts liability for research activity that has been managerially approved by the R&D Department.

The Trust therefore accepts liability for the above research project and extends indemnity for negligent harm to cover you as principal investigator and the researchers listed on the R&D approval form provided that each member of the research team has an employment contract (substantive or honorary) with the Trust. Should there be any changes to the research team please ensure that you inform the R&D Department and that s/he obtains an employment contract with the Trust if required.

Yours sincerely

Dr D R Norfolk
Associate Director of R&D

Approved documents
The documents reviewed and approved are listed as follows

Document	*Version*	*Date of document*
Protocol		Received 04/05/07
SSI Form	5.3	07/03/07
NHS REC Application Form	5.3	07/03/07

Conditions of R&D Approval

- Approval from your local Clinical Management Team must be obtained before starting the study.

- Approval of the appropriate Research Ethics Committee, where necessary, must be obtained before starting the study. Any changes made to the project during ethical review must be reviewed and approved by the R&D Department to maintain R&D Approval status.

- Arrangements must be made to ensure that all members of the research team, where applicable, have employment contracts with the Trust (either full or honorary).

- Agreements must be in place with appropriate support departments regarding the services required to undertake the project and arrangements must be in place to recompense them for the costs of their services.

- Arrangements must be in place for the management of financial and other resources provided for the study, including intellectual property arising from the work.

- Priority should be given at all times to the dignity, rights, safety and well being of participants in the study

- Healthcare staff should be suitably informed about the research their patients are taking part in and information specifically relevant to their care arising from the study should be communicated promptly.

- Each member of the research team must be qualified by education, training and experience to discharge his/her role in the study. Students and new researchers must have adequate supervision, support and training.

- The research must follow the protocol approved by the relevant research ethics committee. Any proposed amendments to or deviations from the protocol must be submitted for approval to the Research Ethics Committee, the research sponsor, regulatory authority and any other appropriate body. The R&D Department should be informed where the amendment has resource implications within the CMT and the CMT research lead/clinical director notified.

- Any adverse events/adverse drug reactions must be reported to the appropriate research ethics committee, research sponsor and any other regulatory authority. Adverse events in clinical trials of investigational medicinal products must be reported in accordance with the Medicines for Human Use (Clinical Trials) Regulations 2004. All serious adverse events as defined in the research protocol,

that occur within LTHT should be reported via the IR1 reporting system to the Risk Management Department, Trust Headquarters, St James's University Hospital, Beckett Street, Leeds LS9 7TF.

- Reports on the progress and outcomes of the work must be produced on time and to an acceptable standard. Please send a copy of the progress report produced for the Research Ethics Committee to the R&D Department for monitoring.

- Procedures should be in place to ensure collection of high quality, accurate data and the integrity and confidentiality of data during processing and storage.

- Arrangements must be made for the appropriate archiving of data when the research has finished. Records must normally be kept for 15 years.

- All data and documentation associated with the study must be available for audit at the request of the appropriate auditing authority. Currently 10% of REC approved projects are randomly selected for audit inspection by the R&D Department each year. You will be informed by letter if your study is selected.

- Findings from the study should be disseminated promptly and fed back as appropriate to research participants.

- Findings from the study should be exposed to critical review through accepted scientific and professional channels.

Commercially Sponsored Trials
If the study is commercially sponsored approval is given subject to provision of the following documents.

- Clinical Trials Agreement - agreed and signed off by the R&D Department, the Principal Investigator and the Sponsor.

- Indemnity agreement, if not included in the Clinical Trials Agreement- (standard ABPI no fault arrangements apply) signed by the R&D Department and the Sponsor

It is essential that all the responsibilities set out in the Research Governance Framework and outlined above are fulfilled. The Trust reserves the right to withdraw R&D approval for a study, and therefore the provision of indemnity cover (for negligent harm) for its employees, where it is found that the above criteria have not been met. The Trust will not accept liability for any activity that has not been fully approved.