PROBLEMS WITH INHALATIONAL DRUG DELIVERY

Thesis submitted for the degree of

Doctor of Philosophy

at the University of Leicester

by

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December 1999

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Acknowledgements

The work in this thesis was supported by the Astra Foundation.

I would like to thank Peter Richardson, and the Trustees of the Astra Foundation for their support.

Part of this work was also supported by Fisons, Glaxo Wellcome and Pari GmbH

I would like to acknowledge the help of many people:

First and Foremost, Chris O'Callaghan, my supervisor and good friend.

Professors Hamish Simpson and Mike Silverman, Heads of the Department of Child Health at the University of Leicester.

Judith Jackson, Judy White, Paul Johnson, Bijal Morjaria and Liz Marriott, technicians in the Aerosol Laboratory. Judith, especially, has been a stalwart of patience and good humour.

Barbara Barbour and Juliet Jackson, Departmental Chief Technicians, for their support of the Aerosol Laboratory. Paul Goodenough, Senior Scientific Officer in the Department of Child Health. Anne Gilbert and Pam Walker for 'organisational assistance'.

Anne Hollingworth, who taught me to use the multi-stage liquid impinger. Elna Berg, Dave Hallworth, Michelle Clay, Martin Knoch, Jo Lim for advice and support.

John Chubb of JCI Ltd. and Peter Powell of Static Safe Environments for help with the static charge measurements.

Sy Mangat, Sales Development Specialist, Kodak Ltd. for help with the high speed video.

Much of the HPLC equipment used was donated by various pharmaceutical companies, including Glaxo Wellcome and Astra Draco. The breathing simulator was donated by Pari GmbH.

Many spacer devices, nebulisers and drugs were donated by their manufacturers, especially Astra, Fisons, Glaxo Wellcome, 3M Healthcare, Medicaid and Pari GmbH.

Many of the MSLI runs described in this thesis were undertaken by the Aerosol Laboratory Technical Staff.

Lucy Foster helped with the temperature and humidity measurements (Chapter 5.1).

Judy White calibrated the breathing simulator (Chapter 5.4).

Paul Goodenough wrote the computer program for recording the static probe measurements (Chapter 5.6).

Nick Blackwell helped with the computer enhancement of the spacer images from Chapter 6.7.

Abstract

Inhalational therapy is used to deliver medication to the lung, either to treat diseases or, less commonly, for systemic absorption. A number of devices have been developed to aid or improve inhalational therapy, and this thesis deals with metered dose inhalers, used with spacer devices, and nebulisers. Despite their seemingly simple construction and concept, the correct choice and use of an inhalational drug delivery device can dramatically alter the amount of drug available for inhalation. Studies in this thesis, supported by emerging pharmacokinetic evidence, have highlighted areas where the device can affect the drug delivery.

In vitro methods were used to quantify and characterise the different devices, including inertial impaction for aerosol particle sizing, sinus flow pumps for breathing simulation and high speed video recordings to analyse aerosol plume geometry and spacer function.

The results these studies reveal a number of factors that may affect drug delivery. Firstly, delay between metered dose inhaler actuation into a spacer and inhalation can reduce the amount of drug available to the patient. Secondly, multiple actuations of the metered dose inhaler into the spacer prior to inhalation also reduce the amount of drug available. The size of the spacer may also affect the amount of drug available for inhalation, and this will vary with the drug prescribed. Different formulations may differ in their aerosol cloud speed and volume, and this may alter the amount of drug delivered from different spacers.

Plastic and polycarbonate spacers may be highly charged with static electricity. Such spacers deliver less drug than those where the static charge has been reduced by an anti-static lining, or where the spacer is constructed from static dissipative materials. Washing the spacer also reduces its charge, but the optimum washing regime for spacers is not known.

Different nebulisers deliver different amounts of drug, and the assessment of nebulisers varies with the method used. Accurate assessment should include direct measurement of the mass of drug released, and should incorporate simulated patient breathing. Effective nebulisation of drug ends after a few minutes. This time will depend on the nebuliser and drug being used, but for some medications administered for asthma, little drug may be delivered after five minutes, and patients should be advised to stop nebulisation after this time.

In conclusion, studies in this thesis support the hypothesis that the method of use or choice of inhalational drug delivery device affects the amount of drug that is available for inhalation by the patient.

Introduction

"It ain't what you give, its the way that you give it".

The therapeutic options for inhaled drug delivery are increasing. When bronchodilators were the principle drugs delivered by inhalation, scientific evaluation was relatively limited. With such a small airway dose of bronchodilator needed to achieve maximal bronchodilation, variations in drug delivery between devices was not felt to be critical. By contrast, use of expensive drugs such as DNAse, and the use of inhaled corticosteroids, where side effects may be significant, demand greater knowledge of the drug delivery system used.

This thesis describes a series of experiments into the use of the two most commonest types of inhalational drug delivery device; metered dose inhalers, used with or without spacer devices, and nebulisers.

In the ***Background***, the history of inhalational therapy is briefly described, and some of the medications currently used or proposed for inhalational therapy listed. The factors determining the deposition of inhaled particles in the lung are then discussed.

Metered dose inhalers are the commonest inhalational drug delivery device prescribed, with over 400 million manufactured each year. Their deceptively simple method of actuation belies the fact that less than fifteen percent of the metered drug dose is deposited in the lungs. The majority of patients are unable to use their metered dose inhaler optimally, and will deposit even less drug in their lungs.

Metered dose inhalers are pressurised by chlorofluorocarbons. The mechanisms by which these compounds damage the Earth's ozone layer are described, and some of the alternatives discussed. Replacement propellants for chlorofluorocarbons have different physico-chemical properties, and their use may affect drug delivery from new formulation metered dose inhalers.

Spacer devices, described next, have been manufactured to overcome some of the problems of metered dose inhalers, and are becoming increasingly popular for the delivery of inhaled drugs in the treatment of asthma. They reduce the problems of poor inhaler technique which may lead to treatment failure with metered dose inhalers, largely eliminate oral absorption of inhaled steroids and have been shown to be as effective as nebulisers in the treatment of acute severe asthma. By the attachment of facemasks they can be adapted to treat patients of all ages. Popularity has lead to a rapid increase in the number of different types of spacer available.

As with other inhalation devices, the dose of drug available for a patient to inhale may vary greatly depending on which spacer is used. Despite their seemingly simple construction and concept, the correct choice and use of a spacer can dramatically alter the amount of drug available for inhalation. Studies outlined in this thesis, supported by emerging pharmacokinetic evidence, highlight areas where the incorrect use of a spacer device can affect the drug output.

Nebulisers are used by patients who cannot use metered dose inhalers, who require higher doses of medication than can be conveniently administered by other methods, or who require drugs that are not available in other forms. Nebulised therapy has also been used for systemic therapy of some drugs.

Effective nebuliser therapy requires a device that repeatably and quickly delivers sufficient drug to the site of action, with minimal wastage, at a low cost. Clinicians are bombarded with competing claims about different nebuliser systems. In many cases, however, insufficient details are available to make the most appropriate choice. Conventional jet nebulisers are highly inefficient as much of the aerosol is wasted during exhalation. Recent designs have attempted to reduce these inefficiencies. The rapid increase in the number of nebulisers marketed and significant differences in design may result in drug delivery to patients varying by a factor of 2 or more. Experiments in this thesis investigate how drug output from nebulisers is measured, and compares the output of the corticosteroid budesonide from a number of different nebulisers.

The experiments described in this thesis are all 'in vitro', measuring the output of

the clinical situation. In the final part of the ***Background***, various methods of assessing inhalational drug delivery devices are described, and their relationship with lung deposition and clinical parameters discussed.

In the ***Methods*** section, the experimental techniques used in this thesis are described in detail. The mainstay of the experimental methods is the assessment of aerosol particle size using the glass multi-stage liquid impinger, with subsequent drug analysis using high pressure liquid chromatography.

The individual experiments and their results are presented in the ***Results*** section. The effect of delay between metered dose inhaler actuation and sampling; the effect of multiple actuations into the spacer prior to sampling; and the use of different spacers are investigated. The effect of altering spacer length and volume is determined, and the emptying pattern of spacers recorded on high speed video. Different formulations of the metered dose inhalers have different properties, and this is investigated for inhalers containing chlorofluorocarbons compared with newer propellants, and for generic formulations of the same drug and propellant.

Static charge accumulates on the walls of many polycarbonate and plastic spacers, attracting drug particles, which become charged when they are produced at the metered dose inhaler valve stem. The factors affecting spacer static charge and the effect this has on drug output are investigated.

Inhalational drug delivery devices are often tested at a constant flow rate. A sinus flow pump is used to simulate breathing, and the output of spacers and nebulisers determined under different breathing patterns. The particle size output of budesonide from different nebulisers and compressors is also measured.

Finally the results are discussed in the light of in vitro and in vivo work from other authors. The work in this thesis suggests ways to optimally use spacer devices and nebulisers. In general this work is supported by others, and should lead to improved

Research question to be answered.

Does the method of use or choice of inhalational drug delivery device affect the amount of drug that is available for inhalation by the patient?

Inhalational Therapy

The inhalational route has many advantages in the treatment of diseases of the respiratory tract. Medication may be delivered directly to its site of action, giving a faster onset of action and allowing lower doses of drug to be administered (Larsson and Svedmyr, 1977). Systemic absorption of drug is diminished, reducing systemic side effects of the medication (Hochhaus *et al.*, 1992). However, the respiratory tract has a number of features that are designed to prevent particulate matter from entering the lungs, and inhalational therapy, especially to children who are unable or unwilling to co-operate, is difficult and inefficient.

Historical background.

Inhalational therapy has a long history, with vapours and smokes being used in the treatment of respiratory disease and other ailments more than four thousand years ago (Miller, 1973; Yernault, 1994; Hill, 1988; Sakula, 1988). In Ayurvedic medicine, a dried paste was prepared from plants such as *Datura ferox* and smoked in a pipe. The length of the pipe could be altered to modify the strength of the vapour inhaled. Hippocrates is reported to have recommended the inhalation of hot vapours through a reed inserted into the lid of a boiling pot, the patient protecting his mouth from scalding with moistened sponges (Hippocrates, 1657). Galen, in the second century, also proposed this treatment, and the ancient Egyptians relied on the inhalation of vapours given off from extracts of the plant *Hyoscyamus* placed on hot bricks (Parks, 1970). Other ancient texts describe the inhalation of steam from hot springs, of aromatic vapours and of smoke from plants such as *Atropa belladonna* and *Datura ferox* in the treatment of bronchial asthma.

This latter remedy was introduced to Europe in the early nineteenth century. The Physician-General of Madras in India, finding that smoking the root of the plant was useful in treating his own asthma, had some sent to an Edinburgh physician, Dr Sims, for him to try. He confirmed the plants effectiveness, and, substituting the British thorn apple (*Datura stramonii*), published his findings in 1812 (Sims, 1812). Stramonium acts as an anticholinergic, and found wide acceptance as an anti asthma

drug, being incorporated into proprietary remedies of the day, such as 'Potters Asthma Cure'.

Another plant with anticholinergic properties, *Atropa belladonna*, also became a standard remedy for asthma in the nineteenth century, either by mouth, as a nasal spray or in cigarettes. 'Asthma cigarettes' became very popular, normally consisting of tobacco combined with other medicaments, such as cannabis ('Grimauds'), arsenic ('Cigarettes de Joy') and camphor ('Savory & Moore's'). In other brands the cigarette paper was impregnated with chemicals, such as potassium nitrate, which gave off bronchodilating substances on burning. Tobacco smoking itself was also thought to relieve acute asthma, the patient being instructed to smoke until he 'felt sick or faint, or got other miserable sensations'. A cigar at night was considered preventative!

The pharmacological effects of extracts of the adrenal gland were first described in 1895 (Oliver and Sharpey-Schafer, 1915), and these extracts were used in the treatment of asthma and hay-fever in 1900. Within a few years adrenaline (epinephrine) was extracted, and its successful use in the treatment of asthma was first reported in 1903 (Bullowa and Kaplan, 1903). Adrenaline soon became the mainstay of asthma treatment, given by injection, with the first use of inhaled adrenaline in the treatment of asthma described in 1929 (Camps, 1929). Combinations of adrenaline, atropine and stramonium were available, but the cardiac side effects of adrenaline limited its usefulness. Isoprenaline was introduced in metered dose inhaler form in the 1950s, and proved an effective therapy for asthma. Unfortunately, an increase in asthma deaths in the 1960s was thought to be due to the excessive use of isoprenaline inhalers by patients, leading to cardiotoxic complications (Speizer *et al.*, 1968). The problem was to develop drugs that would act on the adrenergic receptors in the lung, but not those in the heart. In 1948, Ahlquist described the α and β effects of adrenaline (Ahlquist, 1948), and in 1967 the β receptor was subdivided into β_1 and β_2 receptors (Lands *et al.*, 1967), and selective β_2 receptor stimulants, such as salbutamol and terbutaline, were developed, which had much less cardiac effects.

In 1817, Sir Alexander Crichton published an account of the use of the vapours of boiling tar in the treatment of tuberculosis, a treatment continued in Berlin in the first half of the nineteenth century. In 1859 a portable device was marketed in France which generated an aerosol of liquid tar for medicinal use. Many types of nebuliser have since been developed. Early devices used mouth pressure to draw air through the liquid to be nebulised, with more complex, steam driven nebulisers being produced in the nineteenth century. These were replaced by mechanical pumps, such as those constructed of glass and operated manually by compressing a hand bulb to generate air flow. In 1946 electrical pumps providing a continuous flow of air were advocated and the Collinson nebuliser (Collinson, 1937), constructed of ebonite with a plate baffle to filter out large drug particles became the most popular nebuliser in the United Kingdom. In 1958 Wright (1958) described a new nebuliser which was considerably more compact than the Collinson. The nebuliser was constructed of perspex with a removable liquid feedtube, allowing it to be cleaned easily. Now discontinued, it found widespread use in bronchial challenge testing where it became part of a standardised technique for the administration of broncho-provocative agents such as histamine. With the advent of portable, oil free compressors and injection moulding of plastics, a wide variety of disposable nebulisers have become available. Recent advances in design have improved their efficiency by increasing the amount of drug delivered to the patient, and by increasing the proportion of drug released in small particles which can enter the lung (Chapter 4.4).

In 1955, the five year old daughter of George Maison, experiencing difficulty using her hand held nebuliser, suggested that the system used to spray paint should be modified to aerosolise her medication. Within a year, the first pressurised metered dose inhaler (the Medihaler, Riker Laboratories) was released (Freedman, 1956). In 1962, the glass inhaler was changed to an aluminium can delivering up to 300 doses. This cheap and easily manufactured delivery system became the mainstay of inhalational therapy, complemented by the development of add on spacer or reservoir devices in the 1970s, and the release of breath actuated devices such as the Autohaler in the 1980s. Dry powder inhalers, which do not contain propellants and rely on the patient's inspiratory flow to aerosolise the medication, were developed in the 1970s and 80s, and now sophisticated, multiple dose dry powder inhalers are available to

deliver a variety of medications. Nebulisers, metered dose inhalers, with or without spacers, and dry powder inhalers are the main types of inhalational drug delivery devices currently used.

Medications available and proposed for inhalational therapy.

Almost any drug may be aerosolised and inhaled, and a wide range of medications are currently formulated for inhalation. Many more have been proposed or are used on an experimental basis. The accompanying table details some of these. The commonest inhaled medications are those used in the treatment of asthma and chronic obstructive airways disease, for instance β_2 sympathomimetics (such as salbutamol and terbutaline), anti-cholinergics (such as ipratropium bromide) and anti-inflammatory medications (such as sodium cromoglycate, or the corticosteroids budesonide and beclomethasone diproprionate).

Asthma and chronic obstructive airways disease
Bronchodilation - β_2 agonists, anticholinergics, $MgSO_4$.
Prophylactic therapy - corticosteroids, cromoglycate.
Emergency treatment of acute asthma

Cystic fibrosis
Prevention/treatment of infection - Inhaled antibiotics.
Reduction of sputum viscosity - DNAse
Secretion hydration - Amiloride.
Protease inhibition - α-1-antitrypsin.
Gene therapy - via liposomes or viruses.

Autoimmune Deficiency Syndrome
Prevention of pneumocystis carinii pneumonia - Inhaled pentamidine.

Dyspnoea - Morphine, inhaled opiates.

Treatment of hyperkalaemia
Nebulised salbutamol is as efficacious as intravenous insulin and glucose in lowering plasma potassium levels in uraemic patients.

Immunisation
Measles vaccination - High rates of seroconversion in young infants vaccinated by the inhalational route.

Treatment of croup.
Reduction of inflammation -
Nebulised adrenaline
Nebulised steroids

Inhalational drug delivery on intensive care
Treatment of Bronchiolitis - Ribavirin
Adult respiratory distress syndrome - Surfactant,
Prostacyclin, NO related vasodilators
Pulmonary hypertension - Nitroprusside, $MgSO_4$
Bronchopulmonary Dysplasia - Steroids

TABLE. Some indications and medications proposed for inhalational delivery

The prevalence of asthma appears to be increasing (Weiss, 1996), and asthma is currently one of the most important diseases of childhood in the United Kingdom

(Lenney *et al.*, 1994). Over four hundred million metered dose inhalers are prescribed each year. Eighty five percent of the costs of mild to moderate asthma are due to drug therapy (Jonsson, 1995). It has been estimated that the total expenditure in the UK primary care sector on anti-asthma medications was £275 million in 1990, and that the total National Health Service costs of childhood asthma range between £79 and £136 million (Lenney *et al.*, 1994). For these reasons there is substantial interest in improving the efficiency of inhalational drug delivery.

Factors affecting lung deposition of aerosols.

Inhaled particles deposit in the body by five different mechanisms, of which inertial impaction, sedimentation and diffusion are the most important. Interception and electrostatic attraction are less significant.

The airway consists of a system of branching tubes of increasing cross sectional area. The velocity of air flow through the airway decreases markedly beyond the lobar bronchus due to this increase in cross sectional area. The speed of inhaled aerosol laden air and turbulent flow is greatest in the nose/mouth, pharynx, trachea and larger bronchi, encouraging mixing of inspired and residual air, and deposition of aerosol particles. In the smaller bronchi and alveolar region beyond this, air flow is laminar.

Generation (airway)	Diameter (mm)	Length (mm)	Total Cross sectional area (cm^2)	Velocity (cm/s)	Residence Time (ms)
0 (Trachea)	18	120	2.5	390	30
1 (Main Bronchus)	12	48	2.3	430	11
2 (Lobar Bronchus)	8.3	19	2.1	460	4.1
8 (Smallest Bronchi with wall cartilage)	1.9	6.4	6.9	140	4.4
11 (Terminal Bronchus)	1.1	3.9	20	52	7.4
16 (Terminal Bronchiole)	0.6	1.6	180	5.4	31
21 (Alveolar Duct)	0.43	0.7	3.2×10^3	0.32	210
23 (Alveolar Sac)	0.41	0.5	12×10^3	0.09	550

Table. Airflow in the lungs. Adapted from (Hinds, 1983).

Based on Weibels (adult) lung model. Velocities and residence times assume a flow rate of 1l/s

Inertial impaction

During inhalation, the airflow negotiates a series of bends and bifurcations in the airway. At each of these changes in direction, aerosol particles tend to travel a short distance in their original direction before conforming to the new airflow. This distance is dependent on their inertia, a product of their mass and velocity, and is quantified by the stopping distance.

Particles from the metered dose inhaler are emitted at high velocity and a large proportion deposit in the oropharynx by inertial impaction. Within the lung, maximum deposition by inertial impaction occurs at the carina, and to a lesser extent at other airway bifurcations.

Sedimentation

Within the smaller airways and alveoli, where air velocity is low and the airway diameters are small, deposition is mainly by sedimentation or gravitational settling. This effect is largest in horizontal airways, and is enhanced by breath holding after

inhalation. The importance of sedimentation can be expressed by comparing the settling distance (the settling velocity multiplied by the time spent in that part of the airway) and the airway diameter.

Generation (airway)	Stopping distance / Airway diameter			Settling distance / Airway diameter			RMS displacement / Airway diameter		
Particle size:	0.1μm	1μm	10μm	0.1μm	1μm	10μm	0.1μm	1μm	10μm
0 (Trachea)	0	0.0008	0.068	0	0	0.0052	0.0004	0.0001	0
1 (Main Bronchus)	0	0.0013	0.109	0	0	0.0041	0.0003	0.0001	0
4 (Segmental Bronchus)	0	0.0031	0.272	0	0	0.0022	0.0005	0.0001	0
11 (Terminal Bronchus)	0	0.0017	0.149	0	0.0002	0.021	0.0029	0.0006	0.0002
16 (Terminal Bronchiole)	0	0.0003	0.028	0	0.0018	0.156	0.011	0.0022	0.0006
21 (Alveolar Duct)	0	0	0.0023	0.0004	0.017	1.52	0.039	0.0079	0.0023
23 (Alveolar Sac)	0	0	0.0007	0.0012	0.047	4.13	0.067	0.013	0.0040

Table. Relative importance of the three main deposition mechanisms (impaction, sedimentation and diffusion) for different size particles in different regions of the lung. (Adapted from Hinds, 1983).

Diffusion

Brownian motion, particularly of sub micron particles, may lead to deposition on the airway walls. Like sedimentation, this effect is greatest in the smaller airways, and is enhanced by factors that lead to prolonged residence times, such as breath holding after inhalation.

Interception

This occurs when a particle strikes the airway surface, because of its size rather than a deviation from the direction of airflow within the airway. It is greatest when the particle is large relative to the airway, and where the streamlines of the airflow are close to the airway wall. Interception is an important mechanism of deposition for fibres and irregularly shaped particles, which may penetrate the small airways

because of their small aerodynamic size (see chapter 4.5), but then deposit by interception because of their large size in one dimension.

Electrostatic attraction

Particles generated by inhalational drug delivery devices are often electrostatically charged. This charge can induce an equal and opposite charge on the airway wall (a 'mirror' charge), leading to attraction of the particle and electrostatic deposition (Melandri *et al.*, 1983; Prodi and Mularoni, 1985; Yu, 1985; Chan *et al.*, 1978). This is most apparent in particles less than 1μm in diameter and for long thin particles, such as fibres (Vincent, 1985). Aerosols consisting of large numbers of like charged particles may also deposit because mutual repulsion drives the particles onto the airway wall. These mechanisms are thought to be of less importance for the deposition of therapeutic aerosols in the lung, although it has been suggested that artificial charging of aerosols may be used to enhance drug delivery to the lung (Hashish and Bailey, 1987)

Particle size

The deposition characteristics of an aerosol in the airway depend largely on the particle or droplet size. Generally the smaller the particle the greater its chance of peripheral penetration and retention. However, for very fine particles below 0.5μm in diameter there is a chance of avoiding deposition altogether and being exhaled (Moren and Andersson, 1980). In 1966, the Task Group on Lung Dynamics, concerned mainly with the hazards of inhalation of environmental toxins, studied experimental and theoretical models and proposed a model for deposition of particles in the lung (Anonymous, 1966). The model suggested that particles larger than 10μm in diameter are most likely to deposit in the mouth and throat. Between sizes 5 and 10μm a transition from mouth to airway deposition occurs (Stahlhofen *et al.*, 1980). Particles smaller than 5μm in diameter deposit more frequently in the lower airways, and would be appropriate for pharmaceutical inhalation aerosols. The Task Group model is based on nose breathing and may underestimate the total lung deposition by

ignoring mouth breathing.

Particle aerodynamic diameter		% Deposition		% Exhaled
μm	Oropharynx	Tracheo-bronchial	Alveolar	
1	0	0	16	84
2	0	2	40	58
3	5	7	50	38
4	20	12	42	26
5	37	16	30	17
6	52	21	17	10
7	56	25	11	8
8	60	28	5	7

Table. Particle size and deposition. From (Stahlhofen *et al.*, 1980)

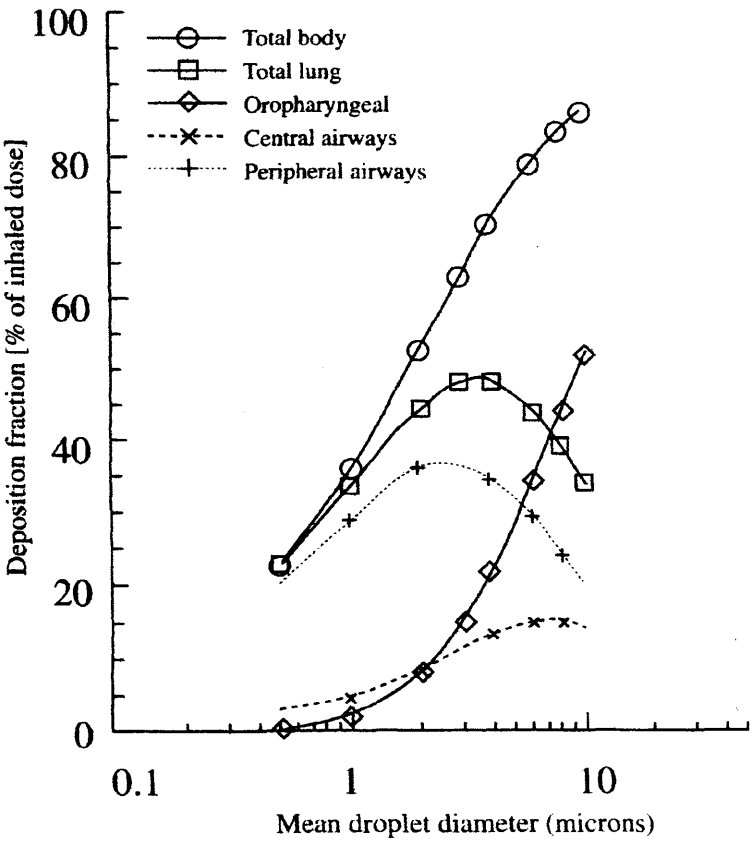


Figure. Particle size and deposition

The deposition profiles for an inhaled nebuliser cloud as predicted from Rudolph's (Rudolph *et al.*, 1990) model, which assumes oral breathing, are shown in the figure. Assuming the model is correct for healthy subjects the following conclusions may be drawn from it. Firstly, oropharyngeal deposition decreases with decreasing median droplet diameter, falling from 60% of the inhaled dose at 10μm to virtually zero at 1μm. Central airway deposition peaks at 6-7μm and peripheral airway deposition at 2-3 μm. Interestingly the dose reaching the peripheral airways, as a fraction of that

inhaled, varies by less than 8% over the median droplet diameter range 1-5μm. Particle deposition in the lung periphery is diminished in patients with bronchoconstriction (Love and Muir, 1976), with greater amounts landing in central airways, and the optimum particle size for lung deposition in children and those with airways obstruction is less clear. In Rudolph's model the curves in the figure would be moved to the left by greater impaction in the central and upper airways of such patients. It may be necessary to use finer aerosols when a high degree of airway obstruction is present. Small individual particles carry very little mass (i.e. one thousand 1μm particles have the same mass as one 10μm diameter particle) and depending on size may be exhaled. Generation of small particles in high concentration is difficult and delivery time is prolonged.

The ideal particle size to achieve deposition in the lung is subject to continuous speculation, but the largest particles capable of penetrating into the lung are considered to offer the greatest therapeutic advantage and the range 1-5μm diameter particles have been accepted as the pharmaceutical industries target. This value is not universally accepted, and the relevance of in vitro measurements of particle size to lung deposition and clinical effect has been widely debated (see Chapter 4.5)

Breathing pattern

As noted above, breathing pattern may affect particle deposition in the airway. Fast inspiration encourages inertial impaction of drug in the upper airways and more central deposition (Dolovich *et al.*, 1981). Fast inspiration increases the threshold levels of response in bronchial provocation tests (Laube *et al.*, 1992; Cardellicchio *et al.*, 1989), possibly due to decreased lung delivery.

A breath hold or pause after inhalation increases lung deposition of drug by allowing longer for particles to deposit by sedimentation or diffusion.

The effect of breathing pattern on the amount of drug available for inhalation from nebulisers is discussed in Chapter 4.4, and the effect of breathing pattern on the output of drug from spacers and nebulisers investigated in Chapters 6.10 and 6.13 respectively.

Upper airway

The nose is an excellent filter of inhaled particles, and nose breathing reduces the lung deposition of aerosols by half (Everard *et al.*, 1993), most of the aerosol being deposited in areas of changing flow direction or turbulent flow in the anterior third of the nose (Itoh *et al.*, 1985; Newman *et al.*, 1987). Data are available only for adults, however, and little is known of the particle retaining properties of the nose in childhood.

Another problem with nasal breathing was highlighted in older children breathing through the mouthpiece of a spacer device, where therapeutic failures were attributed to inappropriate inhalation through the nose rather than the mouthpiece (Pedersen and Ostergaard, 1983).

There is some evidence that asthma improves in children after tonsillectomy, and it has been hypothesised that this is due to an increase in lung deposition of medication after the obstructing tonsils are removed. The effect of changing upper airway parameters has been investigated in vitro (Miller and Purrington, 1996), but little is known of the dynamic nature of the throat and oropharynx during inhalation from different drug delivery devices. Studies of mouth and throat deposition have shown wide inter-individual variation (Svartengren *et al.*, 1991; Anderson *et al.*, 1990), with some individuals having reproducibly high oropharyngeal drug deposition, perhaps related to functional narrowing of the posterior pharynx (Svartengren *et al.*, 1994).

Age

A number of models have been proposed to calculate the deposition of particles within the respiratory tract of children. These make a number of assumptions about breathing pattern and the structure of the upper and lower respiratory tracts. Thomas (1988) assumed nasal breathing at rest, estimated nasal dimensions from the tracheal cross sectional area (assuming the infant nose to be a scaled down adult one), and made further assumptions about age related tidal volume and respiratory rate. He predicted that nasal deposition would rise with age, so that 0.2% of 2μm diameter

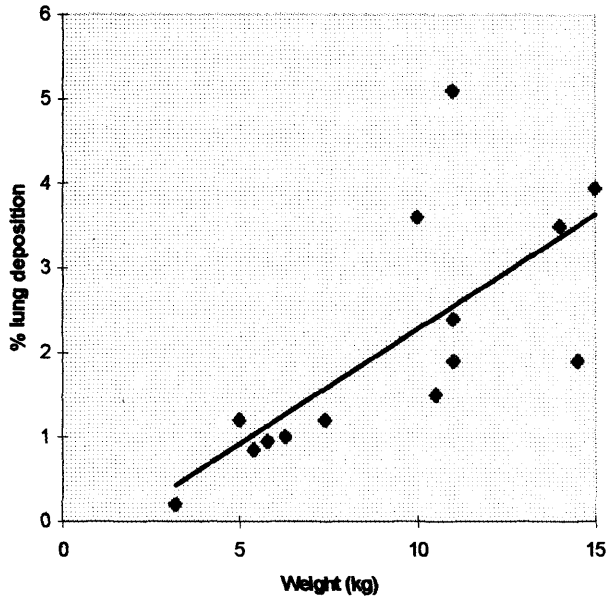
particles would deposit in the nose at one month, rising to 37.8% at 10 years. Xu and Yu (1986), making different assumptions, predicted the opposite trend with oral deposition, and that 6% of 2μm diameter particles would deposit in the mouth at one month, falling to 0% at ten years. To improve these models, age related measurements of upper airways dimensions, the relative amounts of nasal and oral breathing at different ages, and the effect of airways obstruction on particle deposition in the upper airways and lungs are needed.

There have been few clinical studies of deposition of nebulised aerosols in children. Alderson et al (1974) used a Devilbiss 900 ultrasonic nebuliser and face mask to study radiolabelled aerosols in eleven children with cystic fibrosis aged from eighteen months to seventeen years. They found large extra-thoracic deposition in the younger children, and that lung deposition increased with age. In addition the subjects underwent ventilation scans. Those with normal ventilation scans had uniform deposition of labelled aerosol, whereas those with areas of reduced ventilation had corresponding areas of reduced deposition. The mode of inhalation was not noted in this study, and if the younger children nose breathed, this may explain the differences.

In a group of eight children aged eight to thirteen years, inhaling technetium-99M labelled albumin from a Respirgard nebuliser and mouthpiece, O'Doherty and colleagues (1993) found total lung deposition of pentamidine to be similar (2.5% of the nominal dose) compared to a group of adults. There was no relationship between age and total deposition, but the children had more central deposition than the adults.

Conversely, Mukhopadhyay and colleagues (1994) failed to show a significant relationship between indices of pulmonary damage and total lung deposition of radio-labelled tobramycin inhaled via a mouth piece in a group of twenty-seven children and young adults aged four to twenty three with cystic fibrosis, although higher Crispin Norman scores and lower FEV_1 were associated with reduced peripheral deposition. The mean dose delivered to the lungs was 8mg (6.7% out of a nominal 120mg placed in the nebuliser) and there was wide variation between patients. The authors also failed to demonstrate any relationship between age and lung deposition. Chua et al (1994) also found a median lung deposition of 6% in eight children with cystic fibrosis aged six to eighteen years during mouth breathing. Nose breathing in

the same group reduced lung deposition to 2.7%, while the median deposition in infants aged 0.3 to 1.4 years, who breathed nasally during quiet sleep, was 1.3%.



Tal (1996) studied a heterogenous group of fifteen infants and children with respiratory disease aged between 2.5 months and five years. Technetium labelled salbutamol was administered via MDI and Aerochamber spacer plus facemask. Lung deposition varied from 0.2% to 5.1% (mean 2%, SD 1.4%), increasing with weight (Figure).

The mean lung deposition was 0.2% per kilogram body weight, excluding one child noted to be crying during the study in whom the deposition was 0.036%.

This compares with two adults in the same paper who inhaled radiolabelled salbutamol from the Aerochamber with a mouth piece. Lung deposition was 0.21% and 0.25% respectively.

In conclusion, the amount of aerosol inhaled may be independent of patient size after approximately six months of age and the amount of inhaled aerosol deposited in the lung does not appear to increase with age in children aged four or above. The situation in infants remains unclear, but the amount of drug deposited in the lungs per kilogram body weight may be similar for infants and adults. Total deposition and deposition pattern may be altered by disease. The wide variation in deposition between individuals and possible differences due to the different drug delivery systems used must be borne in mind when interpreting studies of inhaled therapy.

Face mask or mouthpiece

The use of a face mask with a nebuliser or spacer device has been shown to be an effective method of drug delivery to children too young to use a mouthpiece (O'Callaghan *et al.*, 1989; McCarthy, 1990). Potential problems with face masks are that some of the drug will land on the face, some may be inhaled through the nose, or a seal may not be achieved, leading to leakage of the drug. It has been shown in vitro, using a lung model to represent the breathing pattern of a child, that holding the face mask only 2 cm from the face may reduce drug delivery by 85% (Everard *et al.*, 1992).

Compliance

Patient compliance with inhaled medication is poor. In studies using electronic timer devices attached to metered dose inhalers, where subjects knew that compliance was being monitored, on only half of the study days was the prescribed medication taken, whether this was self-administered by adults or children (Spector *et al.*, 1986; Coutts *et al.*, 1992) or where administration was supervised by a parent (Gibson *et al.*, 1995). Poorly compliant patients are at increased risk of exacerbations (Milgrom *et al.*, 1996). The most effective inhaler for any given patient is the one that the patient will use on a regular basis and in an effective manner. Although there is no evidence that compliance is improved by changing to a different inhaler device, small, unobtrusive devices are often marketed on the basis that they are more acceptable to the patient, and will therefore be used more. There is increasing interest in drug delivery devices that can both monitor and prompt patient use.

Summary and conclusions

The administration of drugs by inhalation was first described in antiquity, and although the world-wide market for inhaled asthma medications is in excess of 4.5 billion pounds per annum, inhalational therapy is still not optimal, with the delivered dose of medication varying by 400% depending on the drug delivery system chosen and how it is used.

A wide range of drugs are available or have been proposed for inhalational therapy, which allows small doses of medication to be delivered to the lungs, minimising systemic side effects. Particle size is the major determinant of drug particle deposition in the lungs, although this is modified by breathing pattern, upper airway anatomy, patient age and disease severity. The ideal particle size to achieve deposition in the lung is subject to continuous speculation, but particles in the range 1-5μm diameter particles have been accepted as the pharmaceutical industry's target.

Metered Dose Inhalers

Metered dose inhalers (MDIs) were first introduced in 1956 by Riker Laboratories (Freedman, 1956; Riker Laboratories Inc, 1960) and are now the commonest inhalational drug delivery device prescribed in the world. Used mainly in the treatment of asthma and allergic diseases, many different drugs have been formulated for use with them. MDIs are cheap, unobtrusive and easy to actuate. Unfortunately, they are also easy to use incorrectly, reducing drug delivery to the patient. Even with optimum use, only 10-15% of the emitted dose is deposited in the lungs (Newman *et al.*, 1982).

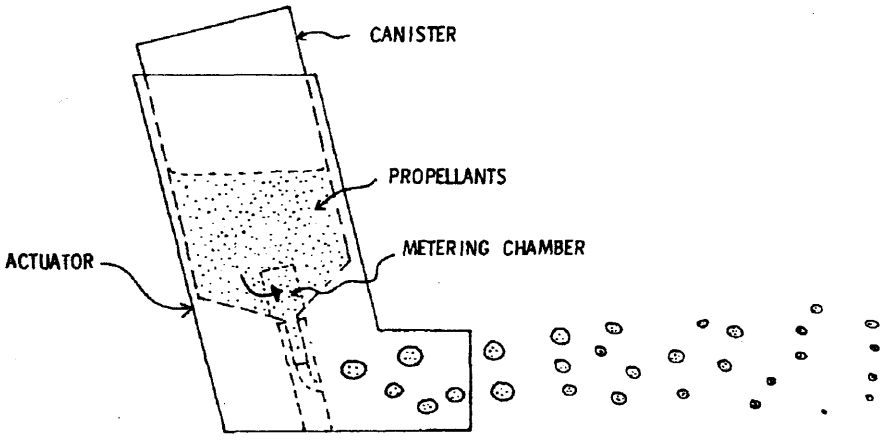


Figure: Schematic metered dose inhaler.

Description of MDIs

The MDI consists of a container, which holds the drug, a volatile propellant, variable surfactants and other excipients under pressure. Most drugs are not soluble in the commonly used chlorofluorocarbon propellants, and are therefore formulated as suspensions. The container is capped by a metering valve, which contains a single dose of drug

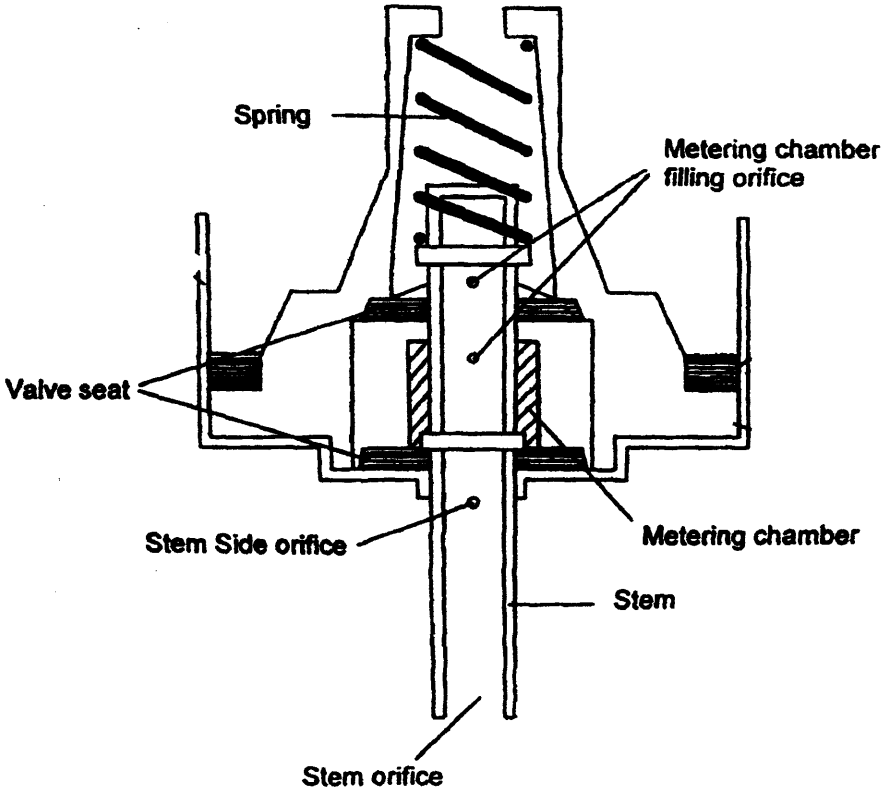


Figure: The metered dose inhaler valve.

At rest, the metering chamber is in contact with the main drug reservoir, or a smaller reservoir (the tank retaining cup) which contains the next few doses. As the valve stem is depressed during actuation, the metering chamber is sealed off from the rest of the MDI, isolating the contents. As the valve stem is depressed further, the side stem hole enters the metering chamber, exposing the chamber to atmospheric pressure. The pressurised contents of the metering chamber vaporise, and the rise in pressure forces the drug and propellant through the valve stem. The size of the metering chamber determines the amount of drug/propellant mixture released at each actuation (and hence the dose emitted). The metering chamber size, vapour pressure of the propellants and the characteristics of the MDI adapter determine the size and speed of the aerosol cloud produced (Chapter 6.5). When the MDI is released, the valve returns to its resting state and the metering chamber fills with drug/propellant mixture ready for the next actuation.

Until recently, MDIs have used a mixture of chlorofluorocarbons (CFCs) as propellants. These compounds are hazardous to the Earth's ozone layer, and are therefore being replaced by MDIs containing hydrofluoroalkanes (see below and Chapter 6.9), or by other types of inhaler.

Table: Characteristics of CFC propellants (Hickey, 1996).

Propellant	Boiling Point (°C)	Vapour Pressure (psia)	Liquid Density (g/cm^3)
CFC - 11 CCl_3F	23.8	12.7	1.49
CFC - 12 CCl_2F_2	-29.8	81.5	1.33
CFC - 114 $CClF_2ClF_2$	3.77	26.0	1.47

These three propellants are used in varying combinations to achieve the desired characteristics of vapour pressure, density and solvency for the particular drug being administered.

Most current CFC containing MDIs are formulated using micronised or spray dried drug particles held in suspension. Surfactants are used to disperse the drug particles in suspension, and for valve lubrication. Surfactant molecules interact with each other, with drug particles, and the propellant to stabilise drug particles in the predominantly non-polar propellant milieu (Bower *et al.*, 1996). Oleic acid, sorbitan mono-oleate (Span 80), sorbitan tri-oleate (Span 85) and phosphatidyl choline are the commonly used surfactants in CFC containing MDIs in concentrations typically around 0.1% w/w, but sometimes up to as high as 2% w/w. High concentrations of (non-volatile) surfactants increase emitted particle size as they do not evaporate from the surface of drug particles, and may slow the evaporation of volatile propellants.

The metering chamber volume and actuator geometry have a significant effect on the output from a metered dose inhaler, dictating the volume and rate of emission of the aerosol formation. Propellant expands in the space between the MDI valve and the actuator orifice, forming a mixture of vapour and liquid phases before exiting. The dimensions of this space and the adapter orifice may be altered to effect droplet formation. In addition, actuator design may be used to slow the aerosol cloud (Newman and Clarke, 1993), which may be beneficial, reducing oropharyngeal deposition of drug. Others have inserted baffles into the MDI actuator (Byron *et al.*, 1989) which, although they reduce total drug output from the MDI, increase the proportion of drug delivered in smaller particles.

The production of droplets from MDIs may be estimated mathematically, given information on the composition of the drug formulation, details of the actuator design, and the ambient conditions (Hickey, 1996). Simplistically, the linear velocity is given by the ratio of the volumetric flow rate and the orifice area, or:

$$U = Q/a_0$$

The volumetric flow rate Q is given by:

$$Q = C_d a_0 \left[\frac{2\Delta p}{\rho\left(1 \cdot \left(a_0/a_1\right)\right)} \right]$$

where C_d is the coefficient of discharge, a_o is the orifice area, a_1 is the inlet area, ρ is the density of the fluid and ΔP is the pressure drop across the nozzle.

The initial particle size of droplets is given by the equation:

$$D_m = 6\, d_0\, (Re_1)$$

Where D_m is the mass median diameter, d_0 the orifice diameter and Re_1 the Reynolds number for flow. This is given by Hinds (1983):

$$Re_1 = \frac{\rho d_0 U}{\eta}$$

where η is the viscosity of the fluid. Solution of these equations gives an estimate of the mass median diameter and forward velocity of aerosol particles at the MDI actuator orifice

Following emission of the propellant/drug mixture, and formation of droplets, the propellants evaporate, reducing droplet size. This is given by the equation (Hinds, 1983):

$$\frac{dD_m}{dt} = \frac{4D_c M}{R\rho\, D_m}\left[\frac{P_\infty - P_d}{T_\infty - T_d}\right]$$

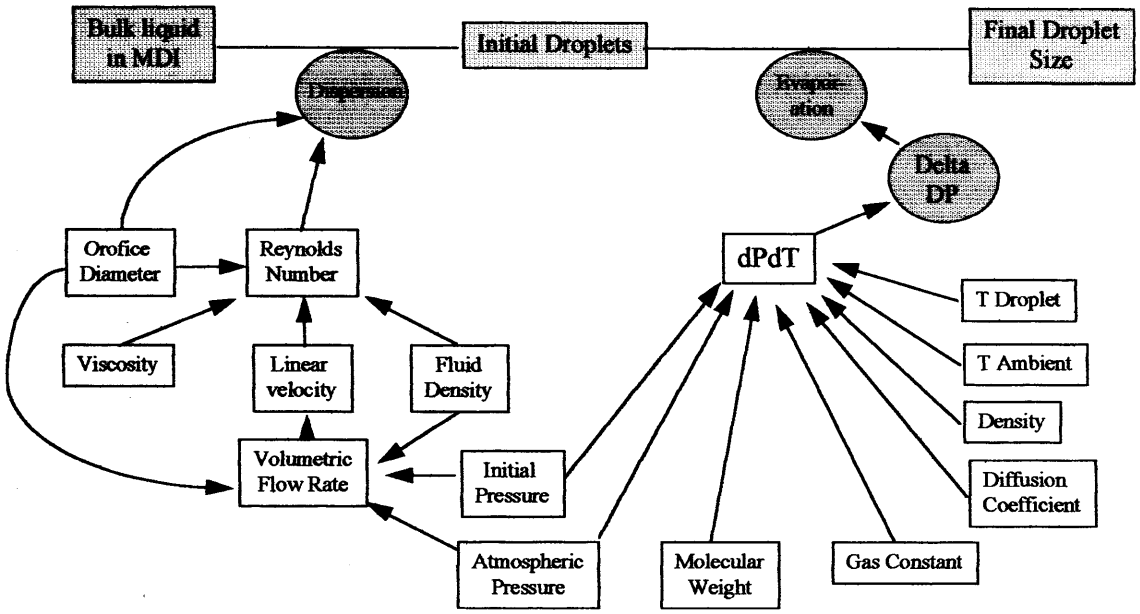
Where D_c is the diffusion coefficient, M is the molecular weight, P_ω and P_d are the ambient and droplet surface pressures, T_ω and T_d are the ambient and droplet surface temperatures, and R is the gas constant.

The diffusion coefficient is given by Hickey (1996):

$$D_C = \frac{BT^{3/2}\left[(1/M_1) - (1/M_2)\right]^{1/2}}{P(r_{12})^2 I_D}$$

Where T is the absolute temperature, M_1 and M_2 are the molecular weights of propellant and air, P is the absolute pressure in atmospheres, r_{12} the collision diameter and I_D the collision integral.

Taking all these factors into account, a simple flow diagram illustrating the factors affecting droplet size from a MDI may be drawn (Hickey, 1996):



Clearly the drug containing particle size output of suspension formulations cannot be smaller than the original size of the micronised drug particles. At low drug concentrations, emitted droplets will contain a single drug particle and approximate to the original, micronised size (Gonda, 1985). However, at higher drug concentrations, droplets containing multiple drug particles will be produced, leading to an increase in droplet size, and reducing drug delivery to the lungs.

The final size of solution formulations, in contrast, will depend on the initial droplet size, the concentration of non-volatile components (drug and excipients) in the droplet, and the ambient conditions. Thus it is possible to use the drug concentration to alter the final particle size. In practice, it is often difficult to dissolve drug in the CFC propellants, solution formulations may be less stable, and drug may be lost to the elastomers in the MDI valve (Atkins, 1991).

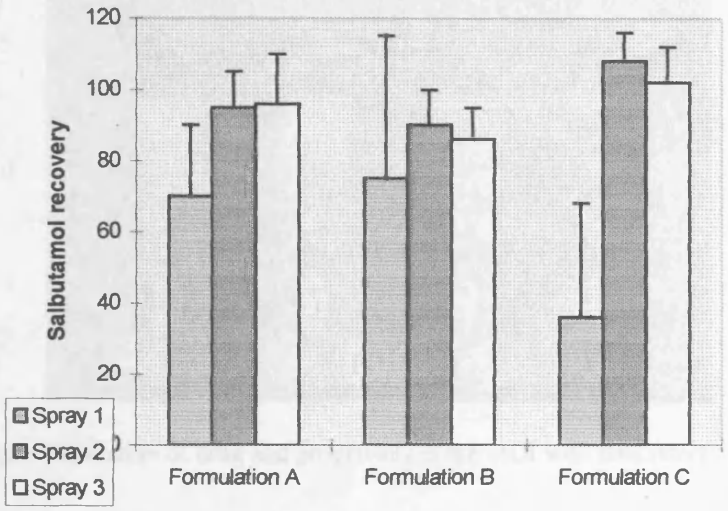
Factors affecting the output and dose uniformity of MDIs.

A number of factors affect the output and dose uniformity of MDIs. Some of these are described below:

Loss of prime occurs when the MDI has been left for a period of time without being

actuated. The propellant leaks from the metering chamber, at a rate dependent on the construction of the valve, and the first actuation contains little or no drug. Subsequent actuations are delivered as normal, until the MDI is again rested for some time. Loss of prime typically occurs over a few days (Schultz, 1995).

MDI output is also dependent upon the *storage orientation*. Storing the MDI valve stem up overnight reduced the output of the first actuation by 25% (Everard *et al.*, 1995). Cyr et al (1991) determined the output of salbutamol MDIs from three manufacturers after a short period of storage. Each MDI was primed by firing nine shots to waste, then stored valve down for three hours. The drug content of the next three actuations was measured, the MDI being shaken for five seconds before each actuation. There were considerable differences between the first and subsequent actuations, and between the different manufacturers' inhalers.



Recovery of salbutamol from unprimed MDIs, as a percentage of label claim (Cyr *et al.*, 1991). Values are the mean recovery (μg) and error bars the standard deviation. The first actuation shows greater variability, both within each formulation, and between formulations

Blake et al (1992) compared the change in FEV_1 after two actuations from a generic salbutamol MDI and two actuations from the innovator product (Ventolin, Allen & Hanburys). In a single blind, randomised crossover study, subjects received the salbutamol as the first two doses from a new MDI, with spirometry ten times over the ensuing eight hours. The maximum improvement in FEV_1 was 12% higher after the Ventolin. Eleven (out of seventeen) subjects repeated the study, but on this occasion the MDIs were primed by firing two actuations to waste. No difference in FEV_1 was

observed. Although the study had a low power to detect a difference between the MDIs, the authors concluded that the importance of priming the MDI was dependent upon the individual valve and formulation used, a conclusion supported by a prior in-vitro study (Fiese *et al.*, 1988).

The MDI should be shaken before use. Suspension formulations tend to separate out with time, all the drug creaming at the top or bottom of the inhaler, depending on the relative densities of the drug and propellants. The first actuation, already contained in the metering chamber, may have a normal output, but the second and subsequent actuations, filled from the separated suspension or concentrated drug, will contain a highly variable amount of drug.

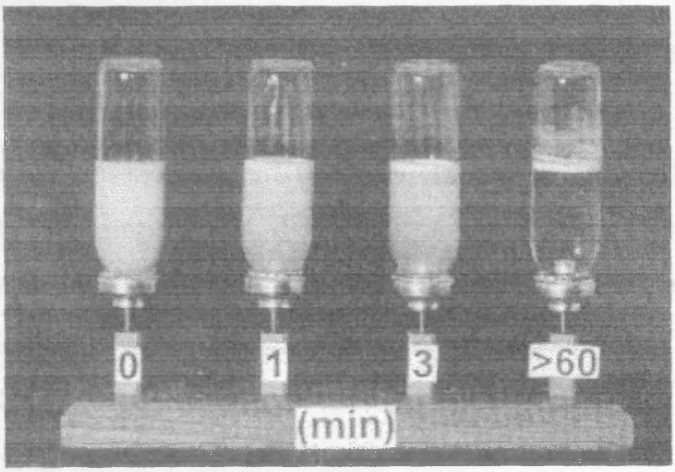


Figure: Separation of drug and propellants in the MDI with time (Berg, 1995).

Berg (1995) calculated the theoretical effect of a failure to shake the MDI once every ten actuations, based on assumptions that the drug creamed rather than settled, that the tank retaining cup held two doses, and that an hour or more occurred between each dose. The results are shown in the figure.

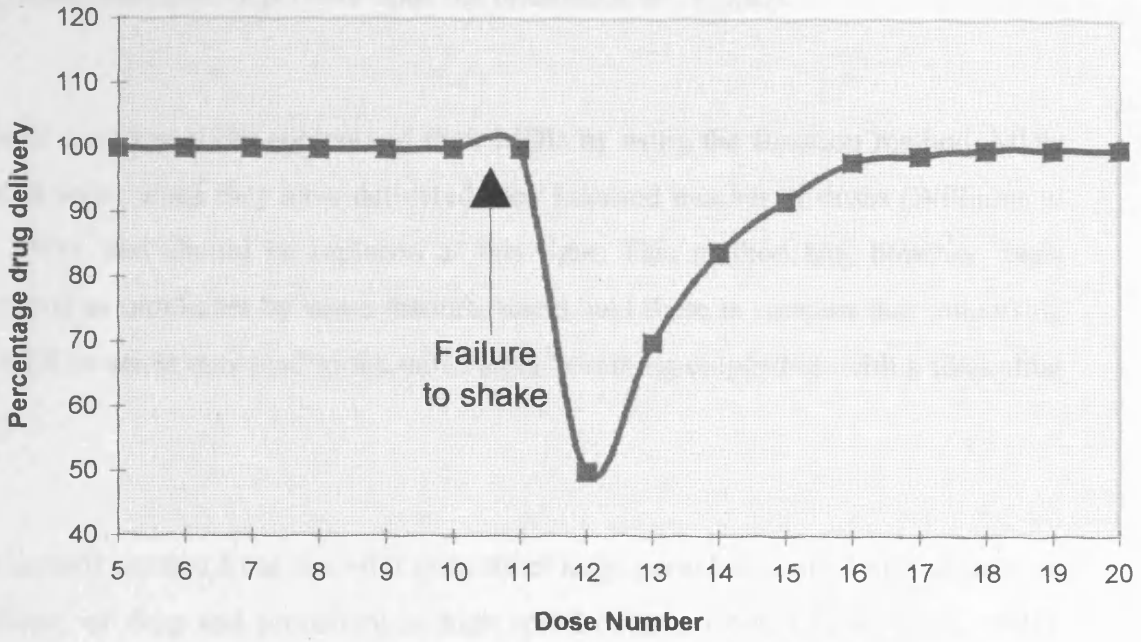


Figure: Theoretical effect of failure to shake MDI on drug output (Berg, 1995).

The output from MDIs is dependent upon the vapour pressure of the propellants (Moren, 1978). At low temperatures, the vapour pressure falls and the mass output is increased (Wilson *et al.*, 1991), although the drug is in larger particles and in-vitro deposition in a lung model is reduced. Warming the MDI to body temperature has been advised prior to actuation (Hampson and Mueller, 1989).

As the MDI begins to run out of formulation, the delivered dose can become highly variable, a phenomena known as 'tail-off'. Current MDIs are filled to allow more than the claimed number of actuations before tail-off occurs, but the inhalers will still have propellant and drug them at this stage, and the patient may continue to use the inhaler. In this case variable dose delivery may occur, resulting in poor control of asthma symptoms. Tail-off may be rapid over a few actuations, or may occur unpredictably over twenty or more actuations before the MDI is completely empty, depending on the valve design and position of the entrance to the metering chamber. Where this is at the base of the metering chamber, it is covered by propellant until the final actuations, ensuring relatively constant filling of the metering chamber. Where the entrance is higher in the metering chamber, filling may become variable as the

propellant level falls, dependent upon the orientation of the MDI.

Patients may assess the contents of their MDIs by using the flotation method. MDIs float in water when they have delivered their licensed number of doses (Williams *et al.*, 1993), and should be replaced at this time. This method has, however, been described as unreliable by some manufacturers, and there is concern that immersing the MDI in water may lead to the valve stem becoming clogged up with a thick drug paste.

The aerosol emitted from the MDI consists of large particles, some 20μm or more in diameter, of drug and propellant at high speed (Clark, 1996; Dhand *et al.*, 1988). These particles rapidly decelerate and shrink as the propellants evaporate, as described above, but they have considerable momentum, and many impact on the throat and oropharynx. This drug contributes to local and systemic side effects, but not to the therapeutic effect of the medication.

Slowing the aerosol cloud might therefore be beneficial, and the Spacehaler (Evans Medical Ltd, Leatherhead, UK) is a new, compact, pressurised aerosol device that uses the same canister as a conventional metered-dose inhaler. The actuator design, however, reduces the velocity of the aerosol cloud that emerges from the inhaler, reducing impaction of the aerosol in the oropharynx, and hence the amount of the non-respirable drug delivered to the patient (Newman and Clarke, 1993). The device appears to be as effective in the delivery of salbutamol to adults as an MDI and large volume spacer (Gunawardena *et al.*, 1997). Its advantage is that it is compact and unobtrusive. Despite evidence of similar efficacy to properly used standard MDIs, unpublished studies suggest that the side effect profile of the Spacehaler may, paradoxically, be worse (Anonymous, 1993). There are no published studies of this device used by children or for the delivery of steroids.

The replacement of chlorofluorocarbon propellants

In 1974, Molina and Rowland hypothesised that the release of inert halocarbons, such as the chlorofluorocarbons (CFCs) used in current metered dose inhalers, into the atmosphere could propose a threat to the environment (Molina and Rowland, 1974). It was proposed that the released halocarbons would reach the stratosphere, where they would catalyse the destruction of ozone (O_3).

Simplistically, the stratosphere contains a layer of ozone, which absorbs ultraviolet light, according to the reactions:

1. $O_2 + UV \rightarrow 2O\cdot$
2. $O\cdot + O_2 \rightarrow O_3$
3. $O_3 + UV \rightarrow O\cdot + O_2$
4. $O_3 + O\cdot \rightarrow 2O_2$

Thus stratospheric oxygen is broken down by UV light (180-240nm) to two oxygen radicals ($O\cdot$, Equation 1). These then combine with oxygen to form ozone (O_3 Equation 2) which absorbs more UV light (UV-B, 210-310nm) and is broken down to oxygen once more (Equations 3 & 4). The consequence of this process is a reduction in the amount of UV light reaching the lower atmosphere.

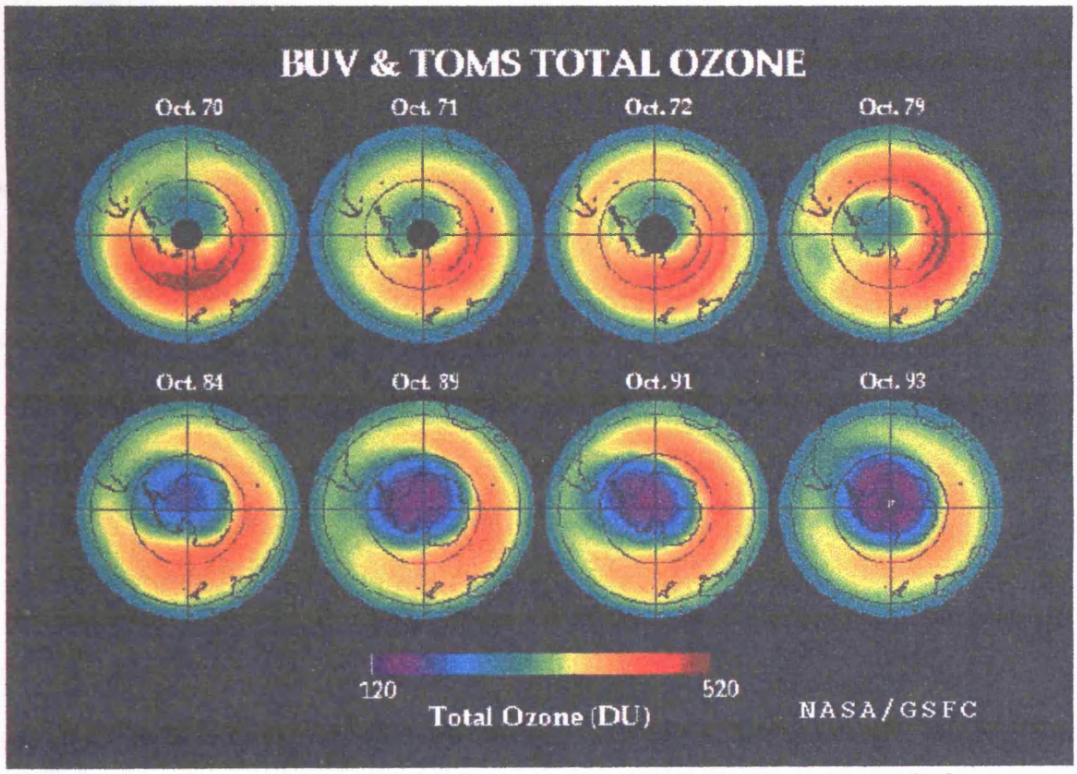
Stratospheric halocarbons interfere with this process by destroying ozone without any absorption of UV light, according to the reactions:

$$O_3 + X\cdot \rightarrow XO\cdot + O_2$$
$$XO\cdot + O_3 \rightarrow 2O_2 + X\cdot$$

Where $X\cdot$ is a free radical, such as naturally occurring hydroxyl radicals or halogens, or man-made halocarbons. The low level of production of the naturally occurring compounds is matched by their breakdown, keeping the system in balance. However, Molina and Rowland postulated that the additional load of man-made compounds

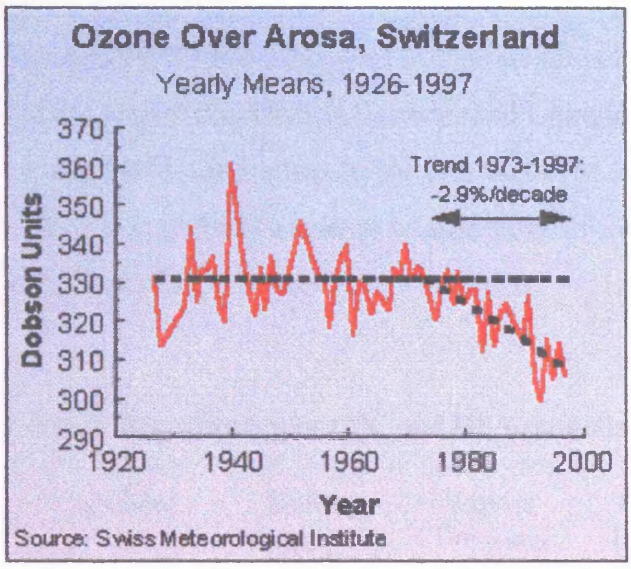
would lead to significant extra destruction of stratospheric ozone, which would lead to severe environmental and health effects, such as crop failure, increase in melanoma and other cancers, increase in the incidence of cataract and inhibition of the immune system (Coldiron, 1992).

Their hypothesis was strengthened in 1985, when the British Antarctic Survey revealed the presence of the Antarctic ozone 'hole' (Farman *et al.*, 1985), a marked increase in the normal level of ozone depletion occurring in the Antarctic spring. Repeated measurements have shown that the level of ozone above the Antarctic is continuing to fall.



Satellite pictures of the Antarctic ozone 'hole' from 1970 to 1973.
(from http://jwocky.gsfc.nasa.gov/)

Depletion of the ozone layer is not confined to the poorly populated Antarctic region:



This graph shows long-term ozone levels over Arosa, Switzerland, and is taken form the US environmental protection agency website (http://www.epa.gov/docs/ozone/science/arosa.html). It demonstrates constant average ozone levels from 1926 until 1973, with a sustained fall beginning in 1973, since when ozone levels have dropped at an average rate of 2.9 percent/decade. Similar depletion is seen over other northern temperate and Arctic areas.

Increasing concern over the effects of ozone depletion have led to a ban on the use of CFCs for most purposes, according to the terms of the Montreal Protocol (Montreal Protocol, 1987) and subsequent amendments (London Amendment, 1990; Copenhagen Amendment, 1992; Montreal Amendment, 1997). Alternative propellants which are not ozone depleting, such as hydrofluoroalkanes (HFAs), are being developed (Partridge, 1994; Newman, 1990), but until these are more widely available CFC use in MDIs is exempted from the terms of the protocol (D'Souza, 1995). A non CFC formulation of salbutamol (Airomir, 3M) has been released in the UK, and further formulations of salbutamol (Sultanol, Glaxo Wellcome), beclomethasone diproprionate (Baker Norton) and fluticasone (Flutide, Glaxo Wellcome) have been released in Europe. It is expected that the major part of the transition to non-CFC MDIs will occur in 'industrialised' nations by the year 2000, and that there will be minimal need for CFCs in MDIs after 2005 (Montreal Amendment, 1997). It cannot be assumed that CFC and the replacement HFA inhalers are equivalent, and this is investigated in chapter 6.9. Furthermore, some of these drugs have been formulated to be equivalent in particle size and drug output to their CFC containing precursors (i.e. Airomir and Sultanol with Ventolin). In contrast, 3M's CFC free beclomethasone diproprionate emits drug in much smaller particles than the CFC containing equivalent product, resulting in more peripheral deposition of the medication (Leach, 1998). It is not clear what effect this will have on the dose

equivalence or side effect profile of the new formulation.

Formulations containing the HFA propellants have been shown to be non toxic and stable (Alexander, 1995; Alexander and Libretto, 1995; Thompson *et al.*, 1998; Lester *et al.*, 1996). HFA propellants are, however significantly different from their CFC precursors, and this has necessitated extensive design and formulation changes to the CFC free MDI.

Table: Characteristics of CFC and HFA propellants (Hickey, 1996)

Propellant	Boiling Point (°C)	Vapour Pressure (psia)	Liquid Density (g/cm^3)	ODP	GWP
CFC - 11 CCl_3F	23.8	12.7	1.49	1.0	4,000
CFC - 12 CCl_2F_2	-29.8	81.5	1.33	1.0	8,500
CFC - 114 $CClF_2ClF_2$	3.77	26.0	1.47	1.0	9300
HFA - 134a CF_3CFH_2	-26.2	83.7	1.22	0	1,500
HFA - 227 CF_3CFHCF_3	-17.3	58.6	1.42	0	--

ODP - Ozone depleting potential. GWP - Global warming potential (see text for definition)

The similarity of the physical properties of the two HFA propellants mean that it is not possible to achieve a range of propellant characteristics by altering the propellant mixture, as it is with CFCs. New manufacturing processes have had to be introduced to cope with the lower boiling point of the new propellants, and the MDI valves and actuators have been redesigned to work with the different chemicals. The surfactants commonly used in CFC MDIs are not soluble in HFAs, and are included in much smaller quantities for valve lubrication rather than keeping the drug in suspension. Ethanol is used as a co-solvent, and one of the new steroid preparations has been

formulated as a solution MDI, resulting in a much finer aerosol than the CFC precursor.

The emitted aerosol from some HFA 134a formulations is warmer and less dense than that from the CFC containing metered dose inhalers, and patients may experience a different feel and taste when taking their inhalers (Bamber, 1996; Bell *et al.*, 1991). They should be warned about this, and reassured that it does not mean that the inhaler is not working (Partridge and Woodcock, 1995).

A further advantage of the non-CFC MDI is its enhanced dose reproducibility (June *et al.*, 1996), and a reduced effect of storage orientation or ambient temperature, compared to the CFC inhalers described above (Schultz *et al.*, 1994).

Although the HFA propellants have no ozone depleting potential, concern has been expressed that they contribute to global warming. It has been known for many years that gases in the Earth's atmosphere absorb reflected infra red energy (Tyndall, 1861), and that these gases cause the 'greenhouse effect' making the surface of the Earth many degrees warmer than it would otherwise be. It was subsequently discovered that gases produced by human activity, notably carbon dioxide emitted from the internal combustion engine, but also CFCs, contribute to the 'greenhouse effect' and lead to global warming (Ramanathan, 1975). It has been estimated that CFCs contribute to a quarter of the annual increase in the greenhouse climate forcing (Hansen *et al.*, 1989). The amount of global warming caused by a substance can be expressed as its Global Warming Potential (GWP), the ratio of the warming caused by a substance to the warming caused by a similar mass of carbon dioxide. Thus, the GWP of CO_2 is defined to be 1.0 . CFC-12 has a GWP of 8,500, while CFC-11 has a GWP of 5,000. Relative to CFC-11, HFA-134a has a GWP of between 0.25 and 0.3 (Fisher *et al.*, 1990), or approximately 1,500 relative to CO_2. Thus 'ozone friendliness' does not mean 'greenhouse friendliness' (Shine, 1990), leading some to call for the regulation of HFAs, and to question their use as replacements for CFCs.

Use of MDIs

Metered dose inhalers are easy to actuate, but difficult to use properly. Many adults and most children use their MDIs incorrectly (Larsen *et al.*, 1994; Saunders, 1965; Paterson and Crompton, 1976; Epstein *et al.*, 1979; DeBlaquiere *et al.*, 1989; Pedersen *et al.*, 1986; van Beerendonk *et al.*, 1998), and most healthcare workers are similarly uncertain about their correct use (Kelling *et al.*, 1983; Kesten *et al.*, 1993; Hanania *et al.*, 1994; Amirav *et al.*, 1995; Amirav *et al.*, 1994; Guidry *et al.*, 1992; Interiano and Guntupalli, 1993). The most common errors are the inability to co-ordinate inhalation with metered dose inhaler actuation, to inhale too quickly, and to exhale without a breath hold (Dolovich *et al.*, 1981; Newman, 1985; Crompton, 1982). Nasal inhalation is also a common error amongst children (Pedersen and Ostergaard, 1983). The 'cold freon affect', where the patient stops inhaling when the propellants impact on the oropharynx, and inhalation through the nose (Pedersen and Ostergaard, 1983) are also common causes of failure of MDI use.

A further source of confusion is that the optimal method of MDI use is not known. Newman et al (Newman *et al.*, 1981; Newman *et al.*, 1982) demonstrated maximal bronchodilatation and lung deposition with the MDI in the mouth, slow deep inhalation and a ten second breath hold. In contrast, Dolovich (1981) obtained the largest delivery of radiolabelled aerosol to the lungs and Thomas (1984) the greatest degree of bronchodilatation using the 'open mouth' technique, with the MDI held some 4cm from the wide open mouth, although others have not found this to be beneficial (Newman *et al.*, 1981; Lawford and McKenzie, 1982; Chhabra, 1994). The closed mouth technique has been assessed in detail in a study (Hindle *et al.*, 1993) using urinary salbutamol excretion. A greater relative lung bioavailability of the drug was observed with exhalation to residual volume, slow inhalation with MDI actuation, and a ten second breath hold following inhalation.

Breath actuated MDIs

Breath actuated metered dose inhalers were introduced in the late nineteen-eighties (Baum and Bryant, 1988). They remove the need for patients with poor co-ordination to inhale and actuate the MDI at the same time, improving lung deposition (Newman *et al.*, 1991). They are actuated at low flow rates (Fergusson *et al.*, 1991), and may be

used by adults and children over seven years of age after instruction (Schecker *et al.*, 1993; Pedersen and Mortensen, 1990). They are not, however, suitable for younger children who cannot breath deeply when using inhalational drug delivery devices.

Metered dose inhalers	
Advantages	**Disadvantages**
Easy to actuate	Difficult to use correctly
Small, Portable.	Variable drug delivery
Many different drug formulations available	No dose counter
Inexpensive	Expensive (when environmental costs included)
	Contain damaging chemicals

Table: advantages and disadvantages of metered dose inhalers.

Summary

MDIs are convenient and widely used inhalational drug delivery devices. Despite their simple appearance and ease of actuation, patients find MDIs difficult to use optimally. The requirement to co-ordinate inhalation and actuation make them unsuitable for use on their own by young children and infants, and by many elderly patients.

Details of their storage and operation may have a large affect the delivered dose of drug, which is also affected by ambient conditions. Dose reproducibility may be poor at the end of the metered dose inhaler's life.

Chlorofluorocarbons, damaging to the Earth's ozone layer, are used as propellants in metered dose inhalers, and new propellants are currently being developed and marketed to replace them. These new formulations may have different characteristics and interact differently with adjuncts used for inhalational therapy.

Spacer Devices

In Chapter 4.2, the high speed and large particle size of droplets emitted from metered dose inhalers (MDIs), the high oropharyngeal deposition of drug and the difficulties patients have co-ordinating MDI actuation and inhalation were discussed. To overcome some of these problems *spacer devices* were introduced (Levison *et al.*, 1985). These consist of variously shaped structures into which aerosol is emitted prior to inhalation.

Spacing devices may be used with pressurised metered dose inhalers and dry powder inhalers. They provide a 'space' between the inhaler and the patient, trapping large particles in the spacer and, for MDIs, allowing the aerosol to slow and propellants to evaporate, reducing particle size. *Holding chambers* provide a reservoir of drug from which the patient breathes. The drug may be generated by MDI (Konig, 1985), dry powder inhalers (Crompton, 1991) or nebulisers (Thomas *et al.*, 1988). Typically holding chambers also have the properties of spacing devices, but in addition store the aerosol until the patient inhales, reducing the need for co-ordination between inhaler actuation and patient inhalation. *Valved holding chambers* allow the patient to breathe tidally from a reservoir of drug. In practice, valves are not totally effective, allowing exhalate into the chamber, and may be difficult for the patient to open (Cox *et al.*, 1984). The necessity for a valve in a holding chamber has never been demonstrated (Levison *et al.*, 1985), and in very young infants, non valved spacers may be more effective (Fok *et al.*, 1997). The use of spacer devices with face mask attachments, which give a seal around the face (e.g. the Aerochamber; the Volumatic with Laerdal face mask; the Nebuhaler with McCarthy mask; the Babyhaler) are becoming increasingly popular (O'Callaghan *et al.*, 1989). Treatment of young asthmatics with inhaled steroids via spacer devices with a face mask attachment has been particularly successful in a group that is otherwise difficult to treat (Noble *et al.*, 1992; Connett *et al.*, 1993). Less drug is deposited in the mouth and oropharynx than with nebulisation and treatment time is shorter. However, delivery of drug by a mouthpiece is more

efficient, and patients should use this in preference to a facemask as early as possible.

Many different spacers have been described and are available for the prescribing physician. The following section gives details of some of them, and their physical characteristics are summarised in the accompanying table. The spacers have been evaluated using a variety of in vitro methods, which are discussed further in Chapter 4.5, and by in vivo studies. These typically take the form of a comparison of the spirometric response to an inhaled bronchodilator from the spacer in comparison with the MDI alone, or with other drug delivery devices (i.e. Fuglsang and Pedersen, 1988; Cushley *et al.*, 1983; Munch *et al.*, 1983; Gomm *et al.*, 1980; Lee and Evans, 1984 and others). Many of these studies have failed to show a difference between delivery devices, possibly because the studies had insufficient power to detect a difference, or because the dose of bronchodilator used is at a relatively flat part of the dose response curve. For instance, in one study (Newman *et al.*, 1991), both lung deposition and spirometric response were measured after inhalation of radiolabelled salbutamol from a MDI and MDI plus spacer. Despite almost doubling of the radiolabelled lung deposition (from 12.3% to 23.8%) there was no difference in FEV_1 response between the two methods of inhalation.

An alternative explanation is that the forced expiratory manoeuvres used as the end point of many studies may affect the result. Fairshter (1987) found no difference in FEV_1 or FVC between metaproterenol delivered by MDI alone or MDI plus spacer, although there was a significant difference in partial flow volume curves between the delivery methods, favouring the spacer. This was attributed to airways hysteresis following the forced expiratory manoeuvre.

Further differences may occur due to the study design. Cumulative dose response studies, for instance, may give a different response than studies using single doses of drug (Britton and Tattersfield, 1984). Another problem arises in determining the 'commencement time' when comparing MDIs (where the dose is rapidly delivered), with nebulisers, where delivery takes five minutes or more.

	Manufacturer	Shape	Length (cm)	Volume (ml)	Valve	Flow monitor	Compatability	Comments
"Spacing" Devices								
Boehringer spacer	Boehringer Ingleheim	Cylindrical	--	50	✗	✗		*
Inhalet	Boehringer Ingleheim	Tube	10	80	✗	✗		Collapsible *
Azmacort Spacer	Rhone Poulenc Rorer	Cylindrical	--	113	✗	✗	Integral to MDI	Collapsible
Microspacer	Schering Corp	Cylindrical	--	15	✗	✗		*
Syncroner	Rhone Poulenc Rorer	Open spacer	10	--	✗	✗	Integral to MDI	
"Holding Chambers" - Small								
Aerochamber	Trudell Medical	Cylindrical	11	145	✓	✗	All	
Optichamber	HealthScan	Cylindrical	13	220	✓	✓	All	*
Spacechamber	Medical Developments	Cylindrical	14	250	✓	✗	All	*
Babyhaler	Glaxo Wellcome	Cylindrical	23	350	✓	✗	Glaxo Wellcome MDIs only	
Rondo	Leiras Oy	Spherical	--	270	✓	✗	All	
Nebuchamber	Astra Draco	Pear Shaped	14	250	✓	✗	Astra MDIs only [1]	Stainless steel
Microchamber	Ferraris Medical	Cylindrical	7	50	✓	✗	All	*
Easivent	DEY Laboratories	Conical	--	--	✓	✓	All	*
Integra	Allen & Hanburys	Rectangular	13	350			For Becloforte MDI	Collapsible *

"Holding Chambers" - large								
Nebuhaler	Astra Draco	Pear Shaped	23	750	✓	✗	Astra MDIs only	
Fisonair	Rhone Poulenc Rorer	Diamond	19	800	✓	✗	Fison's MDIs only	
Volumatic	Allen & Hanburys	Diamond	23	700	✓	✗	A&H MDIs only [3]	
Reverse Flow devices								
Inspirease	Schering Corp.	Cylindrical	11.3	650	✗	✓	All	Collapsible
Aerosol Cloud Enhancer	DHD	Pear Shaped	17.5	160	✓	✓	All	*
Dynahaler/Optihaler	HealthScan	Cylindrical	12	60	✗	✗	All	*
EZ Spacer	Vitalograph	Cylindrical	12	700 (approx.)	✗	✗	All	Collapsible *

* - Limited or no peer reviewed data available on spacer performance.

[1] 3M Airomir (CFC free Salbutamol) may fit the Nebuhaler.

[2] Other MDIs may fit the Fisonair.

[3] Most generic salbutamol MDIs fit the Volumatic.

Table: Characteristics of commonly used spacer devices.

One of the simplest spacers described is the *coffee cup*. A hole large enough to accept the metered dose inhaler adaptor is made in the bottom of a plastic or styrofoam disposable cup. This is then held over the patients mouth and nose, and the MDI actuated. The method has been successfully used to administer inhaled bronchodilators and sodium cromoglycate to preterm infants with respiratory symptoms (Yuksel *et al.*, 1990; Yuksel and Greenough, 1992), although there is no data for this method compared to other spacers or drug delivery devices.

Various other household items have been used as aids for inhalational therapy. (Freigang, 1977; Woodcock *et al.*, 1984; el Kassimi, 1987; Lee and Evans, 1984; Lee and Evans, 1987). One of the first spacers described was a 1.2l vinegar bottle adapted to accept the MDI and fitted with a paediatric face mask (Freigang, 1977).

Woodcock (1984) used a one litre disposable rebreathing bag to administer a combination of fenoterol and ipratropium bromide to a group of ten adult asthmatics with good inhaler technique. There was a statistically significant improvement in PEFR following drug administration via the 'Blo-Bag' compared to the unmodified MDI. A cheaper version (el Kassimi, 1987) incorporates a paper or plastic bag taped to the MDI adapter. The patient actuates the MDI into the bag, removes the canister from the adapter and inhales the aerosol through this. The effectiveness of spraying bronchodilator aerosol into a plastic bag was assessed in one study (Lee and Evans, 1984) of children aged 7 to 15 years with good MDI technique, inhaling two 90μg actuations of salbutamol or placebo via an MDI alone, or via a plastic freezer bag. There was no difference in change in FEV_1 following salbutamol administration by either of the two methods. Such 'home made' spacers have been advocated for use in poorer communities, although in one study there was a trend for the Siriraj spacer, cheaply made from local materials, to be less effective than the Volumatic spacer in the delivery of salbutamol (Vichyanond *et al.*, 1992).

Hayden irreverently described the *Chocuhaler*, 'sweet deliverance in asthma management' (Hayden *et al.*, 1995), a chocolate Easter egg modified to accept a metered dose inhaler and mouthpiece. The device proved to be an effective holding

chamber, although not very robust, as the device either melted in the Western Australian sun, or was eaten by the subjects!

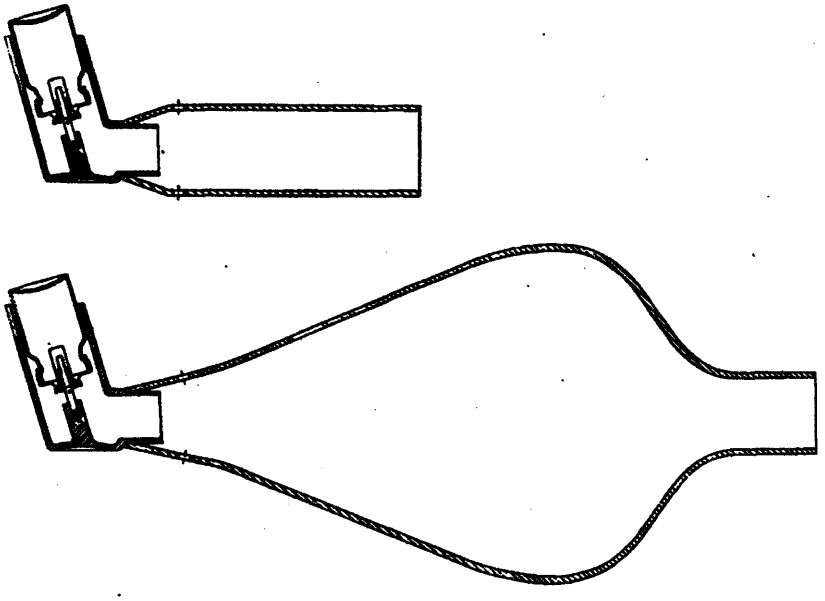
The *Tube Spacer* was first described by Moren in 1978 (Moren, 1978) who administered 250μg of terbutaline by metered dose inhaler to nine adult subjects via a variety of extension tubes made from glass, 50mm or 100mm in length, 24mm or 32mm in diameter, or from a pear shaped glass tube, 250mm in length and 130mm maximum diameter. The spacers were open ended, had no valve, and were fitted with entrainment holes near the actuator insertion, to allow air to enter as the aerosol was inhaled. Eight actuations of the MDI were each inhaled in one 'deep inhalation' by the subjects, with a one minute interval between each actuation, and inhalation commencing at the time of MDI actuation. The amount of drug collected in the MDI actuator, the spacer and from subjects' mouth washings, was then determined by UV spectrophotometery. The amount of drug recovered was expressed as a percentage of the total MDI output (table).

	Percentage deposition (mean from nine subjects)			
Spacer	Actuator	Spacer	Mouth	Total
None	6.0	--	47.9	53.9
Tube, 24mm x 50mm	6.0	24.9	17.6	48.5
Tube, 24mm x 100mm	7.1	38.3	8.9	54.3
Tube, 32mm x 50mm	6.8	13.4	22.3	42.5
Tube, 32mm x 100mm	6.1	23.1	10.4	39.6
Pear Shaped	5.4	17.7	8.1	31.2

All spacers reduced the amount of terbutaline collected in the mouth washings. The total recovery of terbutaline from the actuator, spacer and mouth using the narrow (24mm) spacers was the same as from the MDI alone, suggesting that the spacer

trapped drug that would otherwise impact in the mouth. In contrast, the wider spacers, and especially the pear shaped spacer, had a reduced total recovery, which the author suggested implied a greater lung delivery of drug from these devices. There was no difference between spacers of 50mm and 100mm in length.

The assumption that all the drug not collected in the actuator, spacer and mouth washings was delivered to the lungs is questionable, as it would imply a 50% lung delivery from the MDI alone, compared with 10-20% more commonly observed (Newman and Newhouse, 1996), and would also suggest 70% lung deposition from the pear shaped spacer.



The Tube and Pear Shaped spacers (Moren, 1978; Lindgren *et al.*, 1980)

A clinical study using the 34mm diameter, 100mm length tube spacer (Bloomfield *et al.*, 1979) failed to find a difference in lung function following inhalation of 500μg of terbutaline from the MDI alone or from the MDI and spacer, even with a two second delay between MDI actuation and inhalation. No attempt was made, however to determine whether maximal bronchodilatation had occurred, and with only sixteen adult subjects, the study may have been underpowered to detect a difference. A subsequent study with fourteen adult patients (Gomm *et al.*, 1980) also failed to show a difference in lung function after inhalation of 500μg of terbutaline via MDI alone, or

MDI with a 34mm x 100mm plastic spacer. In this study the terbutaline was administered as 125μg, 125μg and 250μg, with 20 minutes between administrations. Spirometry was undertaken over the next 5 hours. The administration of terbutaline produced significant bronchodilatation at each dose level, with no difference between use of spacer or MDI alone. In contrast, the tube spacer was found to be superior to the actuator alone in fifteen adult asthmatics (Lindgren *et al.*, 1980). Three doses of terbutaline (0.25mg, 0.5mg and 0.75mg) were administered at twenty minute intervals to achieve maximal bronchodilatation. The increase in FEV_1 was greater for the tube spacer than the actuator alone, although the two methods were equivalent following the highest dose.

Spicer et al (1980), in a randomised cross over trial, assessed changes in spirometry over a four week period in forty adult subjects (of whom thirty three completed the trial) who received terbutaline via a conventional MDI or MDI plus tube spacer. The dose administered is given in the paper as 'two puffs twice daily and at other times as required' and the use of other inhaled therapy such as corticosteroids was not restricted or reported. The evening peak expiratory flow rate (PEFR) while using the tube spacer was significantly greater than when using the MDI alone. There was no significant difference in the morning PEFR, FEV_1 or FVC. It is assumed that the 'closed mouth' method of MDI use is used in the foregoing studies, although it is not stated in the papers.

The tube spacer was assessed in 22 adult patients with poor MDI technique (Godden and Crompton, 1981). Terbutaline 250 micrograms was administered via an MDI and via the MDI plus tube spacer. In 16 the improvements in FEV_1 were greater after the tube spacer, in four they were greater after the conventional inhaler and in two patients there was no difference. Similar results were seen in another study, in which there was a two second delay between MDI actuation and inhalation (Hidinger and Perk, 1981). Seven children aged 10 to 17 years were also tested with the tube spacer compared with the MDI alone (Van Asperen *et al.*, 1981). Good MDI technique was ensured by one of the investigators actuating the MDI at the beginning of inhalation. In contrast, the MDI was actuated into the tube spacer five seconds before inhalation. Despite this, there was no difference in change in FEV_1 between the two methods, and the absolute

increase in FEV_1 after 500μg of terbutaline by either method was small. The investigators in the study analysed many spirometric parameters, but only reported the change in FEV_1.

Pedersen (1983) compared the bronchodilator response to inhaled terbutaline or placebo administered via MDI or MDI and tube spacer in a group of twenty children aged 6 to 12 years with exercise induced asthma. The children exercised until they had 'acute bronchoconstriction', and then received placebo or terbutaline via MDI or tube spacer, in a randomised, double blind, cross over protocol. FEV_1 was measured five minutes later, when a second dose of placebo or terbutaline was given. FEV_1 was measured again five minutes later. If it had not recovered to the pre-exercise level, aminophylline was given. Both active treatments increased FEV_1 compared to placebo, and the increase was greater following treatment with the tube spacer compared to the MDI alone. One child received aminophylline 'rescue' therapy following terbutaline via the tube spacer, compared with five children following terbutaline via the MDI alone. Errors in inhalation technique, such as submaximal inhalation, and stopping inhalation immediately after actuation (the 'cold freon effect'), were observed less frequently with the tube spacer than with the MDI alone.

Prior et al (1982) compared high dose terbutaline, 4mg six hourly, via MDI and tube spacer, with the same nominal dose administered by jet nebuliser in eight adults with chronic severe asthma. In this open, randomised, cross over trial, patients recorded PEFR and symptom scores for a two week 'run in period, then for two weeks in each treatment arm, before switching to the other modality for a further two weeks. Treatment via both spacer and nebuliser led to a significant increase in PEFR and a decrease in symptom scores compared to the run in period (when patients were taking 200-400μg of inhaled salbutamol six hourly). Although there was no statistical difference between spacer and nebuliser treatments, PEFR was higher, and symptom score lower, in six of the eight subjects when using the nebuliser.

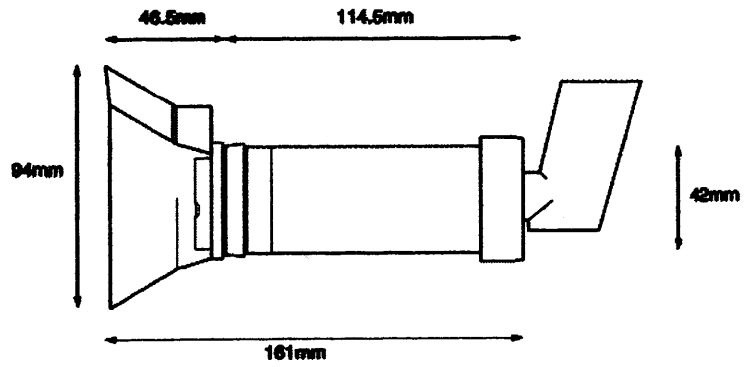
In a randomised, cross over trial (Lulling *et al.*, 1983), 12 adult patients with chronic obstructive airways disease received 0.25mg of terbutaline from a MDI alone, from MDI + tube spacer or MDI + pear shaped spacer. In this study a researcher co-ordinated MDI actuation with the start of patient inhalation. FEV_1 and FVC improved

slightly more with the spacers than the MDI alone, with no difference between the spacers. Although the differences (3-7%) were statistically significant, the authors questioned their clinical relevance.

In summary, the tube spacer is of little additional benefit for the delivery of bronchodilators to patients with good MDI technique, but may be useful in adults with poor technique, in children, and in acute severe asthma. Its use has been largely superseded by other spacers discussed below.

A shortened version of the tube spacer has been incorporated into the MDI actuator to reduce oropharyngeal deposition of steroid aerosols (Sly, 1978). In an eight week comparison of triamcinolone acetonide administered via MDI and short tube spacer versus beclomethasone via MDI alone and placebo (Berkowitz *et al.*, 1998), both formulations were equally efficacious, with no change in the incidence of side effects between the three groups, although the study was too small and too short to detect anything except a very large difference in the side effect profile between the two devices.

The *Aerochamber* was first described in 1980 by Newhouse' group from Hamilton, Canada (Corr *et al.*, 1980), as a 'portable, breath actuated, particle size selective medical aerosol inhaler' (Corr *et al.*, 1982).



Schematic diagram of the Aerochamber

To minimise impaction losses on the end wall of the spacer, the authors calculated the

theoretical cut off diameter for impaction based on formulae from May (1975) and previously published measurements of aerosol velocity and plume width (Rance, 1974). From this they estimated that particles larger than 10.6μm would impact on the end of the spacer if it were 10cm from the actuator. To test this theory, they analysed the output of an MDI alone, and the MDI attached to plastic spacers 3.2cm diameter, and 5.75cm, 8.3cm, 11.2cm and 13.4cm long, and to plastic spacers 11.2cm long and 2.0cm, 3.45cm, 4.1cm and 5.1cm in diameter.

Particle size output was determined by cascade impaction, using an impactor operating at a flow rate of 21l/min. An artificial mouth was added to the sampling system (figure), so that the flow through the spacer during sampling was 41l/min.

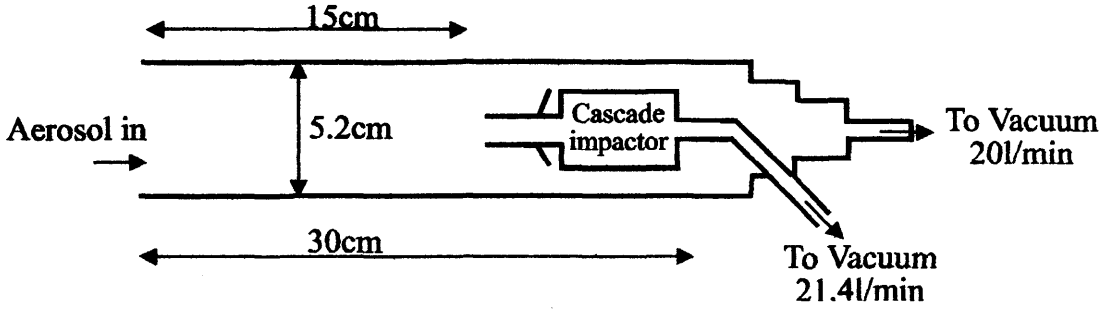


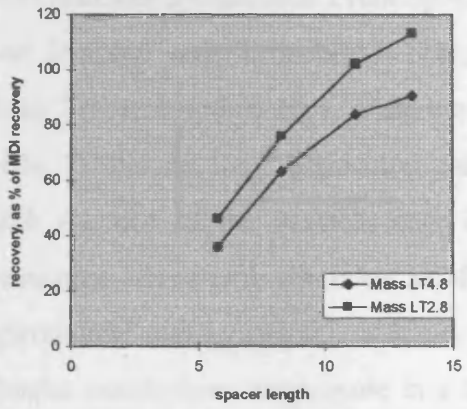
Figure: The 'artificial mouth' (Corr *et al.*, 1982)

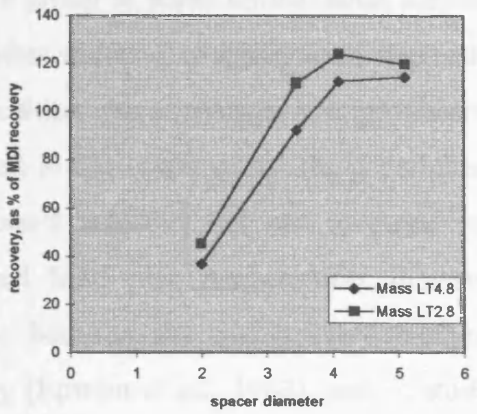
The MDI tested contained the fluorescent marker Uranin Solution (0.3ml of 2.8mg Uranium/ml 0.9% saline), ethanol (4.4ml) and CFC propellants (4.8ml; CFC-12 and CFC-114 in the ratio 1:4). The MDI was actuated into the spacer, which was immediately attached to the artificial mouth and the aerosol sampled.

The results for each spacer and the MDI alone are given as a series of bar charts, giving the relative mass of aerosol in each of eight different size ranges. The percentage of aerosol delivered from each spacer in particles smaller than 2.8μm relative to that delivered from the MDI alone is then given. No other particle size cut offs are discussed. It is not stated in the paper how many times each spacer was tested, and no estimates of the variability of the experiments is given. No statistical analysis was undertaken.

Thus for this solution aerosol, the desired outcome of reducing large particle delivery

while keeping the same or improving the delivery of smaller particles was achieved using a spacer of length 11cm or greater, and diameter 3.5cm or greater (Figures).





Figures: Data from (Corr *et al.*, 1982)

There are, however, a number of problems with this study. Firstly the use of the 'artificial mouth', essentially an added 15cm spacer, allows the aerosol to mature further and makes it difficult to compare the results of this study with others where the additional spacer was not used. The amount of drug impacting in the 'artificial mouth', representing oropharyngeal delivery, was not determined Secondly the solution aerosol used (MMAD 1.7μm from the MDI alone) differs in its characteristics from the suspension aerosols commonly used in MDIs, which have MMADs typically between 2.5μm and 4μm (Ponto, 1983). One therapeutic MDI that does have similar characteristics to the test aerosol used by Corr and colleagues is the HFA formulation of beclomethasone developed by 3M Healthcare (Leach, 1998). Although a commercial (suspension) formulation of fenoterol was tested, and 'very similar results' obtained, no details are given in the paper (Corr *et al.*, 1982). The aerosol used, driven by propellants CFC 12 and CFC 114, would have different characteristics from that used by Rance (1974), upon which the study hypothesis was based, and from other therapeutic MDIs (Moren, 1978).

In a combined radio-isotope deposition and clinical study, Dolovich (1983) studied the deposition pattern of a similar solution aerosol as that used by Corr (1982), and the bronchodilator effect of inhaled fenoterol, from the MDI and the MDI plus Aerochamber (dimensions 11cm by 4.1cm), in a group of seven normal adult subjects, and fourteen with 'bronchitis'. The Aerochamber reduced oropharyngeal deposition from 70% to less than 10%, while the lung deposition was unchanged at approximately 10%. Within the lung, deposition was increased in the 'outer zone' (lung periphery) with the use of the Aerochamber in the normal subjects, but not in those with bronchitis. Fenoterol via both MDI alone and MDI plus Aerochamber improved spirometric measurements, with no difference between the two delivery methods. Similar conclusions were made in a later study (Epstein *et al.*, 1983), and in studies comparing the Aerochamber and nebuliser in the delivery of metaproterenol (Salzman and Pyszczynski, 1986; Berenberg *et al.*, 1985).

In children, Hodges (1981) described the change in peak flow in a group of children aged 5.25 years to 13.8 years, inhaling either fenoterol or placebo through an Aerochamber. The spacer was supplied by Boehringer Ingleheim, and had a length of 7cm and diameter of 3.5cm, dimensions smaller than that used by others. There was no difference in response to fenoterol delivered via the Aerochamber compared to the standard MDI. The authors also described successful Aerochamber use in a group of patients aged 2 to 6 years. Two years later Gurwitz (1983) published a similar study using an Aerochamber 9.7cm long and 3.2cm diameter, and again found no difference in twice daily PEFR or symptom scores with fenoterol delivered via the Aerochamber or via standard MDI alone over a twelve week period. Rachelefsky (1986) also found no difference in the acute bronchodilator response of inhaled metaproterenol via the Aerochamber or MDI alone in sixteen asthmatic children aged 5 - 12 years with good MDI technique. In 1988, Sly used the Aerochamber (Monaghan Medical, Plattsbugh), 9cm in length and 4cm diameter to investigate the change in PEFR in children aged 3 to 6 years following administration of salbutamol or placebo in a non randomised study. PEFR improved in 29 of the 30 children who received salbutamol, and the increase, expressed as percentage of the predicted PEFR, was greatest for the children aged 3 years, possibly because they received a large dose of salbutamol relative to their

The Aerochamber reduces candidal infection and colonisation in patients taking inhaled steroids who do not mouth wash after inhalation (Salzman and Pyszczynski, 1988). An early study (Toogood *et al.*, 1981) suggested that this was at the expense of a reduction in the dose of beclomethasone delivered to the lung, although it was subsequently suggested that the study was flawed due to methodological problems (Newhouse and Dolovich, 1986). In an unblinded study (Salzman and Pyszczynski, 1988), use of the Aerochamber allowed a greater number of subjects to stop taking their oral steroid therapy compared to subjects using the MDI alone. Compared to the MDI alone, the Aerochamber reduces the cardiovascular side effects of inhaled fenoterol in adults (Windom *et al.*, 1989), although again this could be due to reduced drug delivery from the spacer.

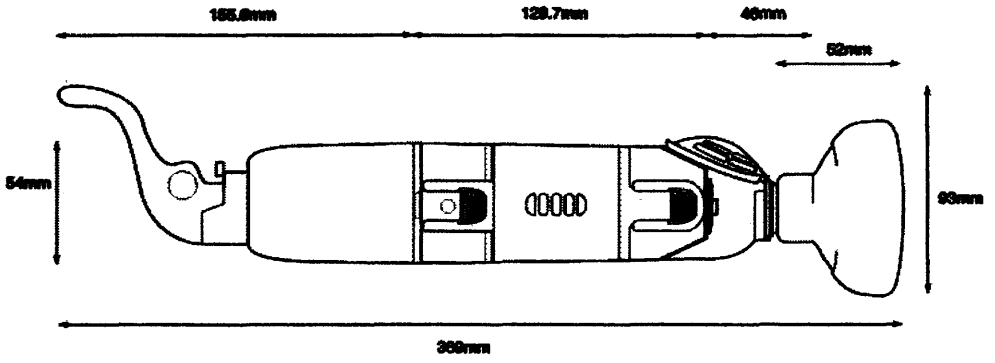
In summary, delivery of bronchodilators appears to be equally efficient when the Aerochamber or MDI with optimal technique is used. The Aerochamber reduces oropharyngeal drug deposition, and may enhance peripheral lung deposition. In children, it provides an effective delivery method in a group who are too young to use the MDI alone.

The manufacturers of the *Optichamber* state that they 'didn't invent the spacer, we optimised it!' (promotional literature, HealthScan Products Inc, New Jersey). At least one of those claims is true. The device is cylindrical, slightly larger than the Aerochamber, 4.6cm diameter and 13cm length, 215ml volume, with a silicone valve similar to the Aerochamber valve. The Optichamber (marketed as the Breath-a-Tech in Australasia) has a similar particle size output profile to the Aerochamber (Dalby *et al.*, 1998), although the Aerochamber delivers slightly less drug than the Optichamber at a sampling flow rate of 28 litres per minute compared to 55 litres per minute. The Optichamber was found to be equivalent to the Volumatic spacer and a jet nebuliser in reversing histamine induced bronchoconstriction in 27 adult asthmatics (Gibson *et al.*, 1995).

The *Spacechamber* (Medical Developments, Melbourne, Australia) is a 250ml volume

and with similar in vitro characteristics (Finlay *et al.*, 1997)

A device developed for use in babies and young children (Ashurst *et al.*, 1992; Kraemer, 1995), the *Babyhaler* is a polycarbonate tube of 350ml volume and 230mm length from MDI to inspiratory valve, and 44mm diameter. Inspiratory and expiratory valves are fitted in an exhalation area of 36ml. The Babyhaler is supplied with a Laerdal silicone facemask which also contributes to the dead space of the device.



Schematic diagram of the Babyhaler Spacer

Designed specifically for use with Glaxo-Wellcome products, for whom it is of optimal length, according to the data of Russi (1988), the Babyhaler is claimed to be ideal for infants who, assuming a tidal volume of 10ml/kg, inspire between 260ml and 340ml in five seconds. It is worth noting that the currently available Babyhaler differs in a number of ways from that used in the early trials (Ashurst *et al.*, 1992; Clarke *et al.*, 1993; Kraemer *et al.*, 1991; Kraemer *et al.*, 1992), specifically in the design of the valves and the size and shape of the exhalation area.

In an uncontrolled study (Kraemer *et al.*, 1992) 600μg of salbutamol given via the Babyhaler improved lung function (decreased thoracic gas volume and increased lung compliance) in a group of 14 infants with recurrent wheeze or chronic lung disease). A number of studies have demonstrated an effect on infant lung function measurements of salbutamol delivered to infants and children compared to placebo, although high doses of salbutamol (400μg to 800μg) were given. In a single dose, double blind study,

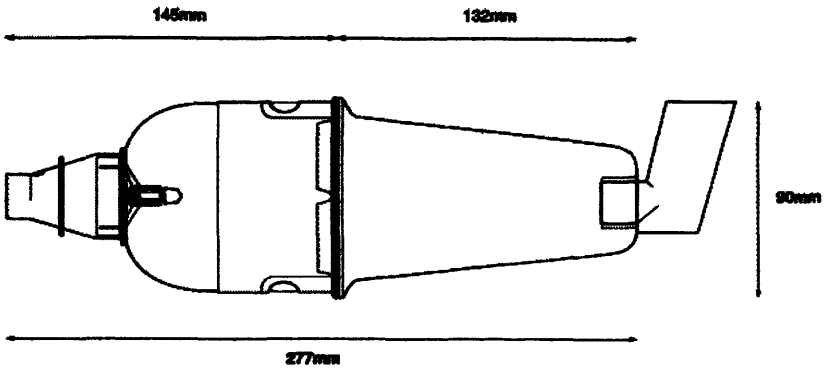
was assessed by the 'squeeze technique' (Clarke *et al.*, 1993). There was no significant change in maximal flow at a lung volume corresponding to functional residual capacity ($Vmax_{FRC}$), functional residual capacity (FRC), oxygen saturation or heart rate with either salbutamol or placebo. This lack of response was also seen in a prior study from the same authors (Prendiville *et al.*, 1987), and was ascribed to the fact that the infants were asymptomatic at the time of testing. In contrast to the baseline tests, the concentration of inhaled methacholine that provoked a 30% fall in $Vmax_{FRC}$ increased following salbutamol compared to placebo from 3.8g/l (95% confidence intervals 1.6 to 6.1g/l) to 12.5g/l (4.9 to 31g/l, $p<0.05$), although data points on two of the ten subjects who completed this part of the study were assumed, following technical difficulties with the measurements. In another study of children aged 2-5 years (Avital *et al.*, 1994), salbutamol administered via the Babyhaler increased the concentration of inhaled methacholine that produced wheeze by 3.7 doubling doses. Two other studies demonstrated improvement in lung function in infants with recurrent wheeze, cystic fibrosis or following neonatal respiratory distress syndrome following salbutamol via the Babyhaler compared to placebo (Kraemer *et al.*, 1991; Dalby *et al.*, 1998).

These studies demonstrate that the Babyhaler is effective in delivering salbutamol to infants with wheeze, but the high doses needed suggest that it is not the most efficient of devices, and there is little evidence to suggest that it is superior to other spacers supplied with face masks. The relatively large dead space of the exhalation area and facemask may disproportionately reduce drug delivery in infants and patients with small tidal volumes.

The *Nebuchamber* or *Non-Electrostatic (NES) Spacer* (Astra Draco, Lund, Sweden) is a 250ml pear shaped spacer made of stainless steel to minimise the effects of spacer static charge (Bisgaard, 1995; Bisgaard *et al.*, 1995). This spacer is discussed further in Chapter 7.8.

The *Nebuhaler* is marketed by Astra Pharmaceuticals for use with budesonide (Pulmicort) and terbutaline (Bricanyl) MDIs. Based on a design from 1976, (Moren

aerosol cloud emitted from the MDI, and early versions of the Nebuhaler were described as the *pear-shaped extension tube* (Cushley *et al.*, 1983; Newman *et al.*, 1981; Lindgren *et al.*, 1980; Lulling *et al.*, 1983; Lewis *et al.*, 1982).



Schematic diagram of the Nebuhaler spacer

Newman (1984) compared lung deposition of radio-labelled Teflon particles delivered via the MDI, or the MDI and Nebuhaler, in a group of adults with chronic respiratory disease. 8.7% of the delivered dose was deposited in the lungs from the MDI, compared with 20.9% from the MDI and Nebuhaler. In this study, there was a one second pause after the MDI was actuated into the mouth or Nebuhaler before inhalation, to simulate uncoordinated MDI use. This explains the low deposition from the MDI alone (8.7%) compared to a similar study from the same group (Newman *et al.*, 1982) in which 14.3% of the nominal dose was deposited in the lungs from the MDI.

The Nebuhaler was compared with a metered dose inhaler in eight adult patients with stable asthma (Morris *et al.*, 1984). The dose - spirometric response curves with cumulative doses of up to 8 mg of terbutaline were significantly greater using the Nebuhaler, whilst side effects were not significantly increased, suggesting improved drug delivery from the Nebuhaler, even in this group of subjects with good inhalation technique. In contrast, no difference was found in a cumulative dose response study between the MDI alone and MDI used with a 750ml spacer in 12 adults with stable asthma (Munch *et al.*, 1983).

Terbutaline delivered via the Pear Shaped spacer leads to a greater increase in FEV_1 and FVC than either the tube spacer or the MDI alone (Lindgren *et al.*, 1980). Systemic side effects of inhaled salbutamol were not altered by the use of the Nebuhaler compared to the MDI in a study of seven healthy adults (Lipworth *et al.*, 1989).

In a double-blind study of 15 adult patients with reversible obstructive airways disease (Dorow and Hidinger, 1982), comparing terbutaline delivered by the Nebuhaler or MDI alone, there was a significant decrease in airways resistance and closing volume following spacer use compared to MDI, although other spirometric parameters were not significantly different. The authors suggest that this demonstrates enhanced peripheral delivery of terbutaline from the Nebuhaler.

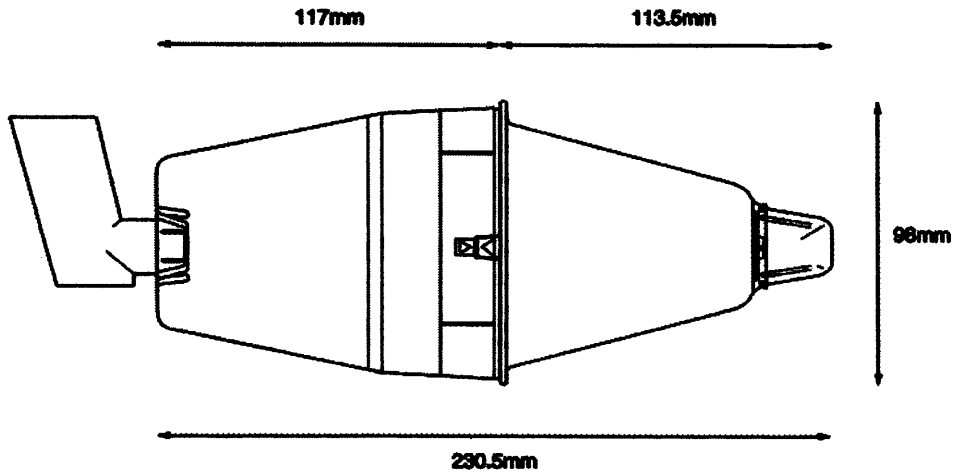
In a cumulative dose response study (Madsen *et al.*, 1982), Terbutaline administered by MDI and Nebuhaler was compared with administration by jet nebuliser in adults with stable asthma. Equal degrees of bronchodilatation, estimated by the change in FEV_1, were found between the two devices with a nominal dose ratio of 1:4. A similar study comparing the MDI and nebuliser found a dosage ratio of 1:8 (Weber *et al.*, 1979). In acute severe asthma (Beasley and ODonnel, 1985) 4mg of terbutaline administered via a Hudson nebuliser with oxygen as the driving gas lead to a greater improvement in FEV_1 and FVC than 1mg via the MDI + Nebuhaler. The authors noted that a number of patients were unable to close the Nebuhaler valve on exhalation, and suggested this as the reason for poor response seen from the Nebuhaler

In children aged 2 to 5 years, the Nebuhaler allows administration of bronchodilators leading to a significant reduction in functional residual capacity, measured by helium dilution (Pool *et al.*, 1988). Terbutaline administered via the 750ml spacer leads to greater improvement in peak expiratory flow rate in children aged 5-14 years than when delivered via the MDI alone (Hidinger and Kjellman, 1984). In a study of 27 children who received salbutamol via MDI and Nebuhaler or nebuliser at a dose ratio of 1:4 (Ba *et al.*, 1989), greater improvement in spirometry was seen following MDI and spacer use, although this was only significant for the change in forced vital capacity. Patients in this study inhaled from the spacer using tidal breathing, rather than inhalation from residual volume to total lung capacity, as is described in many other

studies. The authors conclude that tidal breathing from a spacer is a practical and effective method for delivering salbutamol in acute severe childhood asthma. Inhaled corticosteroids have also been successfully administered to young children with asthma via the Nebuhaler (Gleeson and Price, 1988).

By adding a facemask, such as the Laerdal mask (O'Callaghan *et al.*, 1989) or other soft plastic mask (McCarthy, 1990) to the Nebuhaler, it has been successfully used in the treatment of infants (McCarthy, 1990; McCarthy, 1989; Noble *et al.*, 1992) and young children (Connett *et al.*, 1993). In one trial (Bisgaard *et al.*, 1990) 77 children, aged 11 to 36 months with recurrent wheezing, were treated with budesonide pressurised aerosol 400 micrograms twice daily or placebo via Nebuhaler and face mask for 12 weeks in a double-blind, parallel-group trial. Symptom scores improved, and night disturbance and acute exacerbations were reduced. With the Nebuhaler it is important to administer the aerosol to the infant with the spacer at an angle to ensure that the spacer valve is open, as the infants inspiration may be unable to open the valve. Treatment failure with the Nebuhaler has been ascribed to failure of the patient with severe airflow obstruction to generate sufficient inspiratory force to open the Nebuhaler valve (Cox *et al.*, 1984; Bucknall, 1984).

The *Volumatic* is a 750ml diamond shaped valved spacer produced by Allen & Hanburys for use with their metered dose inhalers. Like the Nebuhaler, it increases fine particle delivery of drug in vitro, and lung deposition of drug compared to the MDI alone (Hassanally and Ganderton, 1987) and reduces oropharyngeal deposition, reducing systemic side effects (Selroos and Halme, 1991).



Schematic diagram of the Volumatic spacer

In ten children aged 8-14 years with asthma, but who had no acute symptoms, Green and Price (1991) found a small but significant increase in FEV_1 following salbutamol via the Volumatic compared to placebo, but no difference between the Volumatic and the MDI alone. In this small study, however, the MDI was actuated by the researcher, reducing the effect of patient inco-ordination.

The Volumatic was used to deliver high dose inhaled steroids to 24 asthmatic children aged 1.6 to 4.9 years in a randomised, double blind trial (Wilson and Silverman, 1990), with a reduction in day and night time symptoms during treatment compared to placebo.

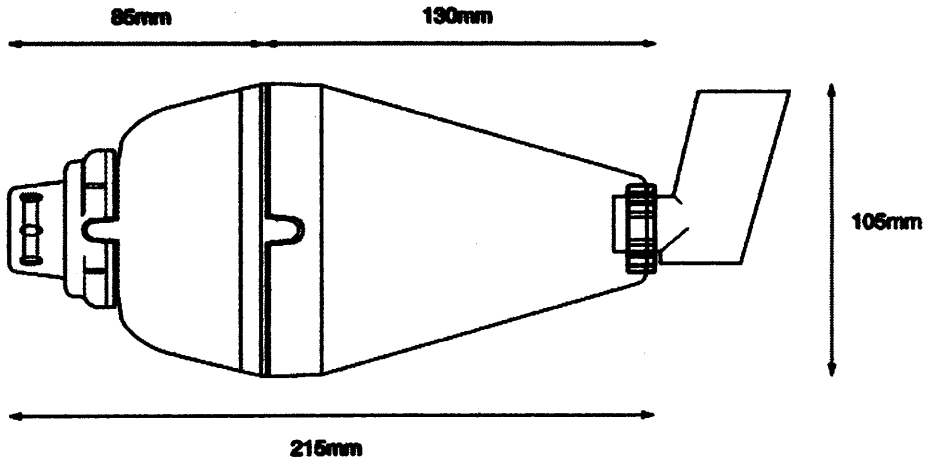
In a large randomised trial of children with acute asthma (Robertson *et al.*, 1998) the Volumatic was found to be less effective in reducing clinical scores or increasing peak expiratory flow than an unnamed jet nebuliser at a salbutamol dose ratio of 1:4.2, a dose ratio previously found to be effective (Kerem *et al.*, 1993). However, the salbutamol was administered as three actuations into the spacer prior to inhalation, and a new spacer was used for each patient. Both of these factors may have reduced drug delivery, as discussed in Chapters 6.2 and 6.8 respectively.

Both the Nebuhaler and Volumatic spacers increase the pulmonary delivery of salbutamol compared to the MDI. Greater relative lung bioavailability is obtained from the Nebuhaler compared to the Volumatic spacer (Hindle and Chrystyn, 1994),

MDI adapter, which may have altered the aerosol plume characteristics. Similar results were found in a pharmacokinetic study of healthy adults inhaling a CFC free salbutamol preparation (Lipworth and Clark, 1998). In 30 adult asthmatics, 100μg of salbutamol via the Volumatic gave greater bronchodilatation (increase in FEV_1) than 250μg of terbutaline via the Nebuhaler (Chapman and Crompton, 1990).

Instruction leaflets for the Nebuhaler suggest that aerosol should be inhaled with one or two slow, steady breaths, although in children tidal breathing sufficient to move the spacer valve is equally efficient with the Nebuhaler (Gleeson and Price, 1988) and the Volumatic (James and Masters, 1990).

The Fisonair is a large volume diamond shaped spacer used mainly with Fison's products sodium cromoglycate (Intal) and nedocromil sodium (Tilade). Other metered dose inhalers can be encouraged to fit the spacer. There is little comparative data on the use of the Fisonair compared to other spacers in vivo, although it 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).



Schematic diagram of the Fisonair spacer

In summary, the large volume spacers the Nebuhaler, Volumatic and Fisonair allow effective delivery of their respective drugs to infants, children and adults. The

and reduce both local side effects of inhaled steroids and extrathoracic deposition of drug. Studies comparing these spacers are contradictory, as although there is greater lung bioavailability of salbutamol from the Nebuhaler than the Volumatic, the normally used dose of salbutamol with the Volumatic is more effective than the normally used dose of terbutaline with the Nebuhaler.

The *Inhal-Aid* (Andersson and Boethius, 1984), no longer available, is a device that combines a reservoir aerosol delivery system, two one-way valves, and an incentive spirometer to assist in the delivery of medication from metered-dose inhalers.

The *Inspirease* (also known as the *Reservoir Aerosol Delivery System*, Key Pharmaceuticals, Miami) consists of a 700ml collapsible cylindrical plastic bag connected to a mouthpiece containing a reed that vibrates at inspiratory flows greater than 18l/min, providing an audible warning (Tobin *et al.*, 1982). Earlier versions were of adjustable size between 500 and 1500mls volume, and the authors suggested that after inhaling the medication, the patient expire into the reservoir, allowing them to inhale any expired aerosol with a subsequent breath (Sackner *et al.*, 1981). Newman (1986) compared lung deposition of radiolabelled Teflon particles in ten adults with chronic respiratory disease, comparing the subjects normal MDI technique with 'good' MDI technique and the MDI used with the Inspirease. Nine out of the ten subjects had poor MDI technique, either due to inco-ordination, fast inhalation or a short breath hold following inhalation. One subject deposited no aerosol at all in his lungs following his usual MDI technique. Overall lung deposition was 6.5%. This improved to 11.2% with 'good' MDI technique, and 14.8% with the use of the Inspirease. Most of this improvement was due to an increase in tracheobronchial deposition, with no difference in alveolar deposition between the 'good' MDI technique and the Inspirease.

In a group of adult asthmatics, the Inspirease provided greater bronchodilatation than the MDI alone (Tobin *et al.*, 1982), measured by respiratory impedance plethysmography, or than the Aerochamber (Crimi *et al.*, 1987), measured by spirometry. The differences between the devices were small, but were greater in those

The reed in the Inspirease provides audible warning of an inspiratory flow greater than 18l/min, but is easily damaged and may be inhaled (French and Irwin, 1989).

A collapsible, cone shaped spacer, 750mls volume, was also found to give slightly greater bronchodilatation, measured by PEFR, than a terbutaline MDI alone in a group of twelve children aged 7 to 11 years, in an unblinded cross over trial (Ellul-Micallef *et al.*, 1980).

The *Rondo* spacer (Huhtamäki Oy Leiras, Finland) consists of a small spherical chamber of 270ml volume in which, it is claimed, the circular flow of the aerosol encourages propellant evaporation and reduces particle losses on the spacer walls by impaction (Stenius-Aarniala *et al.*, 1993). In one radiolabelled deposition study, 24% of the administered aerosol was deposited in the lungs of a group of healthy adults (Newman *et al.*, 1991). In a group of adult asthmatics with good inhaler technique, the Rondo spacer was found to be as effective as the Volumatic spacer and the MDI alone (Stenius-Aarniala *et al.*, 1993).

A different type of spacer is the *Syncroner* (Fisons PLC, Loughborough), or 'open spacer' (Altounyan *et al.*, 1983). Designed primarily as a training aid, the spacer has an open section in its upper surface, and folds up like a clasp knife when not in use. When open the Syncroner places the MDI 10cm from the subjects' lips, and if inhalation is not co-ordinated with MDI actuation, aerosol cloud can be seen to escape from the top of the spacer, providing visual feedback to the patient of their poor technique. The spacer increases lung deposition and reduces oropharyngeal deposition of radiolabelled sodium cromoglycate compared to the MDI (Newman *et al.*, 1989). The Syncroner does not function as a holding chamber, and good co-ordination is still needed to use the device.

Do spacers increase lung deposition of drugs?

Although holding chambers improve lung deposition of drug compared to most patient's use of metered dose inhalers, only large volume spacers such as the Nebuhaler, Volumatic or Inspirease give greater lung deposition than the optimally used MDI in adults (Newman and Newhouse, 1996; Newman *et al.*, 1984; Newman *et al.*, 1986) and older children (Levison *et al.*, 1985). Where MDI technique is poor, and in younger children and infants where the MDI cannot be used alone, smaller spacers such as the Aerochamber may also improve lung deposition of inhaled aerosol.

Do spacers reduce side effects of inhaled drugs?

The aim of inhalational therapy is to optimise drug delivery to the lungs, while minimising unwanted local and systemic side effects. As noted earlier, the majority of drug emitted from metered dose inhalers is deposited in the oropharynx, and may be swallowed and absorbed directly or through the gastro-intestinal tract. For salbutamol there is little absorption across the buccal mucosa (Collier *et al.*, 1980) and salbutamol undergoes extensive metabolism in the intestinal mucosa. Systemic effects occur largely from the inhaled rather than the swallowed fraction of drug (Kung *et al.*, 1987). Thus a drug delivery device which improved lung delivery would also increase the systemic effects of salbutamol, especially if delivery were improved to the highly vascular distal bronchial and alveolar regions of the lung.

In contrast to salbutamol and terbutaline, inhaled corticosteroids have significant local side effects in the oropharynx, such as hoarseness and candidiasis, as well as systemic effects, including adrenal suppression, poor growth, and abnormalities of bony metabolism (Geddes, 1992). Although systemic effects will occur from drug absorbed across the lung (Lipworth, 1995), for a given lung delivery of steroids, oropharyngeal and systemic effects will be reduced by minimising extrathoracic drug delivery. There have been a number of recent reviews of steroid side effects which discuss the role of spacers and other inhalational drug delivery systems (Kelly, 1998; Simons, 1998; Toogood, 1990)

In an uncontrolled study hypothalamo-pituitary-adrenal function was tested in 48 adult asthmatic patients taking high dose beclomethasone dipropionate (Brown *et al.*, 1990).

free cortisol excretion was measured while the patients received 1500-2500 μg beclomethasone dipropionate daily via the MDI. Ten patients with evidence of hypothalamo-pituitary-adrenal suppression then changed to inhaling the same dose of beclomethasone dipropionate through a Volumatic spacer. The endocrine tests were repeated and, compared to the initial results, showed that adding the spacer caused an increase in the morning cortisol concentration, an increase in the post-tetracosactrin cortisol concentration and in the 24 hour urine free cortisol excretion. Evidence of persisting hypothalamo-pituitary-adrenal axis suppression was present in only four of the 10 patients; the most pronounced improvements in function tended to occur in those who had never required long term oral corticosteroids. In a randomised, placebo controlled trial in healthy adults, a large single dose of beclomethasone (2mg) caused greater suppression of the morning serum cortisol when given by MDI than by MDI and Volumatic spacer (Farrer *et al.*, 1990). Similar results were seen in a study (Prahl and Jensen, 1987) of children inhaling either beclomethasone or budesonide from the MDI or short spacer (Inhalet) compared to the Nebuhaler, using the 24 hour urinary cortisol excretion to estimate adrenal suppression.

Large volume spacer use also reduces steroid inhibition of bone formation as reflected by a fall in plasma osteocalcin (Meeran *et al.*, 1995). Osteocalcin levels were measured in a double- blind, randomised, placebo-controlled, cross-over study. Twenty-six adults took beclomethasone 500g twice daily for seven days either directly through an MDI or from an MDI and spacer (Volumatic). The fall in osteocalcin when a spacer was used was significantly less than when beclomethasone was taken directly. Local side effects such as candidal infection may also be reduced by small and large volume spacers (Salzman and Pyszczynski, 1988).

Thus, there is evidence that spacer use reduces the side effects of inhaled corticosteroids, and that evidence is best for large volume spacers. Other considerations are also important, however. In an open, parallel group trial of inhaled budesonide via the Turbohaler dry powder device or the MDI and Nebuhaler (Toogood *et al.*, 1997), efficacy was similar between the devices, but systemic side effects were higher with the Turbohaler. The authors suggest that this is due to the increased lung deposition of budesonide from the Turbohaler compared to the MDI.

This emphasises the need to compare clinically equivalent doses of drug rather than just nominally equivalent doses when assessing medication side effects. As suggested above, a highly efficient delivery system may actually increase systemic side effects if appropriate dose reduction is not undertaken. Conversely, a system that reduces overall drug delivery will also reduce drug side effects, and the loss of disease control may not be immediately apparent.

Comparisons of different spacer designs

A number of studies have compared different spacer devices in vitro and in vivo. Some are referenced above, but this subject is addressed further in the Discussion, Chapter 7.

Metered dose inhaler and Spacers compared with Nebulisers

In children and adults with severe exacerbations of asthma several studies have shown that a β_2-agonist delivered from an MDI and large volume spacer device is as effective as a nebuliser for rapidly achieving maximum possible bronchodilation (Kisch and Paloucek, 1992). Studies comparing the two delivery methods are summarised in the accompanying table.

Randomised trials of nebulisers vs. metered dose inhalers used with spacers in the treatment of acute severe asthma

Reference	Number	Age (years)	Study Design	Drug Regime	Primary Outcome Measures	Results	Comments
(Freelander and Van Asperen, 1984)	28	3-13	R, NB	Terbutaline 2.5 or 5mg by NEB, 1.25 or 2.5mg by MDI + Nebuhaler	Symptom Score, PEF	NEB = MDI	Nebuliser group older
(Pendergast *et al.*, 1989)	27	3-6.8	R, NB	Terbutaline 0.2mg/kg NEB + Facemask, 0.05 or 0.1mg/kg MDI + Nebuhaler	Symptom Score	NEB = MDI	
(Fuglsang and Pedersen, 1986)	21	7-14	R, DB	Terbutaline 0.1mg/kg NEB or MDI + Nebuhaler, then cross over	FEV_1	NEB < MDI	12/21 children complained of coughing when using the Nebuhaler.
(Morgan *et al.*, 1982)	18	15-67	R, NB	Terbutaline 4mg, by NEB, then 2mg by Nebuhaler at 30 and 60 minutes, or Terbutaline 4mg, by Nebuhaler, then 2mg by MDI at 30 and 60 minutes	FEV_1, FVC	NEB = MDI	Both groups given O_2 and hydrocortisone
(Salzman *et al.*, 1989)	44	Adults, FEV1<50% predicted	R, DB	Metaproterenol 1.95mg via MDI + Aerochamber (3x I puff every 5 mins.) vs. 15mg by nebuliser over ten mins.	FVC, FEV_1	NEB=MDI	Greater improvement in spacer group, but not statistically significant
(Turner *et al.*, 1988)	75	18-73	R, DB	Metaproterenol 1.95mg via MDI + Inspirease (3x I puff every 2 mins.) vs. 15mg by nebuliser over six mins. Treatments repeated 3 times over 30 min.	Clinical score, FVC, FEV_1	NEB=MDI	
(Colacone *et al.*, 1993)	85	18+	R, DB	Salbutamol 400μg via MDI + Aerochamber vs. 2.5mg salbutamol via nebuliser, repeated every 30 min until maximum bronchodilatation achieved.	FVC, FEV_1	NEB=MDI	
(Berenberg *et al.*, 1985)	100	18+	R, NB	Metaproterenol 1.3mg via MDI + Aerochamber (2 x 1 puff) vs. 1.3mg by Raindrop nebuliser.	FVC, FEV_1	NEB=MDI	Medication administered by respiratory therapist
(Lin and Hsieh, 1995)	111	5-16	R, NB	Terbutaline 2.5mg NEB + mouthpiece, 0.75mg MDI + Aerochamber	FEV_1/PEF/FVC, SaO2, Symptom Score	NEB < MDI	For SaO2, PEF & FEV_1, Desaturation with Nebuliser

Reference	Number	Age (years)	Study Design	Drug Regime	Primary Outcome Measures	Results	Comments
(Parkin *et al.*, 1995)	60	1-5	R, NB	Salbutamol 0.15mg/kg + Ipratropium Bromide 125μg NEB + Facemask, Salbutamol 4-600μg + Ipratropium Bromide 40μg MDI + Aerochamber	Symptom score at 12 hours	NEB = MDI	9 subjects crossed over from Aerochamber to Nebuliser
(Berry *et al.*, 1989)	20	60-91, mean 69.	R, DB	Salbutamol, 2.5mg over 10-15 minutes via Airlife Misty Nebuliser, or 4x 90μg doses over 7 minutes, via MDI + Inspirease	FEV_1, FVC, Dyspnoea Score	NEB = MDI	
(Ba *et al.*, 1989)	27	7-18, mean 11.9	R, DB	Salbutamol, 5mg via Up-Draft II Nebuliser, or 6x 2 puffs (1.2mg) over 1 minute, via MDI + Nebuhaler	FEV_1, FVC, pulse, tremor.	NEB > MDI for spirometry, NEB = MDI for tremor.	Worse baseline lung function in MDI group.
(Morley *et al.*, 1988)	28		alternate assignment NB.	Salbutamol 2.5mg by NEB or 270μg MDI + Inspirease or Metaproterenol 15mg by NEB.	Spirometry, duration of hospitalisation (LOS)	NEB > MDI for spirometry, NEB = MDI for LOS.	Nebuliser group had worse spirometry at outset. No acute improvement in FEV_1 following MDI + Inspirease.
(Beasley and ODonnel, 1985)	20	16-77	R, NB	4mg nebulised terbutaline, then 1mg via MDI + Nebuhaler at 20 and 40 minutes, or 1mg via MDI + Nebuhaler, then 4mg nebulised terbutaline at 20 and 40 minutes	FVC, FEV_1	NEB>MDI	Patients with 'acute severe asthma' but excluded if bronchodilators used in prior 2 hours. Nebulisation time not stated. Nebuliser O_2 driven
(Gervais and Begin, 1987)	10	21-61	R, DB, DD	2x 100μg salbutamol or placebo via MDI + Aerochamber and 2.5mg salbutamol or placebo via Hudson Updraft nebuliser over 10 minutes.	FVC, FEV_1, MMEFR	NEB = MDI	Tidal breathing from spacer for 4 breaths.
(Robertson *et al.*, 1998)	155	4-12	R, DB	Salbutamol 2.5mg via NEB or 600μg via MDI +Volumatic if <25kg. Salbutamol 5mg via NEB or 1200μg via MDI +Volumatic if >25kg.	Clinical score, PEF in subjects >7yrs	NEB>MDI	If no improvement by 30 min, removed from study (27, equal numbers from both groups)
(Benton *et al.*, 1989)	13	5-15	NR, NB, Historical controls	2 x 100μg salbutamol via MDI + Aerochamber, repeated at 1-3 minute intervals until no further improvement in PEF, auscultation or air entry.	PEF, total dose salbutamol required	NEB = MDI for PEF, but at lower dose for MDI	Not really a comparative study. Problems with the use of control data taken from patient's notes.

Reference	Number	Age (years)	Study Design	Drug Regime	Primary Outcome Measures	Results	Comments
(Jasper *et al.*, 1987)	34	Mean age 50	R, NB	Metaproterenol 1.3mg via MDI + Inspirease (2 puffs 5 mins. Apart) self administered vs. 15mg by nebuliser under RT supervision. Treatments repeated four hourly.	FVC, FEV_1, duration of hospitalisation.	NEB = MDI	'Routine use of the MDI....would save $253,487 per year in our institution alone'.
(Idris *et al.*, 1993)	35	10-45	R, DB	Salbutamol 2.5mg by NEB or 360μg MDI + Inspirease. Treatments repeated every 30 min until spirometry >80% predicted	FVC, PEF FEV_1	NEB = MDI	
(Maguire *et al.*, 1991)	16	18-75	R, NB, cross-over.	Metaproterenol 1.3mg via MDI + Inspirease (2 puffs 5 mins. apart) vs. 15mg by nebuliser. Treatments repeated four times from each delivery method per patient	FEV_1, FEF_{25-75}, FVC, lung auscultation	NEB>MDI	Treatments three hours apart.
(Kerem *et al.*, 1993)	33	6-14	R, DB	Salbutamol 0.15mg/kg NEB + Facemask, (max. 5mg) or 6-1,000μg MDI + Volumatic	FEV_1, SaO2, Symptom Score	NEB = MDI	Multiple actuations of MDI into spacer.
(Chou *et al.*, 1995)	152	2-? Median 8.8 yrs.	R, NB	Salbutamol 0.15mg/kg NEB + Facemask, (max. 5mg) 270μg MDI + Aerochamber	PEF, Symptom Score, SaO2	NEB = MDI	Number of treatments determined by attending physician. MDI group had shorter treatment times in ER

Key: R - Randomised; NB - not blinded; DB - double blind; NEB - nebuliser; MDI - metered dose inhaler; PEF - peak expiratory flow
FEV_1 - forced expiratory volume in one second; FVC - forced vital capacity; SaO_2 - oxygen saturation; ER - Emergency room; RT - Respiratory Therapist
NEB = MDI - no difference in outcome between nebuliser and metered dose inhaler groups;
NEB < MDI - outcome measures significantly better in the metered dose inhaler group.
NEB > MDI - outcome measures significantly better in the nebuliser group.

Randomised trials of nebulisers vs. metered dose inhalers used with spacers in the treatment of chronic asthma

Reference	Number	Age	Study Design	Drug Regime	Primary Outcome Measures	Results	Comments
(O'Reilly *et al.*, 1983)	9	24-56yrs	R, NB	Terbutaline 0.5 + 1.0 +2.0 + 4.0mg from Nebuhaler or Acorn Nebuliser	FEV_1, FVC, FEF_{30}	NEB = MDI	NEB < MDI for MEF_{30} . No details of inhalation method given. Multiple t tests used in analysis.
(Gibson *et al.*, 1995)	27	>18 yrs	R, DB, DD	Histamine → 20% drop in FEV_1 then salbutamol 200μg via Optichamber or Volumatic or salbutamol 1mg via Aerflo nebuliser.	FEV_1, FEF_{25-75} at 20 minutes.	NEB = MDI	Greater response from spacers at two minutes, but no difference between spacers at any time, or between spacers and NEB after two minutes
(Blackhall and O'Donnell, 1987)	12	5 - 10	R, NB, Cross-over	Terbutaline 1, 2, 4, 8mg via Bennett Twin Jet Nebuliser + facemask, or 0.5, 1, 2, 4mg via MDI + Nebuhaler	FEV_1.	NEB < MDI (at same μg dose)	Cumulative dose response. Equivalent NEB response at twice MDI dose. Measurements made at different times for the two devices.
(Pierce *et al.*, 1992)	38 Adults, 23 Children	Mean 56 yrs; Mean 9.9yrs	R, NB, Cross-over	Adults: Terbutaline 5mg via Hudson Updraft II Jet Nebuliser, or 2x 3 puff (1.5mg) via MDI + Nebuhaler, 4 times a day for 4 weeks, then cross over. Children: Terbutaline 0.2mg/kg via Hudson Updraft II Jet Nebuliser, or 1 x 0.25mg puff/5kg via MDI + Nebuhaler, 3 times a day for 4 weeks, then cross over.	PEF, FVC, Patient Diary cards	NEB = MDI	Multiple actuations in adult spacer protocol. 2 children withdrew from spacer group due to deteriorating asthma. More patients referred the NEB than the MDI
(Laursen *et al.*, 1983)	12	Mean 53 yrs.	R, DB, Cross-over	Terbutaline 5mg via Sofio Jet Nebuliser, or 6x 1 puff every 2 min (1.5mg) via MDI + Nebuhaler	PEF	NEB = MDI	5 patients did not complete the study
(Salzman and Pyszczynski, 1986)	15	Mean 33 yrs.	R, NB, Cross-over	Metaproterenol 1.3mg via MDI + Aerochamber (2 puff 1 min apart) vs. 15mg by nebuliser over 10-12 mins.	FEV_1, FVC, PEF at 30 minutes.	NEB = MDI	Patients with previously documented poor MDI technique

Reference	Number	Age (years)	Study Design	Drug Regime	Primary Outcome Measures	Results	Comments
(Madsen *et al.*, 1982)	13	Mean 47 yrs.	R, NB, Cross-over	Terbutaline 1.25mg, 1.25mg, 2.5mg via Pari Inhalierboy Jet Nebuliser, or 0.125mg, 0.125mg, 0.25mg via MDI + pear shaped spacer. Doses given at 60 minute intervals	FEV_1, FVC, PEF, FEF_{50}	NEB < MDI (at same μg dose)	NEB:MDI equipotent doses 4:1 for change in FEV_1
(Pedersen and Bundgaard, 1983)	15	20-58	R, NB, cross over	1 mg terbutaline via MDI+pear shaped spacer vs. 1mg and 4mg terbutaline via Hudson Updraft nebuliser.	FEV_1, MEF_{50}, MEF_{75-85}.	NEB = MDI at dose ratio of 1:4	
(Stauder and Hidinger, 1983)				1mg terbutaline via MDI +Nebuhaler vs. 4mg terbutaline via Pari Inhalier Boy nebuliser.	FEV_1, FVC, PEF,	NEB = MDI	1:4?
(Cushley *et al.*, 1983)	16	19-61	PC, NR, NB Cross over	Placebo or doubling doses of terbutaline from 31 to 1,000μg via MDI, MDI+pear shaped extension tube, or similar doses from Inspiron nebuliser over 5 minutes.	Change in sGaw, FEV_1, serum terbutaline levels	NEB < MDI for Change in sG_{aw} (normals & asthmatics), FEV_1 in (asthmatics)	Eight normal and eight asthmatic patients. Nebuliser dose estimated by weight loss. Terbutaline levels greater after metered dose inhaler alone, lowest after MDI + spacer.
(Prior *et al.*, 1982)	8	53-67	R, NB, Cross over	Terbutaline 4mg four times daily via jet nebuliser or MDI + Tube spacer.	PEFR, Symptom score	NEB = MDI	One patient significantly better on NEB compared to MDI
(Summer *et al.*, 1989)	36	Mean 63	R, NB	0.5mg terbutaline via MDI + Breathancer vs. Metaproterenol 15mg by nebuliser four times daily until hospital discharge.	FVC, Patient costs	NEB ≤ MDI for spirometry, MDI cheaper.	Patients demonstrated >10% reversibility & were able to perform spirometry. MDI group received more supervision than NEB group.

Key: R - Randomised; NB - not blinded; DB - double blind; DD - double dummy; OC - placebo controlled; NEB - nebuliser; MDI - metered dose inhaler plus spacer; PEF - peak expiratory flow. FEV1 - forced expiratory volume in one second; FVC - forced vital capacity; SaO2 - oxygen saturation; ER - Emergency room; NEB = MDI - no difference in outcome between nebuliser and metered dose inhaler groups;

NEB < MDI - outcome measures significantly better in the metered dose inhaler group.

- *Chronic asthma:*

Pierce et al (1992) found terbutaline delivered by a metered dose inhaler and Nebuhaler spacer provided similar clinical benefit to nebulised terbutaline in the long term domiciliary management of children and adults with stable airflow obstruction. Blackhall and O'Donnell (1987) conducted a dose response study of inhaled terbutaline administered via a large volume spacer or nebuliser in asthmatic children and found that they gave equivalent bronchodilation in children who were not in acute respiratory difficulty at a 1:2 microgram equivalent dose ratio. In a randomised double blind crossover study, Rivlin (1984) compared the same dose of fenoterol given by nebuliser, MDI or MDI and large volume spacer to ten children with asthma. All three methods of drug delivery produced significant changes in lung function compared with placebo, but the increase in FEV_1, FVC and peak flow were greatest when the drug was given by MDI and spacer. Randomised trials comparing nebulisers and metered dose inhalers in the treatment of chronic asthma are outlined in the table.

- *Acute severe asthma*

Observational studies support the use of spacers in acute severe asthma (Benton *et al.*, 1989). Randomised trials comparing nebulisers and metered dose inhalers in the treatment of acute severe asthma are outlined in the table. In two of the studies (Freelander and Van Asperen, 1984; Pendergast *et al.*, 1989) the spacer was less effective in some of the younger children and patients with severe airways obstruction, possibly because they could not produce sufficient inspiratory flow rates to trigger the spacer valve.

A recent analysis of trials comparing metered dose inhalers in the emergency room treatment of acute severe asthma (Kisch and Paloucek, 1992) concluded that there was no significant difference between the two delivery methods. The minority of studies claiming the superiority of nebulisers have compared the bronchodilator response in acute exacerbations using lower doses of β_2-agonists from MDIs than from nebulisers (Maguire *et al.*, 1991), or, where numerical dose equivalence has been maintained (Campbell *et al.*, 1995), the spacer has been used in such a way that most of the dose

administered is not available for inhalation (Barry *et al.*, 1993; Robertson *et al.*, 1998).

The use of a spacer and MDI in preference to a nebuliser is cheaper (Bowton *et al.*, 1992). Newhouse has commented (Newhouse, 1993) that in several studies MDI generated aerosols have been 50-75% less expensive than equivalent nebuliser therapy, although this estimate includes the cost of respiratory therapists, who are generally not used in UK hospitals.

Use of Spacers in special situations

Spacers such as the Nebuhaler have been successfully used to deliver inhaled medication to adults and children with tracheostomies (Webber and Brown, 1984; O'Callaghan *et al.*, 1989; Meeker and Stelmach, 1992), and spacers may be inserted into ventilator circuits to deliver aerosolised medication during mechanical ventilation (Dhand and Tobin, 1997; Denjean *et al.*, 1992; Lee *et al.*, 1994). Grigg and colleagues (1992) have evaluated the delivery of sodium cromoglycate to ventilated neonates via either an ultrasonic nebuliser or MDI + spacer. They first instilled a known amount of drug into the trachea and measured the fraction excreted in the urine over the ensuing 24 hours. They then administered sodium cromoglycate by one of the two systems studied, and by measuring the urinary excretion, extrapolated the dose delivered to the lungs. Despite a 2-3 fold variation in dose delivered between the infants within each group, the MDI and spacer delivered a much higher dose per kilogram than the nebuliser (234μg/kg vs. 107μg/kg). In a parallel study, this group compared their in vivo findings to different in vitro methods of measuring drug delivery from ventilators, and found good agreement with a filter and test lung, suggesting that this may be the method of choice for further in vitro work in this field.

A similar proportion of the nominal dose was delivered to the lungs in studies by O'Callaghan (1992) using beclomethasone in rabbit studies and in vitro by Everard (1992) using sodium cromoglycate. With a 4x11cm cylindrical chamber connected to the inspiratory limb of the ventilator circuit, Everard found better delivery at higher tidal volumes; with longer inspiratory times; by connecting the spacer as close as possible to the ET tube; and by actuating the MDI immediately before the start of the

inspiratory phase. Grigg (1992) subsequently studied the delivery of budesonide from a spacer and MDI in a ventilator circuit, using the same test lung methodology, and reported an encouragingly high percentage drug delivery (14.2% of the MDI dose). These results imply that different drugs may behave differently within spacers and underline the need for devices to be evaluated with each different drug and formulation.

The amount of drug delivered to the ventilated patient is determined by the delivery device used, its position in the ventilator circuit, the ventilator parameters and the use of humidity (Fok *et al.*, 1997; Coleman *et al.*, 1996; Coleman *et al.*, 1996; Dhand and Tobin, 1996).

Summary and Conclusions

Many different devices have been produced to aid inhalational therapy from metered dose inhalers. Many studies, often contradictory, have been undertaken to compare drug delivery from spacers compared to placebo, to the MDI alone, or compared to other spacers. These have shown that some spacer devices improve drug delivery to the lungs, and some reduce it, compared to the optimally used MDI. Most spacers reduce extrathoracic drug deposition. Holding chambers are useful in the management of patients who have difficulty co-ordinating inhalation with MDI actuation - that is, most patients. Spacers are also useful in the treatment of acute severe asthma, and in the delivery of inhalational therapy to patients receiving intensive care. The method of spacer use affects drug output, and an investigation of spacer use forms the larger part of this thesis.

Nebulisers

Effective nebuliser therapy depends upon using a device that will deliver sufficient drug to the site of action, in a repeatable fashion, with minimal drug wastage, in an acceptably short time and at a low cost. Most of the prescribed medication for nebulisers never reaches the lungs (Clay and Clarke, 1987). Of the dose placed in the nebuliser chamber, perhaps two thirds remains there at the end of nebulisation. Two thirds of the dose actually released from the nebuliser may be released during expiration and passes into the surrounding air (Kradjan and Lakshminarayan, 1985). Of that inhaled, some will be in particles too large to reach the lung, and some in particles so small that they do not deposit, but are simply exhaled again. With many nebulisers, only ten percent of the prescribed dose may reach the lung (Zainudin *et al.*, 1990).

For drugs such as bronchodilators, where a small dose may achieve an adequate result, this may not matter. It is clearly more important for drugs with dose related effects (and side effects), such as steroids, and for expensive medications, such as DNAse (Bryson and Sorkin, 1994).

Nebuliser Types - How they work (Mercer, 1973; Dennis and Hendrick, 1992; Newman, 1989)

Nebulisers used in aerosol drug delivery produce a polydisperse aerosol, where the major amount of drug released is contained in particles between 1 and 5μm in diameter. Most nebulisers use compressed air for atomisation (figure 1), whereas some use ultrasonic energy (figure 2). Many types of nebuliser have been developed since their introduction during the last century (see Chapter 4.1). A nebuliser may be distinguished from a simple atomiser by the incorporation of baffle structures which selectively remove large droplets from the outgoing spray (Abramson, 1946).

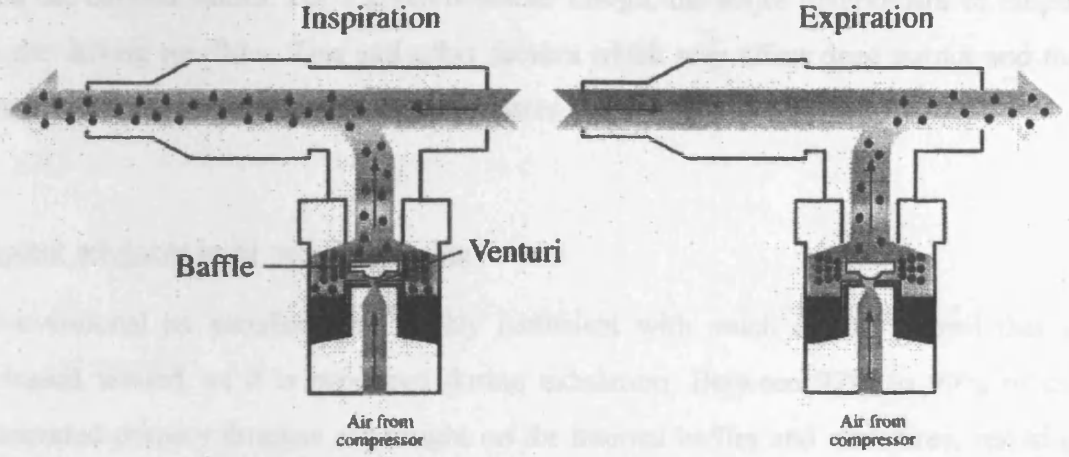


Figure 1. Conventional nebuliser design. Air from the compressor passes through a small hole (Venturi). Rapid expansion of air causes a negative pressure which sucks fluid up the feeding tube system, where it is atomised. Larger particles impact on baffles and the walls of the chamber and are returned for re-nebulisation. Small particles are released continuously from the nebuliser chamber. On expiration the nebuliser continues to generate aerosol which is wasted. From O'Callaghan and Barry, 1997.

The Jet Nebuliser:

In a jet nebuliser the driving gas passes through a very narrow hole, known as a Venturi, from a high pressure system (Figure 1). At the Venturi, the pressure falls and the gas velocity increases greatly producing a cone shaped front. This passes at high velocity over the end of a narrow liquid feed tube or concentric feeding system creating a negative pressure at this point. As a result of this fall in pressure, liquid is sucked up the liquid feeding tube or concentric feeding system (by the Bernoulli effect) and is drawn out into fine *ligaments*. The ligaments then collapse into droplets under the influence of surface tension. This primary generation (atomisation) typically produces droplets between 15 and 500μm in diameter . Coarse droplets impact on baffles while the smaller droplets produced may be inhaled or may land on internal walls returning to the reservoir for re-nebulisation. Baffle design has a critical effect on the size of droplets leaving the nebuliser.

Concentric liquid feeds have been designed to minimise blockage by residual drug build up with repeated nebulisation. A flat pick up plate may allow some nebulisers to be tilted during therapy whilst maintaining liquid flow from the reservoir.

Different jet nebulisers have different output characteristics determined by the design of the air jet and capillary tube orifices, their geometric relationship with each other

and the internal baffles. For a given nebuliser design, the major determinant of output is the driving gas flow. This and other factors which may affect drug output and the amount the patient inspires are discussed later.

Recent advances in jet nebuliser design

Conventional jet nebulisers are highly inefficient with much of the aerosol that is released wasted, as it is produced during exhalation. Between 93% to 99% of the generated primary droplets are caught on the internal baffles and structures, resulting in a low output . Recent developments have been made to reduce these inefficiencies.

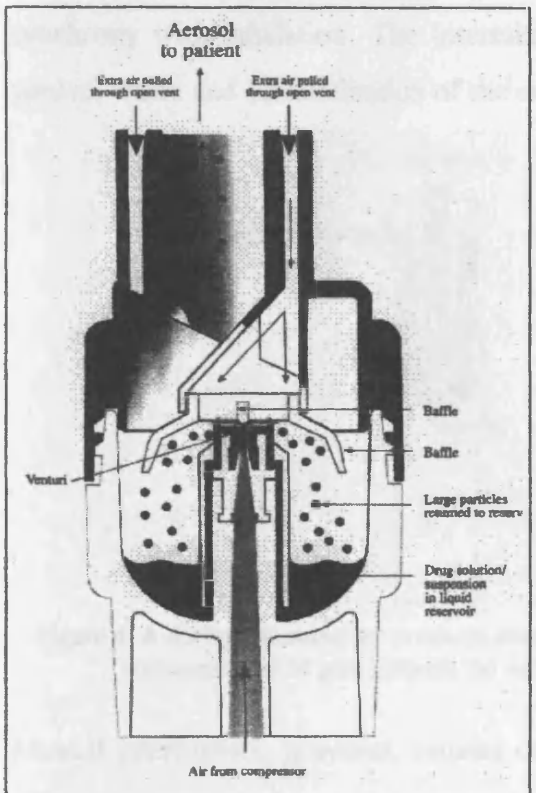


Figure 3. Open Vent Nebuliser. From O'Callaghan and Barry, 1997.

Continuous entrainment of gas through the nebuliser (Open vent nebulisers):

Conventional jet nebulisers produce a fixed flow of gas containing aerosol. As described earlier, the rapid increase in velocity of the compressed air as it passes through the venturi creates a negative pressure, pulling fluid up liquid feeding tubes (Bernoulli effect) for atomisation. Some recent designs (e.g. Sidestream: Medicaid, Pagham, UK) incorporate an extra, open vent into the nebuliser, in such a way that negative pressure generated by the expansion of compressed air, at the venturi, sucks air into the chamber via the vent, as well as fluid from the feeding tubes for atomisation (Figure 3).

This results in a greater air flow through the chamber which pushes more small particles out to be inspired, in a given time, leading to shorter nebulisation times. The contribution of enhanced air flow through the nebuliser in reducing particle size, due to greater solvent evaporation, remains to be determined. Lower compressed air flows are

needed to generate the same respirable output, allowing cheaper, lower specification compressors to be used. If the extra inlet channel (the vent) was blocked, preventing additional flow of air through the chamber, a similar amount of drug would exit the nebuliser and be inspired, but over a much longer time.

Entrainment of gas through the nebuliser on inspiration only (breath controlled, open vent nebulisers):

Continuously operated nebulisers usually produce a constant rate of aerosol, with at least 50% of the aerosol wasted during exhalation. Nebulisers fitted with an interrupter are known to enable economic use of medication, since aerosol is only produced in synchrony with inhalation. The intermittent nebulisation, during inspiration, reduces aerosol waste and contamination of the environment (Figure 4).

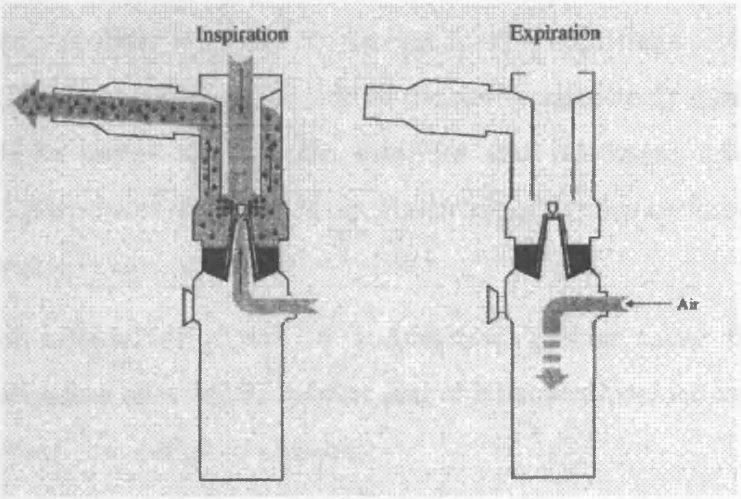


Figure 4. A dosimetric nebuliser produces aerosol only when the patient presses a button allowing compressed air to pass through the nebuliser. From O'Callaghan and Barry, 1997.

Manual interrupters, however, require co-ordination by the patient and the increased efficiency results in longer treatment times. In order to combine the convenience of continuous operation and the efficiency of intermittent nebulisation, the Pari LC Plus (Pari, Germany) (Figure 5) and the Ventstream (Medicaid, UK) were developed.

The Pari LC Plus nebulises continuously, at the fixed compressor flow. During inspiration , a valve situated on top of the device opens allowing extra air to be drawn through the nebuliser. As with the Sidestream, it is claimed that this air will draw a much greater number of aerosolised particles into the inspired air stream. These would

otherwise have been recirculated within the nebuliser chamber. During exhalation the inspiratory valve closes, decreasing the flow of air through the chamber to that from the compressor only. The result is that loss of aerosol during expiration is similar to that from a conventional jet nebuliser.

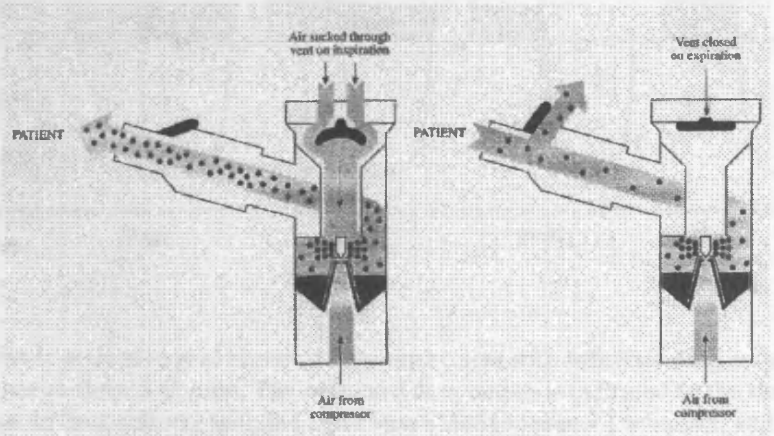


Figure 5. A breath enhanced, open vent nebuliser. From O'Callaghan and Barry, 1997.

The Ventstream nebuliser is similar in design to the Sidestream, but includes an intricate valve system. A valve on the side of the device opens only during inspiration, allowing air to be drawn through the nebuliser and increasing drug output. On exhalation this valve closes and exhaled air passes out of the device through a separate expiratory pathway.

The amount of inspired drug will be considerably greater using these nebuliser chambers. Nebulisation time will be quicker that of a conventional jet nebuliser but not as fast as the Sidestream design of chamber.

The advantages of the 'breath controlled, open vent devices' may be summarised as follows;

- the additional airflow through the nebuliser draws more of the small particles generated out to be inspired. Increased evaporation from droplets may occur so that smaller particles are produced.
- there is an increase in amount of aerosol delivered to the patient and less wastage of aerosol during exhalation (Figure 6). The dose of drug inspired may be doubled.
- lower compressed air flows are needed to generate the same respirable output, allowing cheaper, lower specification compressors to be used.

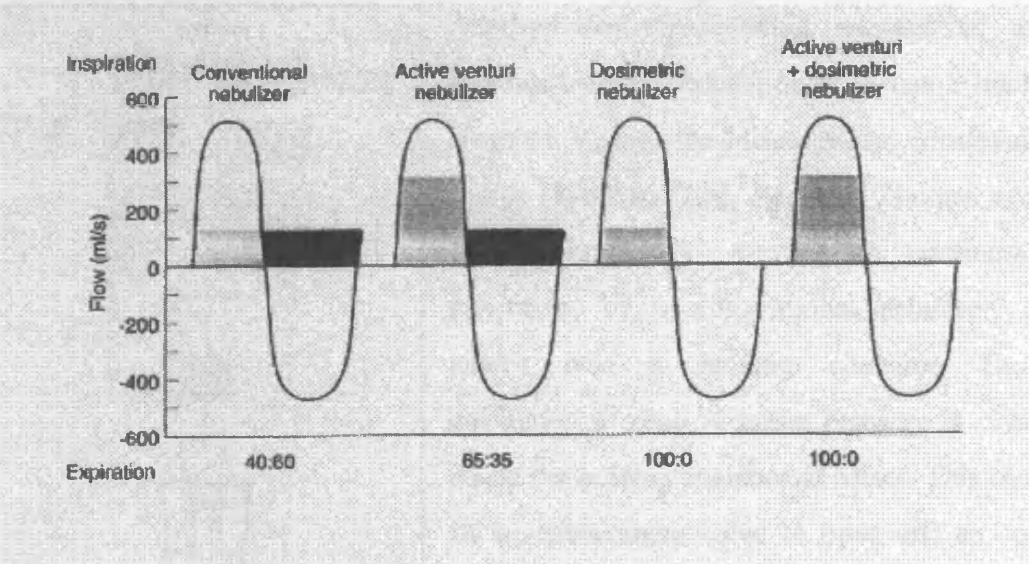


Figure 6. Schematic presentation of identical flow-time traces with nebuliser drug output adapted to a nebuliser/compressor flow of 6L/min. The nebulised drug output is indicated by the shaded area. The improvement in drug delivery with the Open vent ('Active venturi') nebuliser, and the breath enhanced, open vent ('Active venturi + dosimetric') nebuliser is clearly seen. From Nikander, 1994.

These systems do have some disadvantages. Firstly, they are dependent upon the patients inspiratory flow for optimum function and more information is needed before they can be recommended for young children. Viscous solutions (such as ceftazidime) may be nebulised slowly with the less powerful compressor used. As with conventional jet nebulisers, nebulisation of suspensions will depend on the relationship between the size of the suspended particle and the size of the droplet particles produced by the nebuliser.

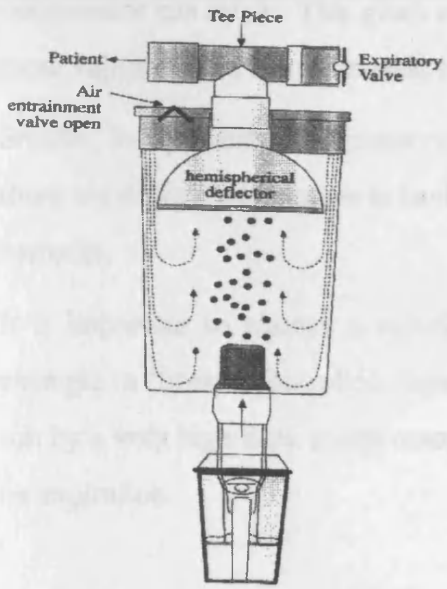


Figure 7. The Mist Miser aerosol conservation device. From O'Callaghan and Barry, 1997.

Another way of reducing wastage of drug produced during exhalation is to use a holding chamber, such as the Miser aerosol conservation device (Medicaid Ltd, Pagham; Thomas *et al.*, 1988; Figure 7). Aerosol is continuously generated, by a conventional nebuliser, and passes into a holding chamber. During inspiration a small negative pressure is created inside the holding chamber of Miser. This causes an air entrainment valve to open and air to be drawn into the device and down the inside walls of the holding chamber. At the bottom of the chamber the entrained air is forced to turn, collect aerosol and exit through a tee piece, delivering the stored aerosol to the patient. The patient inhales concentrated aerosol from the holding chamber during inspiration (as long as the inspired volume is less than the holding chamber volume). Expired air is diverted away from the chamber by a valve, and the chamber fills up with aerosol again.

Compressors

Compressors used to drive gas through the nebuliser chamber vary greatly in power, and some will generate a reasonably high free air flow. However, nebuliser chambers have a resistance to flow, and attaching a nebuliser chamber to a compressor will reduce the flow considerably. Different chambers vary greatly in their resistance. To make valid comparisons between compressors, flow should be estimated with the nebuliser attached and should be measured at the outlet of the nebuliser. This is the *dynamic flow* and is critical in determining the droplet size and nebulisation time. More powerful compressors can generate a higher flow through more resistant nebulisers. Unfortunately, some compressor manufacturers often quote only the maximum static pressure and maximum flow (that is, without the nebuliser chamber in line) that the

compressor can attain. This gives a false impression of the compressors capabilities as these values can be approximately twice the 'dynamic values' obtained.

Smaller, less powerful compressors may not be suitable for all drugs and situations, but there are distinct advantages in having a nebuliser that is small, lightweight and runs on batteries.

It is important to choose a nebuliser and compressor that work well together. For example, a 'breath controlled, open vent' type of nebuliser, such as the Ventstream, run by a very high flow compressor may defeat the object of minimising drug wastage on expiration.

The Ultrasonic Nebuliser:

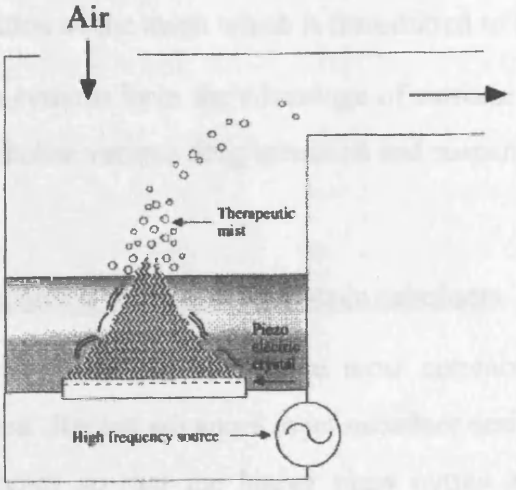


Figure 2. The ultrasonic nebuliser. From O'Callaghan and Barry, 1997.

The *ultrasonic nebuliser* uses a rapidly vibrating piezoelectric crystal to produce aerosol particles (Figure 2). The ultrasonic vibrations from the crystal are transmitted to the surface of the drug solution, where standing waves are formed. Droplets break free from the crests of these waves, and are released as aerosol. The size of droplets produced is inversely proportional to the two thirds power of the acoustic frequency. Like jet nebulisers, baffles within the nebuliser remove large droplets, and much of the aerosol produced impacts on these, falling back into the drug reservoir.

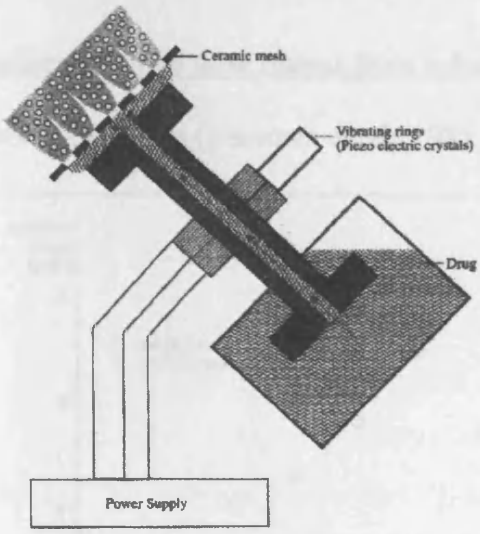


Figure 8. The Omron U1 Nebuliser. From O'Callaghan and Barry, 1997.

A more recent design of ultrasonic nebuliser uses the vibration of the piezoelectric crystal indirectly to generate an aerosol. The Omron U1 (Omron Healthcare; Figure 8) is an example where drug placed in the chamber is pumped, in a peristaltic fashion, up a thin metal tube by vibration from the piezo electric crystals. These are situated adjacent to the metal feeding tube half way down its length. The free end of the metal tube is separated by a small distance from a ceramic mesh with holes that are 4-6μm in diameter, each 100μm apart. The drug solution is pumped through the mesh and droplets break off fluid ligaments produced. Droplet formation is facilitated by rapid vibration of the mesh which is transmitted to the fluid ligaments as they form.

Such systems have the advantage of extreme portability and full details on their ability to nebulise various drug solutions and suspensions are awaited.

Comparison of Jet and Ultrasonic nebulisers

Jet nebulisers are by far the most common type of nebuliser used world-wide at present. Recent advances in jet nebuliser design (discussed above) have improved their efficiency so that the higher mass output and shorter nebulisation times seen with ultrasonic nebulisers (Thomas *et al.*, 1991) may no longer be important discriminating factors. Current ultrasonic nebulisers do not appear to nebulise drug suspensions efficiently, and until newer models are evaluated, they should be avoided for this task. The evidence that they may breakdown complex molecules is conflicting (Waldman *et al.*, 1987; Groth *et al.*, 1989).

Patients may prefer the generally smaller and quieter ultrasonic nebuliser for nebulisation of standard drug solutions to the noisy and bulky jet nebuliser and compressor (Thomas *et al.*, 1991).

Factors affecting drug output from nebulisers (Mercer, 1973; Newman, 1985)

Driving gas flow (Newman *et al.*, 1985; Smith *et al.*, 1992; Clay *et al.*, 1983)

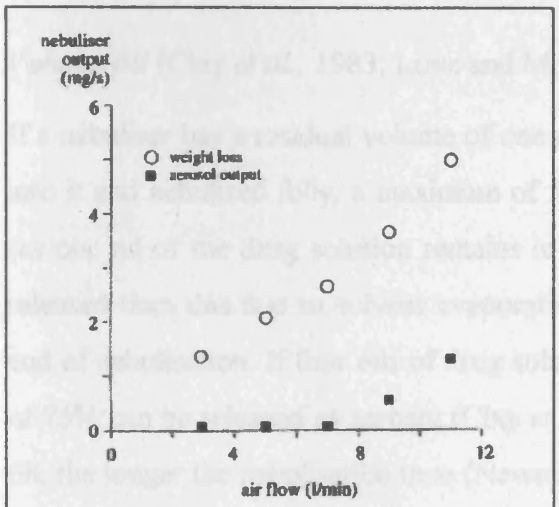


Figure 9. From O'Callaghan and Barry, 1997.

Increasing the driving gas flow will increase the drug output, reduce the particle size (Clay *et al.*, 1983) and decrease the nebulisation time. Below a certain flow, drug output is negligible (Newman, 1985) (Figure 9). Optimum flow will depend upon the nebuliser and drug being used, but is often 6-10l/min. Care should be taken at high flow rates that the tubing does not blow off and that spitting of large aerosol droplets does not occur, which would worsen nebuliser performance.

High driving gas flow rate may not be as important for nebulisers incorporating an air inlet vent, such as the Sidestream, Ventstream or Pari LC Plus nebulisers. When comparing different nebuliser compressors, it is important to compare only the *dynamic* flow (O'Doherty *et al.*, 1990) (see above) produced should be measured.

Residual volume of drug

The term *residual volume* is often used to infer drug wastage (Clay *et al.*, 1983), but **residual mass** of drug is the important factor and may not be directly related to the residual volume of fluid.

Nebulisers which leave a low residual mass of drug are preferable. Internal baffles may be used to reduce particle size, but these increase the surface area of the nebuliser and hence the residual volume. Residual volume may be effectively reduced by tapping the nebuliser intermittently during operation, increasing output (Kradjan and Lakshminarayan, 1985). However, from the clinicians point of view it may be more important to have reproducible output from an efficient device, rather than hoping that

patients, by using such manipulations may receive more drug. By reducing surface tension of a drug solution less will adhere to the nebuliser surfaces with more returning to the nebuliser reservoir for re-nebulisation. Output will, therefore, be increased.

Volume fill (Clay *et al.*, 1983; Love and Muir, 1976)

If a nebuliser has a residual volume of one ml, and two mls of drug solution are placed into it and nebulised fully, a maximum of 50% of the drug will be released as aerosol (as one ml of the drug solution remains in the chamber). In practice less drug will be released than this due to solvent evaporation and increased drug concentration at the end of nebulisation. If four mls of drug solution are placed in the chamber, a maximum of 75% can be released as aerosol (Clay *et al.*, 1983). However, the larger the volume fill, the longer the nebulisation time (Newman *et al.*, 1985; Clay *et al.*, 1983).

Increasing the volume fill will also decrease the drug concentration, and in one study lessened the severity of side effects from inhaled pentamidine (O'Doherty *et al.*, 1990)

Concentration of nebuliser solution

Evaporation of the solvent, usually water, during nebulisation leads to concentration of the drug during nebulisation. This leads to increased drug wastage, as more drug is left behind in the nebuliser at the end of nebulisation and, as mentioned, evaporation invalidates the gravimetric method of measuring drug output. Irritation of the respiratory tract from the inhalation of highly concentrated solutions may occur.

Solution viscosity and surface tension

Theoretically the aerosol particle size is proportional to the surface tension of the drug solution, but experimental work in this area has been conflicting (Davis, 1978; Newman *et al.*, 1987). The primary droplet size is related to surface tension and viscosity, but the baffles in jet nebulisers control the output size. Indeed for most nebulisers a coarser initial droplet size would be seen as an increased nebulisation time, as it takes longer to generate the fine droplets. Certainly highly viscous solutions, such

as certain antibiotics (Newman *et al.*, 1985), nebulise slowly and require powerful compressors. Warming solutions will reduce viscosity and nebulisation time. McCallion *et al* (1995) studying two ultrasonic nebulisers, found droplet size was proportional to the viscosity of the nebuliser fluid and the more viscous fluids had the lowest out puts. While there was a trend for slightly lower mass median diameter values for fluids of lower surface tension, no clear correlation was established.

Solution temperature

Solution temperature may fall by ten degrees or more during jet nebulisation (Clay *et al.*, 1983; Cockcroft *et al.*, 1989). This increases the solution viscosity and reduces the nebuliser output (Dennis *et al.*, 1990) although the aerodynamic size of droplets produced falls with decreasing solution temperature (Phipps and Gonda, 1990). A jet nebuliser, Paritherm, incorporates a heating system allowing warming of the aerosol to body temperature, but does not heat the drug solution itself.

Environmental conditions

Aqueous droplets produced by jet nebulisers can lose water by evaporation causing an increase in the concentration of the solution in the droplet and reduction in droplet size (Phipps and Gonda, 1994). Conversely, increasing the humidity of the inhaled air may increase particle size, depending on the tonicity of the particle (Phipps and Gonda, 1990). Estimation of growth of aqueous aerosols such as produced by a jet nebuliser requires special consideration. The relative humidity of the output of these devices is in the 95-99% range. Consequently, aerosol particles generated from an isotonic solution will probably change very little in size in the respiratory tract, especially when the nebuliser output relative humidity is near 99%. If a hypertonic solution is aerosolised, growth in the lung would occur, while a hypotonic droplet would evaporate towards isotonicity in the lung.

Static charge

Particles produced by both jet and ultrasonic nebulisers may acquire charge by the nebulisation process (Hashish and Bailey, 1987; Hashish and Bailey, 1991). Static charge is an important factor in inhalational drug delivery from spacer devices (O'Callaghan *et al.*, 1993). It is not known whether interaction between charged particles and the nebuliser, face mask or mouthpiece affects drug delivery, and this area deserves further investigation.

Nebulisation time

It is important to consider the effect of drug delivery method on patient compliance. For instance, it has been shown that 80% of the nebulised dose of sodium cromoglycate from most nebulisers is delivered in the first five minutes of nebulisation (O'Callaghan *et al.*, 1989). This sort of calculation should be made for each different drug/nebuliser combination. Lengthening therapy beyond this time may exasperate the patient for little therapeutic gain.

Factor	Positive effect	Negative effect
Increase driving gas flow/compressor rating	Smaller particle size, shorter nebulisation time	More expensive compressor
Increase volume fill	Greater proportion of drug nebulised	Longer nebulisation time
Decrease residual volume	Greater proportion of drug nebulised	Longer nebulisation time
Increase baffles	Smaller particle size	Longer nebulisation time
Decrease solution viscosity (e.g. warm solution)	Smaller particle size	Minor effect
Use an 'open vent' nebuliser	Shorter nebulisation time	Nebuliser cost
Use nebuliser with manual interrupter/assisted 'open vent'	No drug wasted in expiration. Increased dose to patient	Co-ordination required. Longer treatment time.
Use 'breath assisted open vent' nebuliser	Greater (twice or more) drug delivery to patient	Effectiveness has only been shown in adults using bronchodilator solutions

Table: Factors affecting output of drug solutions from jet nebulisers

Ultrasonic nebulisers

Many of the factors described above apply equally to ultrasonic nebulisers. Analogous to driving gas flow is the vibration amplitude and frequency of the piezoelectric crystal. In general, higher frequency nebulisers generate smaller particles, and increasing the nebuliser power (increasing the amplitude of the standing waves), increases the mass output of the nebuliser.

Residual volume affects ultrasonic nebulisers in the same way as jet nebulisers, except that drug solutions are not concentrated as much by solvent evaporation during nebulisation, lessening slightly the effect of larger residual volumes. A more recent ultrasonic nebuliser design, the Omron, described above, has an extremely small dead volume.

Solution characteristics affect ultrasonic nebuliser output. Highly viscid solutions do not form standing waves as easily and are nebulised poorly. Suspensions are also nebulised poorly, perhaps because drug particles in suspension are vibrated away from the area of droplet generation.

Variation between types of nebuliser

Different commercially available nebulisers vary in the size of droplets they produce, and in their nebulisation rate. One study (Nerbrink and Dahlback, 1994) showed that the droplet size of six commonly used nebuliser chambers varies from less than one to more than ten μm, with most in the range 4-6μm. The output of the nebuliser chambers under the test conditions varied by a factor of four.

Variation between nebulisers of the same type and 'nebuliser ageing'

There is a large variation in output between different nebulisers of the same type (Ryan *et al.*, 1981; Merkus *et al.*, 1992; Alvine *et al.*, 1992; Hollie *et al.*, 1991), even when used in the correct way, due to manufacturing. If the jet is blocked with dirt or drug crystals, nebuliser output will be diminished. Repeated use of a singe nebuliser over time may cause a change of the critical points of droplet generation, most significantly the small increases in the diameter of the air orifice. This 'ageing' may be due to mechanical wear from the compressed air source or to excessive cleaning. Depending on the compressor characteristics, increasing the effect of increasing the air orifice diameter may be to decrease driving pressure. With decreasing driving pressure the air velocity is lower, resulting in an increase in droplet size, due to decreased inertial filtering, and output (Merkus *et al.*, 1992). To maintain stable generation of aerosol the driving pressure should be kept constant despite the resulting increase in volumetric flow.

Servicing of compressors:

Servicing is essential to ensure reproducible drug output. Poor compressor and/or nebuliser performance should be included in the `differential diagnosis` of possible causes of failure of nebulised drug treatment.

Factors affecting nebuliser output are inextricably linked with those affecting particle size and nebulisation time. For instance, nebulisers designed to deliver small particles may use extensive internal baffles. This increases the residual volume in the nebuliser, decreasing overall output, and increasing recirculation of the nebuliser solution, lengthening nebulisation time. Increasing the volume fill improves overall output, but lengthens nebulisation time. Increasing driving gas flow rate may help, but for home use a more expensive, higher performance compressor may be needed. Manipulation of the factors discussed above can alter drug output dramatically.

Patient factors affecting drug delivery (see also Chapter 4.1):

Breathing pattern and tidal volume

In conventional jet nebulisers, the aerosol is carried in a volume of air dependent upon the driving gas flow. If this is, say, eight litres per minute, the patient will breath in approximately three litres of aerosol each minute (assuming an inspiratory:expiratory ratio of 2:3). If the patients inspiratory flow is greater than the driving gas flow, air will be entrained, diluting the aerosol (Figure 10).

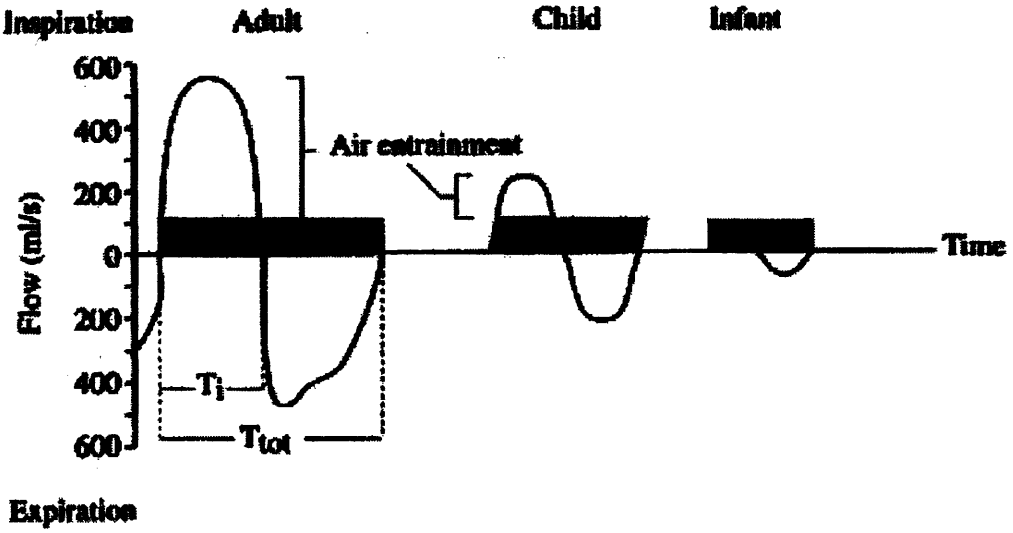


Figure 10. Adapted from Collis *et al.*, 1990.

The effect of this is that infants and children inhale a much larger dose than adults when computed as dose per kilogram (Collis *et al.*, 1990). Once over six months of age, the dose inhaled remains static, and the per kilogram dose inhaled is reduced (Le Souef, 1992). Use of an aerosol holding chamber may reduce the dilutional effect of air entrainment (O'Doherty *et al.*, 1990; Hill, 1988).

Breathing pattern may also affect deposition. Fast inspiration encourages inertial impaction of drug in the upper airways, and more central deposition (Dolovich *et al.*, 1981). Slow inspiration lessens the threshold levels of response in bronchoprovocation tests (Laube *et al.*, 1992; Cardellicchio *et al.*, 1989), possibly due to increased lung delivery. Breath holding at the end of inspiration increases deposition by giving time for particles to settle with gravity, but will be impractical with continuously running nebulisers.

Drug suspensions and solutions

A true molecular solution is defined as a mixture of two or more components which form a homogeneous molecular dispersion in a one phase system. They can be divided into aqueous solutions and non-aqueous solutions. Aqueous solutions, using water for injection or purified water, are the most widely used vehicle for pharmaceutical purposes. Solubility, if poor, may be enhanced by addition of a co-solvent (a water

miscible agent in which the drug is freely soluble), appropriate control of pH and if necessary addition of surfactants. A number of additives may also be included for various purposes. For example, buffers to increase buffering capacity such as citrates, acetates and phosphates. Preservatives may be required such as EDTA and benzylkuronium chloride. Their use and the acidity and tonicity of nebulised solutions have been linked to bronchoconstriction in some patients (O'Callaghan *et al.*, 1986; Beasley *et al.*, 1987).

A suspension is a dispersed system in which insoluble solid particles are dispersed in a liquid medium, often water. Suspensions can be divided into colloidal suspensions, in which the drug particles are less than 1μm in diameter and coarse suspensions in which they are greater than 1μm in diameter.

Suspensions may also be divided into flocculated and deflocculated suspensions (Edmond and Man, 1994). Companies usually try to formulate the former type. In a deflocculated suspension the particles are dispersed as concrete units and sediment down slowly forming a distinct cake in the bottom of the vessel. This cake is difficult to redisperse, and should it form, the suspension is unlikely to be of use. The use of flocculated systems in which the particles form larger aggregates, or flocks, are preferable. These sediment down more rapidly, do not form a clear cake in the bottom of the vessel and are easy to redisperse so that the suspension may be used again.

The production of a flocculated suspension requires the addition of electrolytes, normally potassium chloride or sodium chloride, to reduce or to neutralise the zeta potential around the compound. This means that repelling forces between particles are neutralised and the particles can then interact with each other to form large aggregates which rapidly sediment and are easily redispersed,

It is important to remember that particles of drug within a suspension acquire a 'fluid envelope' during nebulisation, resulting in larger droplets of drug than may be anticipated. Depending on the nebuliser used and the initial size and shape of the drug particles in suspension, droplets containing drug may be too large to escape the nebulisers' baffle system, resulting in very poor output, or drug may be released in large droplets which have a high chance of upper airway deposition. In either case therapy may be ineffective (O'Callaghan, 1990)

Summary

Jet and ultrasonic nebulisers remain of great value in inhalational therapy. Using the appropriate device and conditions it is possible to nebulise virtually any drug and in almost any dose.

Nebulisers are not all the same and vary greatly in the size of droplet they produce, their nebulisation time and drug output. This may have a marked effect on the therapeutic response. Similarly one cannot assume that different drugs nebulised within the same nebuliser, under identical conditions, will have identical output characteristics.

When nebulisers were principally used to deliver bronchodilators, such as salbutamol, scientific evaluation was relatively limited. With such a small airway dose required to achieve maximum bronchodilatation, variation in drug delivery between devices was regarded to be less critical. By contrast, use of very expensive nebulised drugs, such as DNase, and the use of other drugs with important side effects such as nebulised steroids, demands greater knowledge of the delivery systems used.

Evaluation of inhalational therapies

"Why spend $50,000 on a pile of black boxes which often go wrong when for the same money you can get ten years out of a prettier and replaceable assistant who can also make coffee?"

K.R. May, 'A personal note on the history of the cascade impactor' (1982).

Particle size in this thesis generally refers to *aerodynamic particle size*, meaning the size of a spherical, unit density particle that settles with the same velocity as the particle in question. Clearly a very dense particle will have a different aerodynamic behaviour (an increased aerodynamic size) to an equivalently sized but, less dense particle.

Aerosols produced by medical nebulisers and metered dose inhalers are heterodisperse (Mercer, 1973), that is made up of particles of different sizes. Their particle size distribution may be described statistically (Hinds, 1983). Most therapeutic aerosols are not normally distributed (the mean and median values are not the same, and the particle size distribution curve is not symmetrical about the mean). They usually conform to an approximately log-normal distribution. One way of describing such a distribution is in terms of the mass median aerodynamic diameter (MMAD) and Geometric Standard Deviation (GSD).

Aerosols - some definitions

An **Aerosol** - a two phase system made up of a gaseous continuous phase, usually air, and a discontinuous phase of individual liquid or solid particles.

Mass Median Diameter - the diameter of a particle such that half the mass of the aerosol is continued in smaller diameter particles, and half in larger.

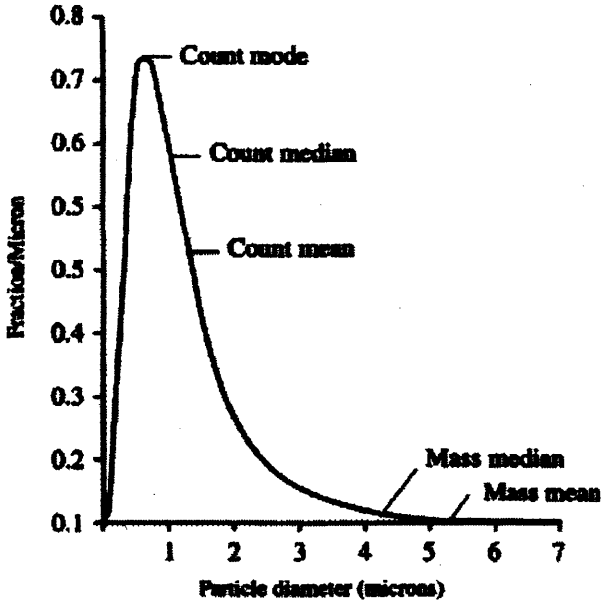
Mass Median Aerodynamic Diameter - the diameter of a sphere of unit density that has the same aerodynamic properties as a particle of median mass from the aerosol.

Geometric Standard Deviation - a dimensionless number which gives an indication of the spread of sizes of particles that make up the aerosol. An aerosol with a GSD of 1 is made up of particles of the same size.

Heterodisperse Aerosol - the aerosol is made up of particles of many different sizes. The GSD is >1.2.

Monodisperse Aerosol - the aerosol particles are all the same (or very nearly the same). The GSD is <1.2

Perhaps a more useful way of describing the aerosol cloud is to determine the total amount of drug contained in particles leaving the drug delivery device and the amount of drug contained in particles smaller than a certain size. Although the drug contained in particles less than 5μm diameter is described as the 'respirable' dose (Lippmann *et al.*, 1980), current evidence to support the idea that all such particles are truly respirable and mainly deposit in the lower respiratory tract is not conclusive. It has been suggested that drug contained in particles smaller than 3μm should also be presented in the results of experimental projects (Dolovich, 1991). The issue of particle size is discussed further below.



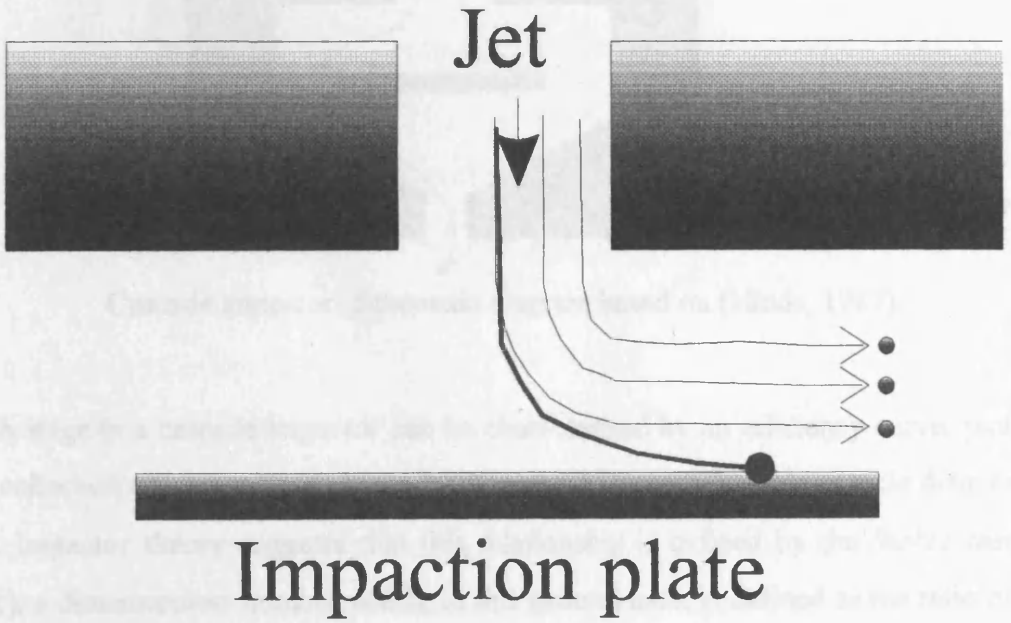
Two pitfalls may arise in the description of aerosol particle size. One is the use of MMAD and mass median diameter (MMD) as if they are interchangeable. MMD is reported by some light scattering devices (see below), and only describes the aerodynamic behaviour of particles if they are spherical and of unit density. The second, and more serious, pitfall is the presentation of **Count** Median Aerodynamic Diameter (CMAD), the diameter of the median **number** of particles in the aerosol cloud, as if it is equivalent to the MMAD. A ten micrometer diameter drug particle contains the same mass of drug as 1,000 particles of one micrometer diameter. Describing the **number** of particles of a certain size may therefore give a very distorted view of the **mass** of respirable drug obtained from a drug delivery device.

Current methodologies for measuring particle size

The two most commonly used methods of pharmaceutical aerosol particle size determination are inertial impaction devices and laser based light scattering devices. Unfortunately neither method is ideal, and each has its own drawbacks. Their results may not be interchangeable. Ideally comparisons of the amount of drug contained in particles of various size from different inhalational drug delivery devices should use identical measurement techniques.

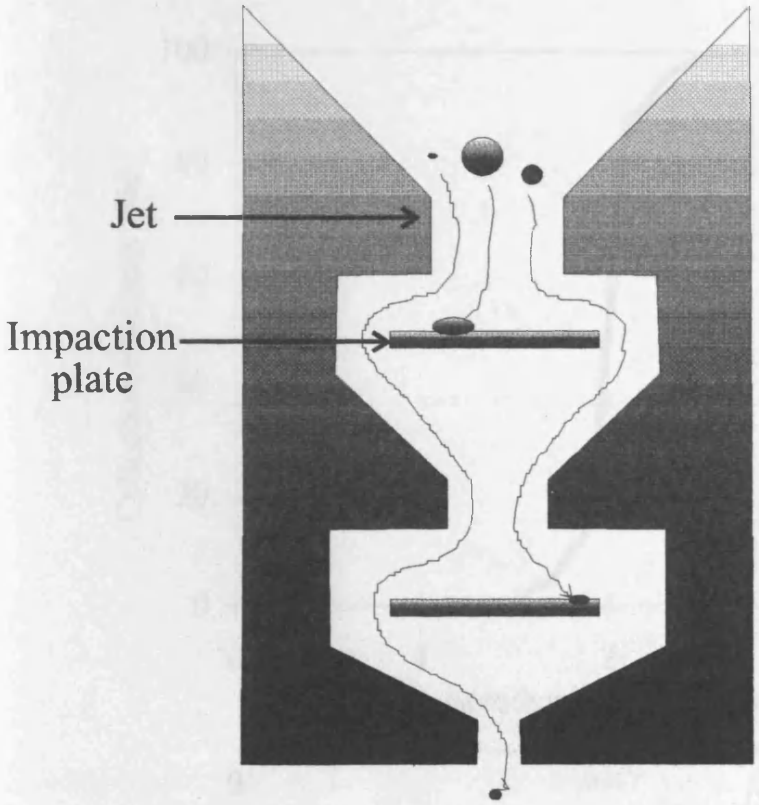
Inertial impaction

Impaction methods of particle size determination include the glass multistage liquid impinger (MSLI) (May, 1966) the Anderson impactor (Andersen, 1966), the high performance multistage liquid impinger (HPMLI) (Asking and Olsson, 1997) and the twin stage impingers described in the British Pharmacopoeia (1988).



Inertial impactors operate by drawing air through a nozzle or jet towards an obstruction, usually a flat plate called an impaction plate (figure). The airflow is forced to make a 90° turn in front of the plate. Aerosol particles with sufficient inertia are

unable to follow the airflow, and instead impact on the plate. Particles with less inertia follow the airflow and are directed around the obstruction. Thus the impactor separates aerosol particles into those larger and smaller than a certain size. *Cascade impactors* consist of a number of impactors (or stages) in series, each separating out sequentially smaller particles (figure).



Cascade impactor. Schematic diagram based on (Hinds, 1983).

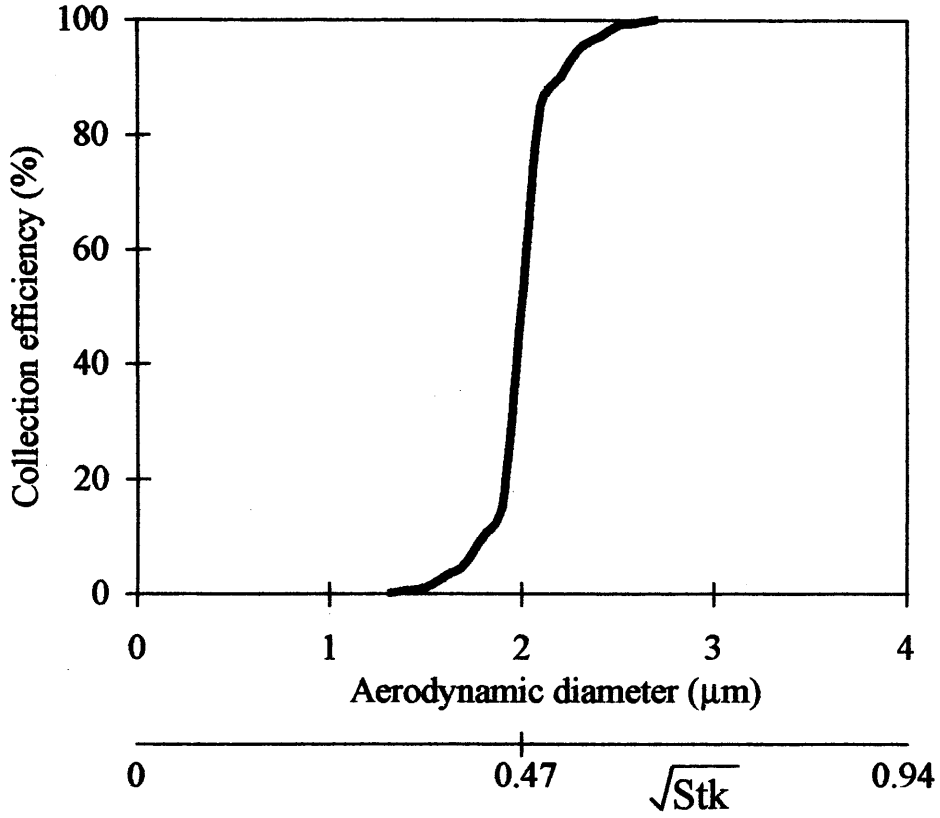
Each stage in a cascade impactor can be characterised by an efficiency curve, plotting the collection efficiency from 0% to 100% against the aerodynamic particle diameter in μm. Impactor theory suggests that this relationship is defined by the *Stokes number* (Stk), a dimensionless number which, in this general case, is defined as the ratio of the particle stopping distance at the average jet exit velocity U to the jet radius $D_j/2$:

$$\mathbf{Stk} = \frac{\tau U}{D_j/2} = \frac{\rho_p d_p^2 U C_c}{9\eta D_j}$$

Where τ is the relaxation time, U the gas velocity, and D_j the jet diameter. P_p is the

particle density, d_p the particle diameter, C_c the slip correction factor, and η the gas viscosity. Thus whether or not a particle of a certain size gets past the impactor is dependent on the (square of the) particle size and the particle density, the jet diameter, and the velocity of the air flow through the jet. For most purposes described here, gas viscosity and slip correction can be ignored (Vincent, 1989).

A typical impactor efficiency curve is shown in the figure.



An ideal impactor has a sharp efficiency or 'cut-off' curve, so that all particles greater than a certain size impact, and all smaller particles pass through. Most impactors do not reach the ideal, but it is assumed that particles larger than the cut off size which get past the impaction plate are compensated for by an equal mass of particles smaller than the cut off size that are retained. The efficiency curve of the impactor is thus defined by the Stokes number that gives a 50% collection efficiency, and, as the Stokes number is proportional to the particle diameter, by the particle size at which the particle has a 50% chance of being impacted (the $d_{50\%}$). This value is usually given in terms of the aerodynamic diameter (where P_p is equal to 1).

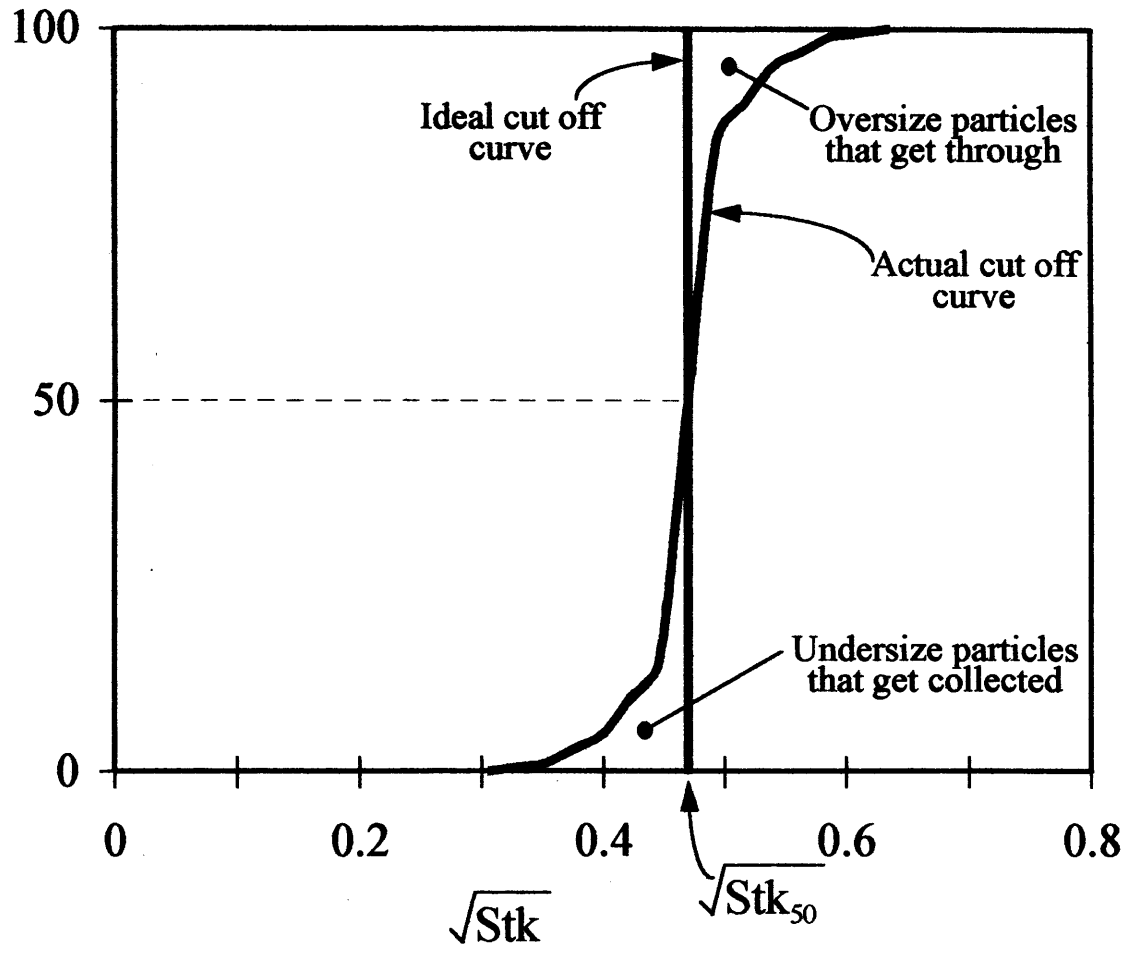


Figure: Actual and ideal impactor efficiency curves (Hinds, 1983)

In the cascade impactor, the stages are arranged in series, the stage with the largest cut off size first, then the next largest, and so on until a terminal filter captures all remaining (smallest) particles. The cut off size is reduced in each stage by decreasing the jet size or number. The same volumetric gas flow passes through each stage, and so as jet diameter decreases, gas velocity increases. Both these factors serve to decrease the $d_{50\%}$ of successive stages.

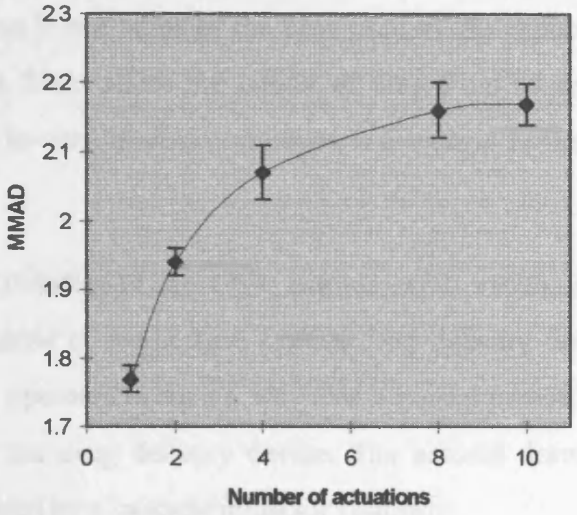
Each stage of the impactor is assumed to capture all particles larger than it's cut-off size that reach it, and the particles on a particular stage represent all particles in the aerosol larger than that stages cut-off diameter, and smaller than the cut-off diameter of the preceding stage. Any particle losses on the walls of the jet or elsewhere in the

impactor 'belong' to the impaction plate immediately below. Ideally these inter-stage losses are kept to a minimum, and in the multi-stage liquid impinger used in this thesis, errors due to wall losses are prevented by careful washing of the internal walls of the device to include inter-stage losses in the collected mass. High interstage losses may lead to errors with some impactors, such as the Andersen impactor (Phillips *et al.*, 1990).

It is assumed that particles which are caught by impaction plates stay there. Solid particles may bounce off, moving on to the next stage, and appearing smaller than they really are (Dzubay *et al.*, 1976). Liquid particles are rarely affected by particle bounce (Pak *et al.*, 1992), and irregularly shaped or soft particles are less affected than smooth, hard ones (Newton *et al.*, 1990; Hinds *et al.*, 1985). This problem can be overcome by coating the surface with a thin film of, for instance, silicone oil (Turner and Hering, 1987; Pak *et al.*, 1992), or bathing the impaction surface in a reservoir of solvent, as in the liquid impinger (May, 1966). When multiple actuations of a metered dose inhaler are actuated into the impactor, surfactants from the inhaler such as oleic acid are deposited on the impaction stages, and these reduce particle bounce (Nasr *et al.*, 1997). Bathing the impaction surface in a reservoir of solvent also solves the problem of stage overload, where if a large mass of aerosol particles are collected on a particular impaction stage, some may be blown off again (re-entrained), being collected further down the impactor and making the aerosol particles appear smaller than they really are.

High loading of impaction plates may modify the collection characteristics of the stage, favouring the premature deposition of particles, making them appear larger than they are (figure). This may be avoided by introducing the minimum aerosol sample possible consistent with the accuracy of the detection method. It has been suggested that multiple actuations of a salbutamol MDI into an Andersen cascade impactor have an apparently higher MMAD than a single actuation due to this effect (Nasr and Allgire, 1995). It must be remembered, however, that sampling a small mass of aerosolised drug may lead to large errors (Fuchs, 1978), especially in the calculation of MMAD and GSD from log-probability graphs. Also, the Andersen impactor has a series of

metal impaction plates, and the problems of stage loading and particle bounce may be less with other impaction surfaces such as the sintered glass of the MSLI.



The effect on MMAD of multiple actuations of a salbutamol MDI into the impactor prior to sampling. Data from (Nasr and Allgire, 1995).

Impactors operate at a constant air flow, typically driven by a vacuum pump, and this flow is critical in determining the cut off diameters for the different stages. Different flows may be used within certain limits (May, 1966), but the cut off diameters ($d_{50\%}$) for each stage are altered, according to the equation:

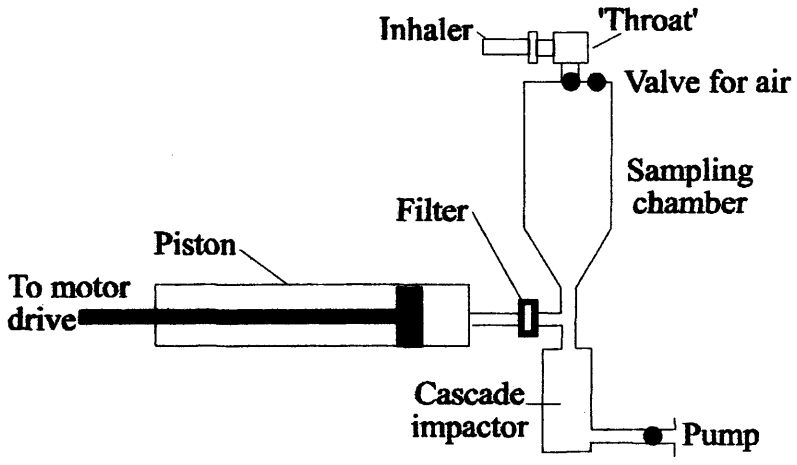
$$\text{New } d_{50\%} = \text{Old } d_{50\%} \times \sqrt{\frac{\text{Old flow (l/min)}}{\text{New flow (l/min)}}}$$

If the new flow is reduced too much, the stage cut offs may all be increased outside the range of interest for inhalational drug delivery (1-5μm). May overcame this problem by designing different sized MSLIs, the smallest being 3.5 inches high and operating at a flow rate of 10l/min (May, 1966). Other impactors are available that operate at different flows (Chan *et al.*, 1986)

Impactors operating at high flows and used to test inhalational drug delivery devices may be criticised on the grounds that the flows are 'unphysiological' (Phillips *et al.*,

1990), especially as recommendations on the optimal use of metered dose inhalers suggest a slow inhalation (see chapter 4.2). However, these criticisms miss the essential point that the impactor is attempting to measure the aerosol particle size, not imitate the lung. The question is not whether the flow used in the impactor is 'physiological' but whether different flows affect the output of drug from inhalational drug delivery devices. The issue of in-vitro/in-vivo correlation is discussed further below.

The electronic lung (Brindley *et al.*, 1994; Burnell *et al.*, 1998) is a device that uses a piston to 'inhale' a dose of medication from a drug delivery device into a sampling chamber. The piston operates in such a way that a typical patient's inspiratory flow is reproduced through the drug delivery device. The aerosol drawn into the sampling chamber is then sampled by a cascade impactor (figure).



The Electronic Lung (Brindley *et al.*, 1994).

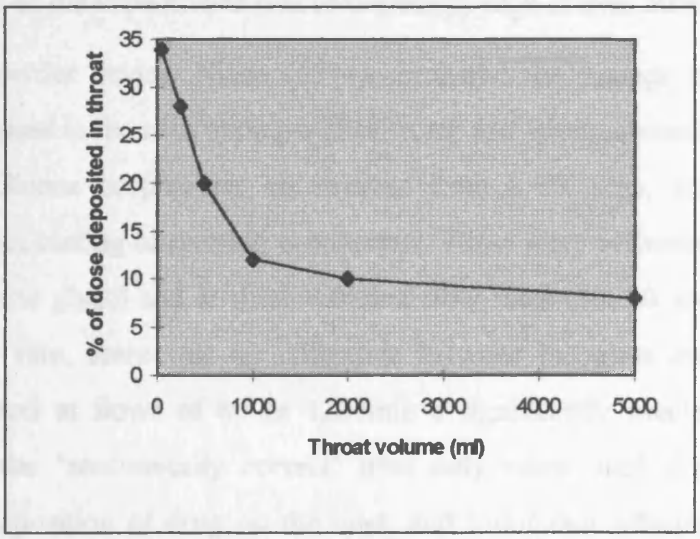
The device does not take into account conditioning of the aerosol particles that may occur in the sampling chamber, and is therefore less suitable for the evaluation of metered dose inhalers or nebulisers than dry powder inhalers.

The impactor inlet or throat affects the measured particle size. For metered dose inhalers, from which the aerosol is emitted at high speed, increasing the distance from the point of actuation to the right angle bend or point where the airflow turns into the impactor increases amount of drug entering the impactor. This distance also allows the aerosol particles to mature, as propellants and (in the case of nebulisers) solvents

evaporate, reducing particle size.

The inlet for the MSLI consists of a glass, right angled tube, approximately 16cm in length and 2.5cm diameter; that for the twin impinger a sphere 50ml in volume; and for the Andersen impactor a metal cylinder, 1.9cm diameter and 20cm in length, with a 90° bend in it. In the past considerably larger inlets have been recommended (Van Oort *et al.*, 1994), although the current recommendation from the US Pharmacopoeia is for the Andersen impactor to be used with the metal inlet (United States Pharmacopeia previews, 1996). The calculated cut off diameter for the MSLI throat is approximately 20μm (Hallworth and Andrews, 1976), based on data for aerosol speed from Rance (1974). Increasing flow through the throat *decreases* throat deposition from a metered dose inhaler, but most of the decrease occurs between 0 and 17.5l/min, with a smaller rate of decrease at higher flows (Hallworth and Andrews, 1976).

Van Oort (1994) compared the particle size output of salbutamol metered dose inhalers and mass of drug depositing on spherical glass inlets of various sizes from 50ml to 5,000ml used with the Andersen impactor. The inlet deposition varied with inlet volume (figure), but was almost constant at volumes over 1,000mls. However, distance from the metered dose inhaler to the wall of the inlet was not independently varied, and this, rather than inlet volume, could be the significant factor.



Surprisingly, Van Oort (1994) found no difference in MMAD with increasing inlet volume up to 1,000mls, but an *increase* in MMAD with inlet volumes greater than this. Intuitively, one would assume that measured particle size would *decrease* with increasing inlet volume, as the aerosol would take longer to reach the inlet wall, allowing propellants to evaporate, reducing particle size. The authors suggest that the

long residence time of aerosol in larger inlets allows particles to re-aggregate due to electrostatic attraction, but present no evidence for this.

Using a metered dose inhaler containing jet milled disodium fluorescein, Fults (1991) investigated the effects of changing inlet size and volume on the amount of drug collected from metered dose inhalers in particles smaller than 5.8μm and 6.4μm (which they termed the 'respirable fraction') using the Andersen impactor and the twin impinger (Hallworth and Westmoreland, 1987) respectively. Four inlet chambers were used, from 40ml to 1,000ml volume, with a distance from the metered dose inhaler inlet to the first impaction surface from 5cm to 18cm. The 'respirable fraction' increased with increasing distance from the metered dose inhaler to the impactor, but was not affected by increasing inlet volume, where the distance was kept constant. The authors also investigated the effect of different inlets on the measured particle size of four different salbutamol inhalers, but found no consistent change in either respirable fraction or MMAD. The results of this study must be treated with caution, as only 3 repeat experiments with each metered dose inhaler and inlet were undertaken, and the coefficient of variation of some of the experiments was astonishingly high at over 50%.

Using the Rotahaler, a dry powder device, Niven (1994) compared the passage of aerosol through the glass inlet used in the twin impinger (Hallworth and Westmoreland, 1987), with that through a silicone oropharynx, constructed from a CT scan, and (presumably post-mortem) direct casting of an adult oropharynx. These were evaluated 'dry' or coated with polyethylene glycol and at three different flow rates (30, 60 and 120l/min). At the lower flow rate, there was no difference between the glass and 'anatomically correct' inlets, and at flows of 60 or 120l/min a significantly smaller passage of powder through the 'anatomically correct' inlet only when used dry. Coating the inlets increased deposition of drug on the inlet, and this factor affected drug passage more than the inlet type.

The penetration characteristics of aerosol through a similar anatomical model were determined with a variety of metered dose inhalers (Miller and Purrington, 1996). In contrast to Niven, there was no change in inlet deposition of drug from a metered dose inhaler when the inlet was coated with mineral oil, and no effect of high ambient temperature or humidity on aerosol penetration. Manipulation of the anatomical throat

to mimic changes in the larynx and upper airway altered the passage of aerosol greatly, and may explain some of the intersubject variability in inhalational drug delivery.

The penetration of six different metered dose inhalers was then studied through four inlets - the rubber anatomical throat, a metal inlet designed to have the same penetration characteristics as the anatomical throat, a glass inlet similarly shaped but slightly smaller in diameter than the MSLI inlet, and the metal inlet recommended in the United States Pharmacopoeia (1996) used with the Andersen impactor. Although there were differences in the absolute values, the four inlets gave remarkably similar results in terms of ranking the formulations, only differing in their assessment of a metered dose inhaler containing HFA propellants and ethanol. In this case the results from the USP and glass inlets were similar.

Dolovich (1995) measured the particle size output from salbutamol metered dose inhalers assessed by the Andersen impactor with each of seven different inlets. The amount of drug recovered from the impactor in particles smaller than 5.8μm varied by a factor of three depending on which inlet was used. Deposition of aerosol in the inlet was dependent upon distance from the metered dose inhaler to the facing inlet wall, rather than the inlet volume.

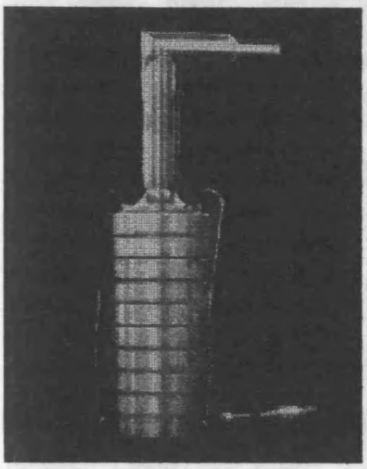
Liquid impingers were first developed in the 1940's for sampling air for bacterial content (Tyler and Shipe, 1959). The use of liquid in the impaction area increases bacterial cell viability by reducing desiccation, and is not essential for the collection of non-viable particles. In this thesis the glass multistage liquid impinger (MSLI), as described by May (1966) and modified by Bell (1973), was used to measure the particle size of various aerosols. This device is fully described in chapter 5.1, but in brief, the MSLI operates by drawing the aerosol through a series of stages, each containing a sintered glass impaction plate and connected by progressively smaller jets. Aerosol velocity therefore increases through each jet resulting in the deposition of smaller particles at each subsequent stage. A filter after the final stage collects the smallest particles. After an experiment, each stage (including the internal walls) is washed and the amount of drug collected in each stage assayed. May (1966) originally

described a three stage impinger, which was modified by Bell (1973) to include an additional stage prior to May's stage one, and a right angled inlet 'throat', 2.5cm in diameter, as suggested by Goddum (1964).

The MSLI determines aerodynamic particle size, and allows the amount of drug contained in particles below a certain aerodynamic size to be computed. The high airflow may be expected to dry out aqueous particles, in contrast to the high humidity of the respiratory tract, and the size of particles produced from aqueous solutions may be underestimated, although there is little experimental evidence for this supposition, and some to suggest that hygroscopic growth of particles from the MDI in high humidity air increases measured particle size by less than 10% (Kim *et al.*, 1985). Adding solvent to the MSLI stages allows measurements to be performed at high humidities, and may reduce this error (Cipolla *et al.*, 1994), which may be important when particles generated by nebulisers or dry powder inhalers are measured.

The MSLI is calibrated by passing an aerosol of known particle size through it, and computing the cut off diameters, above which particles are deposited, for each stage. Owing to the original construction of different impingers, different devices of the same type may give different results with the same aerosol (Phillips *et al.*, 1990; Hallworth and Westmoreland, 1987; Zainudin *et al.*, 1989), and devices, especially the glass impingers such as the twin impinger and MSLI, should be individually calibrated. Calibration of the MSLI is discussed further in chapter 5.1

The 'high performance multistage liquid impinger' (HPMLI, Copley Instruments, Nottingham, UK) is based on the MSLI, and consists of four stages connected by metal, rather than glass, jets. The tangential fourth stage inlet has been replaced with a seven nozzle jet (United States Pharmacopeia previews, 1996). The HPMLI operates at a flow of 60l/min, although it has been calibrated at other flow rates (Asking and Olsson, 1997).



The Andersen impactor (Andersen Samplers Inc., Atlanta) (figure) is an all metal cascade impactor consisting of eight stages and a terminal filter (Andersen, 1966; Vaughan, 1989; Mitchell *et al.*, 1988). It is sometimes used with a pre-separator, and has a metal inlet, described above, although it may also used with a glass, spherical inlet. The impactor operates at a flow of 28.3l/min (1cfm) and is widely used as it allows reasonable discrimination of particles in the size range of interest for inhalational therapy. It has been suggested that the factory supplied calibration is incorrect (Vaughan, 1989), and the impactor should be individually calibrated, rather than relying on calibration based on first principles from the impactor design. An amendment to the Andersen impactor has been described that allows it to operate at different flow rates (Nichols *et al.*, 1998).

Two further devices are described in the British Pharmacopoeia (1988), the all glass twin impinger (Hallworth *et al.*, 1978; Hallworth and Westmoreland, 1987) (Copley Instruments, Nottingham, UK), and the metal single stage impactor (Fisons Research and Development, Loughborough, UK).

The twin impinger consists of a glass inlet, some 50ml in volume, and two impinger stages. Each stage contains solvent (6mls and 30mls in stage 1 and 2 respectively). The twin impinger is easily constructed from readily available scientific glassware and operates at a flow of 60l/min. It has been shown to have a sharp collection efficiency curve, and within certain limits, changes in liquid volume or composition in the stages have little effect on its operating characteristics (Miller *et al.*, 1992). The twin impinger does not have a terminal filter, and the smallest particles are not captured. For particles with an aerodynamic diameter of 1.6μm, approximately 5% are not captured at this stage (Miller *et al.*, 1992).

The metal impactor consists of a narrow metal inlet, 19mm in diameter and approximately 20cm long, with a 90° bend. The inlet opens to a glass sintered disc impaction plate held in a metal chamber. Aerosol passing the plate is drawn through a terminal filter unit entering the side of the chamber. The impactor has a single cut off of 9.8μm when operated at a flow of 60l/min, larger than the currently held 'ideal size' for respiratory drug delivery (1-5μm), and is rarely used now.

In contrast to the other devices described in this section, these latter two devices are dichotomous samplers - they merely divide the aerosol into particles larger or smaller than a certain size, without giving any information on the distribution of particle sizes within the aerosol. Aerosols with widely differing MMAD and GSD could produce the same amount of drug in the upper and lower stages of the impinger (Miller *et al.*, 1992). The twin impinger is also unable to distinguish between aerosols whose particles are predominantly smaller than 6.4μm, even if, for instance, one aerosol is made up of particles 6μm in diameter, and the other 3μm (Kenyon *et al.*, 1995). A similar criticism may be made of the four stage liquid impinger, compared to the eight stage Andersen impactor, especially if differentiation between particles smaller than, for instance, 3μm and between 3μm and 5μm is felt to be important. However, increasing the number of stages may paradoxically *decrease* the resolution of a cascade impactor This occurs where the stage efficiency curves overlap, as a given particle could be deposited on a number of stages. Using a device with a large number of stages is also labour intensive and time consuming.

Evaluating the same metered dose inhaler aerosols gives similar results for the HPMLI and twin impinger (in terms of percentage of drug smaller than 6.4μm), but different results comparing these devices against the metal impactor or the Andersen impactor (Holzner and Muller, 1995). It is clearly important to be cautious when comparing results of studies using different particle sizing devices.

Other inertial impaction devices have been used to characterise pharmaceutical aerosols, including the Marple Miller Cascade Impactor (MSP Corporation, Minneapolis), the Casella Cascade Impactor (CF Casella & Co. Ltd.) (Hallworth and

Andrews, 1976) and the Delron DCI-6 cascade impactor (Delron Research, Powell, Ohio) (Phillips *et al.*, 1990).

Use of particle size measurements to estimate lung deposition are hampered by large inter-laboratory differences in some measurements. Smaldone (1988) measured the particle size output from Respigard and Fisonneb nebulisers, and reported values of 0.8μm and 2.5μm MMD respectively, whereas Thomas (1991) found values of 2.1μm and 5.2μm respectively. Differences can often be accounted for by slight differences in experimental procedure. For instance, results of experiments with spacer devices and an inertial impactor reported from an aerosol research group in Perth, Western Australia, show consistently higher delivered drug masses than experiments with the same devices in the author's studies. In the Perth laboratory, the spacer is attached to the impactor, with the vacuum pump on, when aerosol is actuated into the spacer, whereas in our experiments, the aerosol is actuated into the spacer which is then attached to the impactor (see Chapter 5.1).

Low angle laser light scattering.

Laser diffraction devices, such as the Malvern Mastersizer (Malvern Ltd, Malvern UK), work by passing a laser beam through the aerosol cloud. Particles diffract the light at an angle inversely related to their diameter. This diffracted light is sensed by the machine on one of 30 concentric ring detectors, each representing a particular particle size band. The machine uses light scattering theory to compute the volume of particles present in each band, and from this the particle size distribution.

Aerodynamic particle size is not determined by this method, and all particles produced by the nebuliser that pass through the laser are measured, whether or not they contain drug. This is acceptable for a drug solution but may give erroneous results for a nebulised suspension. With suspensions the laser diffraction device may describe a seemingly excellent particle size distribution, without detecting that only a few of the particles measured, usually larger ones, contain drug. Metered dose inhalers are more

difficult to examine by laser diffraction, as the rapid evaporation of propellants affects the light scattering properties of the aerosols, and leads to erroneous results. Positioning of the aerosol relative to the laser beam is important, as moving the aerosol cloud away from the detection lens in either direction will lead to an initial increase in the measured particle size (Clark, 1995). For comparative experiments especially, it is important that the relative positions of the aerosol and detection lens are maintained and are reported.

Early laser diffraction devices, such as the Malvern 2600, used Fraunhofer theory to estimate particle size from the light scattering pattern. This only approximates particle size for particle smaller than 50μm, and only takes into account diffraction of light at the contour of the particle, assuming it to be completely opaque. Newer devices, such as the Malvern Mastersizer use the more complex Mie theory, which also incorporates the effects of the refraction of light at the boundary between the particle and surrounding air, reflection at the particle surface and absorption of light by the particle. However, to accurately use these instruments, it is important that the refractive index of both the continuous and particulate phase of the aerosol is known or can be estimated.

Results from different laser particle sizing devices (and even different versions of the computational software) are not necessarily interchangeable with each other. The machine and software version used should ideally be reported.

There is good correlation between aerosol particle size measured with the Malvern Mastersizer X and the Andersen impactor or the MSLI (Clark, 1995), at least for non volatile aerosols. However, as described above, aqueous aerosols may desiccate in the impactor, and laser based particle size data may therefore be larger.

Other methods

Optical microscopy has been used to characterise pharmaceutical aerosols in the past (Rance, 1972), but is labour intensive, time consuming and only samples a small part of the aerosol. Computerised image analysis may be used to aid microscopy (Hallworth and Barnes, 1974; Hallworth and Hamilton, 1976)

Aerodynamic particle counters, such as the Aerodynamic Particle Sizer (TSI, St Paul, Minnesota), also known as 'time of flight' instruments, use two parallel laser beams to measure the falling speed of an aerosol particle. Aerosol is introduced into a large holding chamber, and drawn into a jet which divides into an inner and outer tube. Only a small percentage of the aerosol is sampled by passing through the inner tube, drawn in a flow of clean air across two parallel laser beams, momentarily obscuring them. The time between obscuration of the two beams is proportional to the falling speed of the particle, which is dependent on the particle size and density. If data on the aerosol particle density is known, or can be estimated, a computer linked to the particle sizer computes aerodynamic size. Liquid droplets larger than a few micrometers diameter are undersized by the Aerodynamic Particle Sizer, due to distortion of the particle from spherical (Baron, 1986). The device also underestimates the size of non-spherical solid particles (Marshall *et al.*, 1991), and those of high density (Tsai *et al.*, 1998) although these factors may be corrected for by the computer software.

Measurement of nebuliser output

The mass of aerosol released during nebulisation has in the past been measured simply by weighing the nebuliser before and after nebulisation (the gravimetric method; Kradjan and Lakshminarayan, 1985; Douglas *et al.*, 1986). This is highly inaccurate for jet nebulisers, overestimating the drug output as weight loss due to evaporation is not taken into account (Thomas *et al.*, 1988). The use of weight loss as a measure of aerosol output leads to an inaccurate assessment of nebuliser function, and can only be used if the concentration of drug remaining in the nebuliser at the end of nebulisation is also measured. This is then multiplied by the residual volume to give the mass of drug remaining in the nebuliser, which is subtracted from the mass of drug placed in the chamber at the start of nebulisation to give the output, expressed as percentage mass emitted (Newman *et al.*, 1985; Smith *et al.*, 1992).

An alternative in vitro method of assessing nebuliser output is to collect the aerosol output onto a filter, or into an impactor device, and assay the drug or a chemical tracer

added to the nebuliser solution (Dennis *et al.*, 1990). If the latter is undertaken, it must be certain that the tracer behaves in the same way as the drug to be nebulised. In a laboratory situation the most ideal method is to collect the aerosol leaving the nebuliser, assay the amount of the specific drug present and perform particle size analysis as described above. The importance of taking breathing patterns into account when evaluating nebulised drug delivery to the patient is discussed below.

Breathing simulation

Smaldone (1994) coined the term "reality testing" of nebulisers which takes into account the breathing pattern of the patient, which when combined with other basic information, such as particle size, gives a much more accurate reflection of the amount of drug a patient will receive. The importance of reality testing was illustrated by recent studies of nebuliser output using different measurement techniques. The results of an extensive comparison of different nebulisers on their ability to nebulise antibiotic solutions were reported as the percentage mass released from the nebuliser (Hurley *et al.*, 1994). This would appear at first sight a reasonable expression of the nebulisers ability to deliver drug. Using this measurement the Marquest Respigard (6ml volume fill) released 80% of its mass, which may suggest the patient would receive most of the drug placed in the nebuliser. However, these results were obtained using a gravimetric technique, incorporating osmolality measurements, with the assumption that osmolality is directly proportional to drug concentration (see chapter 6.12). A more acceptable technique for assessing output involves collecting the aerosol released from the nebuliser on a filter and assaying the amount of drug collected. This method gives much lower nebuliser efficiencies, at approximately 30%, than those reported using gravimetric methods (McPeck *et al.*, 1993). Both studies fail to take into account the breathing pattern of the patient. Using a breathing simulator with a tidal volume of 750ml, at 20 breaths per minute and a duty time of 0.5, Smaldone et al (1991) found that only 10% of the drug placed in the nebuliser would reach the patient. Breathing patterns are clearly important, as these results are much closer to clinical deposition studies which suggest that approximately 5% of drug placed within the Respigard will be deposited in the patient (Smaldone, 1994). Refinement of in-vitro techniques may

result in an even closer correlation with actual lung deposition, measured by radioisotope deposition and pharmacokinetic methods.

Lung deposition measured by radiolabelled aerosols

Gamma scintigraphy has been used to provide graphical representations of inhaled radio-active particles from a number of drug delivery devices. Results from radiolabeled deposition studies will include the effects of factors other than particle size which are important in determining drug deposition, such as the calibre of the airways and breathing pattern of the patient. Imaging may be two dimensional (planar) or may involve complex three dimensional imaging (ie SPECT scanning).

Problems with scintigraphy include; ensuring that the radiolabel has the same deposition pattern as the drug; quantifying deposition; differentiating between 'central' and 'peripheral' lung deposition; and, in children especially, concern over the use of radioactive substances for non-therapeutic purposes (Everard, 1994).

Radiolabeling of drug preparations may be achieved in a number of ways. With nebulised solutions, radionuclide is simply mixed with the drug solution, and it is assumed drug and radiolabel are present in the aerosolised droplets in proportion to their concentration in the nebuliser. This method is not suitable for nebulised suspensions, as significant amounts of radiolabel may be present in small droplets of solute that contain no drug particles.

The labelling of drug in metered dose inhalers, typically micronised drug particles suspended in a mix of propellants and surfactants, is more complex. It is unusual for the radiolabel itself to be incorporated into the drug molecule (an exception being the labelling of ipratropium bromide with ^{77}Br (Spiro *et al.*, 1984)), but rather it is either loosely bound, or bound to another molecule which, it is hoped, will behave similarly to the drug molecule in vivo.

Early studies used ^{99m}Technetium bound to Teflon or albumin particles (Newman *et al.*, 1984; Newman *et al.*, 1981). These would be dispensed into metered dose inhalers to

which surfactant and propellant mix were added. Although the size of these particles was thought to be similar to the drug crystals when they were placed into the metered dose inhalers, they may not be aerosolised in the same way (owing to the different mix of propellants and excipients) and may not behave the same way as drug particles in the respiratory tract.

An improvement was the method described by Kohler (1988), in which ^{99m}Technetium is added to the metered dose inhaler and is absorbed onto the drug/surfactant particles in a way that depends on the physico-chemical properties of the drug, surfactants, propellants and other excipients present in the metered dose inhaler (Farr, 1996). Kohler's original method added sorbitan treolate and trichlorofluoromethane to the metered dose inhaler as well as the radiolabel, which may affect the aerosol particle size distribution. Subsequent workers (Newman *et al.*, 1989; Summers *et al.*, 1990) improved the method to overcome this, and demonstrated good association of particle size in vitro between the drug and radiolabel. Unfortunately, this association between one drug and formulation is no guarantee of similar association with other drugs. Using an Andersen impactor, Clarke (1992) investigated the particle size output of drug and radiolabel from different formulations of metered dose inhalers. There were significant differences in the particle size distribution of drug and radiolabel from one of the formulations tested, with the radiolabel being associated with the smaller surfactant particles rather than the drug. A knowledge of the physico-chemical properties of the particular drug and surfactants present in the MDI may help to predict similar problems (Farr, 1996; Dolovich *et al.*, 1991), but is no substitute for validation experiments demonstrating concordance of the particle size output of drug and label (Farr, 1996).

The amount of drug deposited in the lungs from a scintigraphic study is normally reported as a percentage of the administered dose - the total activity from all parts of the experimental apparatus, including the patient, at the end of the study. Thus two different devices, or the same device under different conditions, may give the same distribution of radiolabel (i.e. the same percentage delivered to the lungs), but a significantly different mass. Attempts to quantify the absolute mass of drug represented by the level of radioactivity from a gamma camera image are complicated by

attenuation and scatter of the gamma rays by the body and the distance between the patient and the counter. Older studies may not have corrected for tissue attenuation, and different correction factors may be used by different laboratories, making comparisons of different studies difficult (Dolovich, 1993). Whichever method is used, individual correction factors for each gamma camera and patient should be calculated.

An estimate of the pattern of radiolabel deposition within the lung may be obtained by measuring the radioactivity in an arbitrarily defined 'peripheral' region, and dividing it by the activity in an equally arbitrary 'central' region. Methods of measuring this 'penetration index' have not been standardised, and may vary between investigators. Because of the two dimensional nature of scintigraphic scans, the 'central' region contains both large conducting airways, and small bronchi and alveoli, blurring the differentiation between the two regions. Also, it is not entirely clear whether the differentiation of central and peripheral deposition is important. It may appear logical to assume that the efficacy of an aerosolised drug should be related to the local airway dose. In some studies, however, although regional distribution of a radiolabelled aerosol throughout the lung has been carefully determined it has not been related to clinical efficacy of the drug (Smaldone *et al.*, 1994).

Exceptions include a study by Clay et al (1987) who found nebulised bronchodilators achieve better lung deposition with smaller particles (1.8μm) than larger particles (10.3μm) and a correspondingly greater increase in bronchodilatation. Although lung deposition may be optimised by using drug particles of 2-5μm aerodynamic diameter it has been difficult to show that targeting specific airways is crucial for bronchodilator efficacy, as such small doses are required for maximum bronchodilatation. Differences in therapeutic effect of bronchodilators delivered in particles between 2-5μm in size appear to be small. This may not be so with other drugs such as steroids and antibiotics which have a different therapeutic ratio. Research is being undertaken to analyse the dose response relationship of deposited aerosols in terms of clinical response to the drug (Smaldone *et al.*, 1994).

Use of a new technique, single photon emission computed tomography (SPECT) (Perring *et al.*, 1994) provides three dimensional images of an inhaled radio-aerosol, and may be more sensitive than planar scintigraphy in determining distribution of

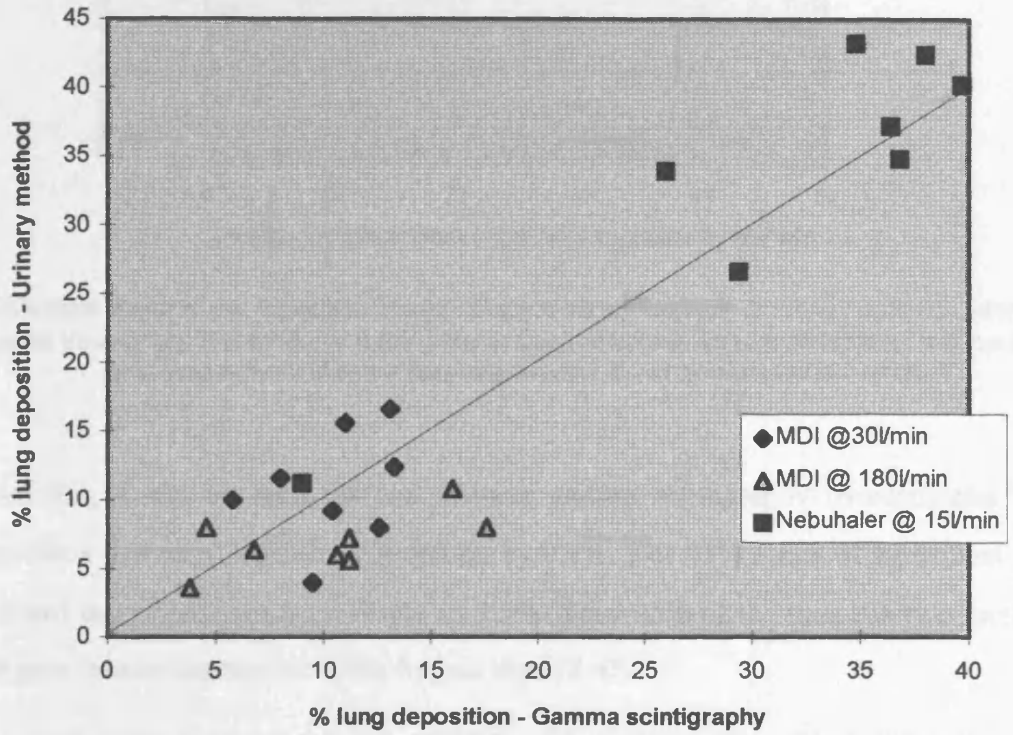
aerosol throughout the lung. Distribution of a range of monodisperse aerosols throughout the lung by SPECT is awaited with interest and may add to information provided by the Task Group on Lung Dynamics and other models. SPECT scanning involves higher radiation doses and longer image acquisition times than planar scanning, and may therefore be more difficult in children and infants.

Exposure to any radiation dose carries some risk, and this has to be weighed against the benefit to the patient or subject. The radiation risk to children is thought to be higher than adults as they may be more sensitive to radiation, and have longer to express the carcinogenic effects of radiation. Although the total radioactive dose in radiolabelled inhalation studies is small, impaction theory and work with other aerosols suggest that high central deposition may occur, and 'hot spots' of deposition may be found over a small area of cells at airway bifurcations (Martonen and Hofmann, 1986; Martonen and Hofmann, 1991; Cohen, 1996; Kinsara *et al.*, 1993). The impact of uneven deposition and hot spots on the calculated risk of radio-isotope administration has received little attention (Everard, 1994).

Radiolabelled deposition studies provide excellent visual images of the two dimensional pattern of radiolabel deposition in the three dimensional patient. It is not always clear how these images relate to clinical effect or drug activity. In one study comparing the conventional MDI with a modified actuator that produced a low-velocity aerosol (Newman and Clarke, 1993), images show a striking reduction in oropharyngeal deposition of drug with the low velocity device, from nearly 70% to 30%, with no difference in the whole lung or peripheral deposition, or in the deposition pattern between the central, intermediate and peripheral lung zones. The study had a power of 90% to detect a 10% difference in whole lung deposition between the two inhalers. It was surprising, therefore, when pharmacokinetic studies showed that systemic absorption of salbutamol was higher from the modified actuator (Newnham *et al.*, 1993), suggesting that it was not possible to extrapolate from radiolabelled lung deposition to drug delivery.

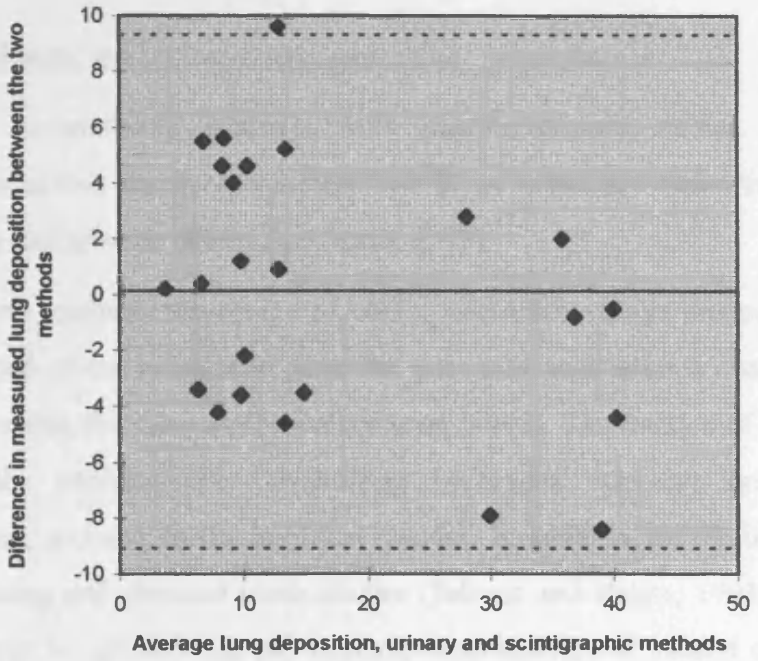
In contrast, two studies (Newman *et al.*, 1995; Borgstrom *et al.*, 1992) compared

gamma scintigraphy with urinary excretion of terbutaline as measures of drug deposition from a pressurised metered dose inhaler, MDI and spacer device, and dry powder inhaler. The urine method, with a 'charcoal block' to minimise absorption of drug from the gastro-intestinal tract, gave lower values for lung deposition than scintigraphy for the dry powder inhaler (21.1% vs. 26.9%) but comparable values for the metered dose inhaler used alone and with the Nebuhaler. The experimental data for the metered dose inhaler and spacer is replotted on the following graph:



Percentage lung deposition of terbutaline from a metered dose inhaler or spacer device, measured by gamma scintigraphy and urinary excretion of drug. The solid line is the line of identity. From (Newman *et al.*, 1995).

Rather than using correlation, levels of agreement between two different methods of measurement are more appropriately assessed using a Bland-Altman plot (Bland and Altman, 1986). This plots the average value of the two measurements against the difference between them, and allows estimates to be made of the bias of one method relative to another, and also allows the limits of agreement to be calculated.



Difference between lung deposition measured by gamma scintigraphy or urinary excretion, plotted against the average. The solid line is the group average difference, and the dotted lines two standard deviations above and below the group average. From (Newman *et al.*, 1995).

From this, it can be seen that on average gamma scintigraphy overestimates lung deposition measured by urinary excretion by 0.1%. The 95% limits of agreement (± 2 standard deviations) are from -9.1% to 9.3%. Thus 95% of the time the two methods will give measurements that differ by less than 18.4%.

The comparison between the two methods of lung deposition of terbutaline from the dry powder inhaler is hampered by small numbers, but here gamma scintigraphy overestimates lung deposition compared to urinary excretion by an average of 5.7%, and the 95% limits of agreement (± 2 standard deviations) are from -3.9% to 15.3%. 95% of the time the two methods will give measurements that differ by less than 19.2%. It is clear from these two studies that the relationship between gamma scintigraphy and measurement of lung deposition by urinary excretion is dependent on the type of inhalational device being assessed, and that the levels of agreement are wide, despite the excellent correlation between the measurements in some cases.

Pharmacokinetic evaluation of lung deposition:

There is accumulating evidence from pharmacokinetic studies to suggest that absorption across the lung vascular bed is an important determinant of systemic bioactivity and adverse effects (Lipworth, 1995).

For example, systemic absorption of inhaled salbutamol occurs predominantly from the vascular bed of the lung rather than the gut, with peak plasma concentrations being achieved within five minutes (Newnham *et al.*, 1993). The fraction of salbutamol which reaches the intestine after swallowing undergoes extensive first pass sulphate conjugation, probably in the intestinal mucosa. In addition, on the basis of data from mouth rinsing and charcoal block studies (Selroos and Halme, 1991; Pedersen *et al.*, 1993) it can be inferred that the systemic bioavailability of inhaled cortico-steroids is mainly determined by absorption across the lung vascular bed. Thus, a drug delivery system which improves lung deposition would, at the same time, be expected to increase lung bioavailability and hence overall systemic absorption.

A non-invasive pharmacokinetic approach to measuring lung deposition of salbutamol involves the measurement of early urinary excretion of native drug and its sulphate metabolite in order to differentiate between lung and gut bioavailability (Hindle and Chrystyn, 1992). The method is based on the premise that inhaled drug absorbed through the lung is rapidly excreted in the urine, whereas swallowed drug is absorbed more slowly, and predominantly excreted in the urine following sulphate conjugation in the intestine. Absorption of salbutamol through the buccal mucosa is negligible (Lipworth *et al.*, 1989). The measurement of salbutamol in the urine collected up to thirty minutes after inhalation was found to be a reproducible and non-invasive means of assessing the relative bioavailability of salbutamol from different formulations or inhalers (Hindle *et al.*, 1993; Hindle and Chrystyn, 1994; Chege and Chrystyn, 1994; Hindle *et al.*, 1995; Chrystyn, 1996; Hindle *et al.*, 1997). A similar method has been described to measure urinary sodium cromoglycate and nedocromil sodium (Aswania *et al.*, 1997; Aswania *et al.*, 1998a; Aswania *et al.*, 1998b). In one comparative study (Clark *et al.*, 1996) measurement of urinary salbutamol excretion gave similar results to

measurement of plasma levels.

Estimation of lung deposition by measurement of plasma salbutamol concentrations is dependent on the availability of a sensitive drug assay, capable of detecting salbutamol concentrations to the order of 1ng/ml. Measurements of plasma salbutamol over the first thirty minutes following inhalation predominantly reflect lung absorption of drug, and lung deposition may therefore be estimated from either the maximum concentration (C_{max}) or the area under the time/concentration curve. This is still an indirect method, since it measures drug concentrations in the plasma, rather than the lung, but short of removing the lungs of volunteers for assay, is the closest to a direct assay that may be obtained. A number of studies have demonstrated a clear relationship between plasma β_2 agonist concentration and bronchodilator effects. However there is a large individual variation in the pharmacodynamic response for a given concentration between different patients (Taburet and Schmit, 1994).

The pharmacokinetic method has been used in the study of two nebulisers, the Hudson Up-draft II and the Ventstream nebuliser system (Newnham and Lipworth, 1994). Lung deposition of drug from the Ventstream should theoretically be greater as it produces a considerable increase in output of respirable particles and matches the nebuliser output to tidal flow. When plasma salbutamol levels were used for pharmacokinetic evaluation approximately double the amount of drug was delivered to the lung using the Ventstream, as assessed by comparison of the peak plasma concentration or area under the concentration-time profile.

Clinical Evaluation

Clinical efficacy studies are ultimately the most relevant measure of the effectiveness of a medication, although they may not be easy to apply to an inhaled drug. Although bronchodilatation is characteristically rapid after administration of a single dose of a $beta_2$ agonist, comparisons between different delivery devices may be obscured by measurements being made on the shallow part of the dose-response curve. Furthermore, with drugs whose onset of action is clinically slower, long term evaluation is required, and appropriate end points more difficult to determine. As

discussed in Chapter 4.3, many past studies have been flawed by inadequate power or inattention to the detail of inhalational drug delivery

In-vitro/in-vivo correlation.

Here we are concerned whether or not measurements of particle size in vitro, using inertial impaction or laser diffraction, show any correlation with lung deposition, measured by scintigraphy or by pharmacokinetic methods, or with clinical effect.

Clearly there is more to estimating lung deposition than particle size. Knowing the engine capacity of a car will tell you something about its potential performance, but will not accurately predict how long it will take you to get round the M25 on a Bank Holiday Monday. Similarly with aerosols, knowing the particle size may allow you to estimate the potential for lung deposition, but the actual deposition observed will vary dependent on the many factors discussed in this thesis, and others. Nevertheless, the question remains, keeping other factors equal, does measured particle size correlate with measured lung deposition or clinical effect?

Metered dose inhalers with or without spacers

A number of studies measuring lung deposition of radio-label have also included details of in-vitro drug or radio-label particle size measurements. The relationship between these has recently been investigated by Newman, using data from his own laboratory (Newman, 1998). These, and a number of other studies using radio-labelling to estimate lung deposition are described in the accompanying table, which gives the radiolabelling method, the % lung deposition and aerosol particle size for a number of studies. Very few of these papers provide sufficient detail to accurately determine the particle size characteristics of the inhaled aerosol, or even, in many cases, the lung deposition pattern. For the purposes of this discussion, only those studies involving metered dose inhalers with or without spacer devices, and direct labelling of the administered drug will be included. Data from these studies are collated below. Further details of the experimental methods are given in the accompanying table:

Study no & Device	Study	WLD %	P%	I%	C%	Particle sizing method	%S4 + F	% < 5μm	% < 3μm	Comments	Ref
1. MDI	Teflon particles, MMAD 3.2μm, GSD 1.2.	8.7 (SEM 1.8)	3.2 (0.6)	5.5 (1.3)		--	--	--	--	9 Asthmatic adults Closed mouth, 1 sec delay	(Newman *et al.*, 1984)
1. Nebuhaler	single puffs	20.9 (1.6)	6.8 (1.5)	14.1 (1.2)		--	--	--	--		
1. Nebuhaler	four puffs	15.2 (1.5)	5.5 (0.8)	9.7 (1.1)		--	--	--	--		
2. MDI	Teflon particles, MMAD 3.2μm, GSD 1.2.	8.8 (SD 3.4)	3.0 (3.1)	5.8 (2.6)		--	--	--	--	8 adults with obstructive airways disease Closed mouth	(Newman *et al.*, 1981)
3. DPI (Turbohaler)	Direct labelling of Budesonide	27.7 (SD 9.5)	--	--	--	MSLI	26*	27.3	--	10 healthy adults 60l/min inspiratory flow	(Borgstrom *et al.*, 1994)
3. DPI (Turbohaler)		14.8 (3.3)	--	--	--	--	--	--	--	35l/min inspiratory flow	
3. Nebuhaler	Teflon particles, MMAD 3.μm Single puffs	21.2 (SEM 2.0)	9.6 (2.1)	11.6 (1.8)		--	--	--	--	10 patients with pulmonary sarcoidosis	(Spiteri *et al.*, 1989)
3. DPI (Turbohaler)	Direct labelling of Budesonide	14.2 (SEM 2.1)	5.3 (1.2)	3.3 (0.5)	5.6 (0.8)	MSLI	23.4	--+	--+	10 adult asthmatics. Inspiratory flow not controlled.	(Newman *et al.*, 1989)

Study no & Device	Study	WLD %	P%	I%	C%	Particle sizing method	%S4 + F	% < 5μm	% < 3μm	Comments	Ref
4. MDI	Radiotracer with ^{99m}Technitium †	15.3 (SD 5.1)	9.1 (3.0)	10.3 (2.5)	8.6 (2.1)	HPMLI	14 (SD 3) *	22 *	14 *	10 healthy adults. Faster inhalation with MDI & MDI/spacer than Respimat	(Newman *et al.*, 1996)
4. Inhacort spacer		28.0 (7.0)	5.4 (1.4)	5.4 (1.9)	4.5 (1.8)	--	--	--	--		
4. Respimat		39.7 (9.9)	14.1 (4.3)	14.9 (3.6)	10.7 (2.5)	HPMLI	48 (SD 8) *	55 *	46 *	Different inhaler nozzle used for In vitro & in vivo study	
5. MDI	Radiotracer with ^{99m}Technitium †	11.0 (SEM 1.4)	4.7 (0.6)	--	--	MSLI	9.5 (SD 4.1)	18	9	Inhalation at 25l/min	(Newman *et al.*, 1989)
5. Syncroner		16.1 (2.2)	6.9 (0.9)	--	--	MSLI	12.6 (SD 4.2)	23	10.7	Inhalation at 25l/min	
5. Syncroner		13.3 (1.7)	4.3 (0.5)	--	--	MSLI at 80l/min ✦	--	20 ✦	--	Inhalation at 100l/min	
6 MDI	Teflon particles, MMAD 3.2μm	6.5 (SEM 1.2)	2.2 *	4.3 *		--	--	--	--	10 patients with obstructive airways dis. Patient's own technique	(Newman *et al.*, 1986)
6 MDI		11.2 (1.3)	5.5 *	5.7 *		--	--	--	--	closed mouth, 'best' technique	
6. Inspirease		14.8 (1.8)	5.0 *	9.8 *		--	--	--	--		

Study no & Device	Study	WLD %	P%	I%	C%	Particle sizing method	%S4 + F	% < 5μm	% < 3μ m	Comments	Ref
7. MDI	^{99m}Technitium pertechnetate solution, MMAD 2.2, GSD 2.9	10.4 (SD 4.2)	4.5	2.6		--	--	--	--	Haelthy Adults. Open mouth technique.	(Dolovich *et al.*, 1983)
7. Aerochamber		9.9 (4.0)	3.1	3.3		--	--	--	--	Solution aerosol from Aerochamber MMAD 1.32 ✣	
8. MDI	^{99m}Technitium hexamethylpropylen eamine solution, MMAD 2.2, GSD 2.9	38 (SD10)	14 (4)	11 (3)	14 (4)	Andersen	--	50 *	--		(Ashworth *et al.*, 1991)
8. Spacer	10cm long, 45ml volume non-valved spacer	57 (25)	23 (11)	16 (7)	18 (7)	--	--	--	--	Spacer reduced Andersen inlet deposition from 24% to 1%	
9. MDI	co-precipitation of SCG with ^{99m}Tc, MMD 3.8μm (Spray drying) ❖	9.2 (SD 5.9)	--	--	--	MSLI+	--	8.8 +	4 +	Haelthy Adults. Inspiratory flow 55-70l/min	(Vidgren *et al.*, 1987)
9. Inhalet	Single actuations/inhalations	16.1 (5.7)	--	--	--	--	--	--	--	Inspiratory flow 55-70l/min	
9. Nebuhaler	Two actuations per inhalation.	14.4 (5.2)	--	--	--	MSLI	7.5 +	9.8 +	4.7 +	Inspiratory flow 55-70l/min Single actuations 'in vitro'	
9. Inspirease	Two actuations per inhalation.	20.4 (3.3)	--	--	--	MSLI	6.2 +	8.0 +	4.1 +	Inspiratory flow 20l/min Single actuations 'in vitro'	

Study no & Device	Study	WLD %	P%	I%	C%	Particle sizing method	%S4 + F	% < 5μm	% < 3μm	Comments	Ref
10. MDI	Salbutamol labelled with ^{99m}Tc (1)	21.6 (SD 8.9)	9.5	12.1		Andersen. MMAD 2.8, GSD 1.5 ϕ	--	--	--	10 normal subjects	(Melchor *et al.*, 1993)
10. Volumatic		20.9 (7.8)	10.3	10.6		--	--	--	--		
10. DPI		12.4 (3.5)	4.9	7.5		Andersen @60l/min MMAD 2.8, GSD 1.7 ϕ	--	--	--		
11. MDI	Salbutamol labelled with ^{99m}Tc (1)	24.1 (SD 8.5)	--	--	--	Andersen. MMAD 2.8, GSD 1.5 ϕ	--	--	--	6 normal subjects. Apparently same Andersen data as (Melchor *et al.*, 1993),	(Biddiscombe *et al.*, 1993)
11. DPI		11.3 (2.2)	--	--	--	Andersen @60l/min MMAD 2.8, GSD 1.7 ϕ	--	--	--	and possibly same patients included in both studies?	
12. MDI	Co-precipitation of SCG with ^{99m}Tc, MMD 2.4μm (Spray drying) ❖	9.2	--	--	--	--	--	--	--	8 normal volunteers. Inhalation method not explicit	(Vidgren *et al.*, 1990)
12. DPI	-- " --	20.9	--	--	--	--	--	--	--	Ingelheim DPI	(Vidgren *et al.*, 1990)
13. DPI Easyhaler	Co-precipitation of salbutamol with ^{99m}Tc, (Spray drying) (2)	24 (SD 6)	--	--	--	Andersen	--	29 (<5.8μm)	--	12 Asthmatic patients	(Vidgren *et al.*, 1994)

Study no & Device	Study	WLD %	P%	I%	C%	Particle sizing method	%S4 + F	% < 5μm	% < 3μm	Comments	Ref
14. MDI	^{99m}Tc labelled Teflon, MMD 2.1μm	12.7 (sd 4.1)	--	--	--	Twin impinger	--	37.1 (sd 6.8) <6.4μm	--	6 subjects, 5 asthmatic	(Zainudin *et al.*, 1989)
15. DPI (Turbohaler)	Direct labelling of Budesonide	26.9 (SD 3.8)	12.9 (2.2)	8.4 (1.0)	5.7 (0.9)	--	23.4 (3)	--	--	6 healthy adults. Mean inspiratory flow 55l/min.	(Borgstrom *et al.*, 1992)
16. MDI	Radiotracer with ^{99m}Technitium †	10.7 (SD 2.6)	4.5 (1.5)	3.4 (0.7)	2.8 (0.8)	MSLI	19.6	26.2	18.9	8 healthy adults. Inhalation at 30l/min	(Newman *et al.*, 1995)
16. MDI	Radiotracer with ^{99m}Technitium †	10.4 (SD 5.0)	3.1 (1.1)	3.2 (1.4)	4.1 (2.7)	MSLI	19.6	26.2	18.9	Inhalation at 180l/min	(Newman *et al.*, 1995)
16. Nebuhaler	Radiotracer with ^{99m}Technitium †	31.6 (SD 10.1)	12.6 (3.9)	10.6 (3.7)	8.4 (3.0)	--	--	--	--	Treated to reduce static charge	(Newman *et al.*, 1995)
17. MDI	Albumin labelled with ^{99m}Tc	11.2 (SEM 0.8)	--	--	--	Twin impinger	--	37.1 (sd 6.8) <6.4μm	--	Difference in siae and in vitro deposition between drug and albumin	(Zainudin *et al.*, 1990)
17. Rotahaler	Albumin labelled with ^{99m}Tc	9.1 (0.6)	--	--	--		--	11.5 (sd 3.4) <6.4μm	--	Different in vitro & in vivo technique for delivering drug	(Zainudin *et al.*, 1990)
18. MDI	Labelled salbutamol	12.3	--	--	--	--	--	--	--		(Newman *et al.*, 1991)
18. Rondo	Labelled salbutamol	23.8	--	--	--	--	--	--	--		(Newman *et al.*, 1991)
19. Aerochamber	Radiotracer with ^{99m}Technitium †	1.97 (SD 1.4)	--	--	--	--	--	--	--	15 children aged 2.5 mnths-5yrs with chronic respiratory disease.	(Tal *et al.*, 1996)
20. Azmacort	Labelled Triamcinilone Acetonide	9.9	--	--	--	--				corrected for tissue attenuation (Dolovich, 1993)	(Dolovich, 1989)

Study no & Device	Study	WLD %	P%	I%	C%	Particle sizing method	%S4 + F	% < 5μm	% < 3μm	Comments	Ref
20. Nebuhaler	Radiotracer with ^{99m}Technitium †	26.7 (6.2)	8.1 (2.2)	9.6 (2.3)	9.1 (2.7)	--	--	--	--	Ten mild-moderate adult asthmatics. Data for new spacers. Metal spacer and	(Kenyon *et al.*, 1998)
20. Volumatic		22.1 (10.1)	6.6 (3.5)	8.1 (3.8)	7.5 (3.1)	--	--	--	--	'primed' spacers also evaluated	
21. Nebuhaler + terbutaline	Radiotracer with ^{99m}Technitium	32.6 (5.7)	--	--	--	--	--	--	--	Five normal subjects.	(Matthys, 1990)
21. Volumatic + salbutamol	?method	28.3 (4.9)	--	--	--	--	--	--	--	Very few experimental details given	
21. Aerochamber + fenoterol		20.5 (6.5)	--	--	--	--	--	--	--		
21. Aerochamber + salbutamol		11.6 (3.3)	--	--	--	--	--	--	--		
21. Aerochamber + terbutaline		6.6 (2.1)	--	--	--	--	--	--	--		
22. MDI	Direct labelling of ipratropium bromide with ^{77}Br	11.2 (4.0)	5.5	3.4	2.3	--	--	--	--	Seven normal adult subjects	(Spiro *et al.*, 1984)
23. Microprocessor controlled MDI - Smartmist.	Radiotracer with ^{99m}Technitium †	14.1 (SEM 2.0)	7.5 (1.1)	--	--	Andersen. MMAD 2.8, GSD 1.8 γ	26.7 % (5.5) <5.8μm	≈25 % <4.7μm *	≈20 % <4.7μm *	Nine normal adult subjects Data for slow inhalation & early MDI actuation given	(Farr *et al.*, 1995)
24. MDI	Radiotracer with ^{99m}Technitium †	8.8 (SE 1.1)	4.4 (0.5)	--	--	MSLI	6.3 (SE1.1)	--	--	Ten normal adult subjects	(Fisher *et al.*, 1990)
Aerotube @30l/min		11.3 (SE 1.9)	5.5 (0.8)	--	--	MSLI (60l/min)	7.7 (SE1.4)	--	--	10cm tube spacer	
Aerotube @100l/min		7.1 (SE 1.3)	3.2 (0.5)	--	--						

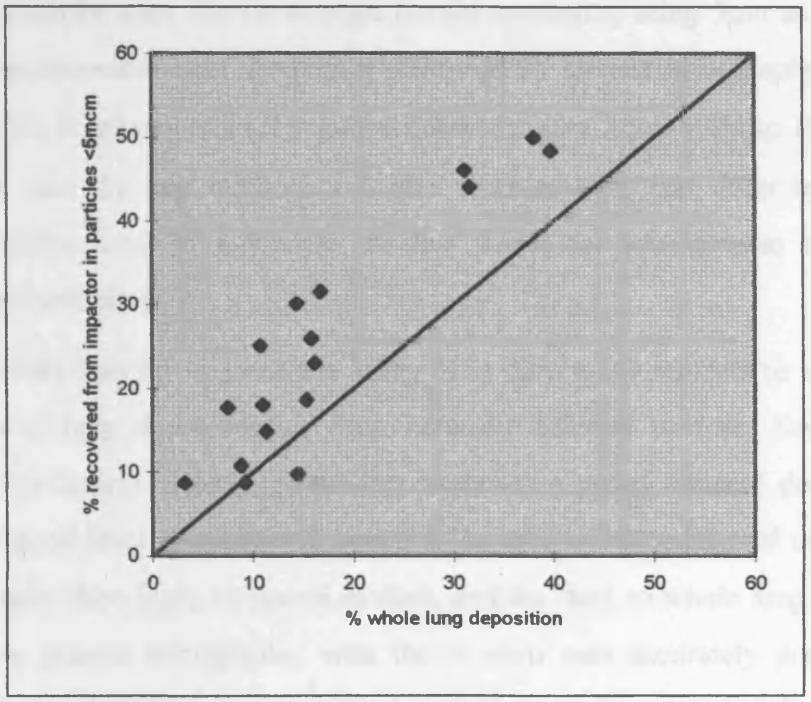
Individual studies are grouped by double lines.
Dashes imply that the relevant data was not available from the published paper.
Values are mean and (SD) unless otherwise stated.
* Estimated from chart or comment in paper
+ Particle size distribution not log normal - parameter therefore not calculated.
γ Excludes ballistic component recovered from throat.
† After the method of (Kohler *et al.*, 1988), or modified according to Newman *et al.*, 1989; Summers *et al.*, 1990
✦ MSLI run at 80l/min. Barry & O'Callaghan, unpublished data.
At 30l/min: Drug <5μm = 10.4%; Stage 4 recovery (D50%=6.1μm)=16.7%
At 80l/min: Drug <5μm = 20%; Stage 4 recovery (D50%=3.7μm) = 14.3%

✣ From (Corr *et al.*, 1982)
❖ From (Vidgren *et al.*, 1987)
✢ From (Barry and O'Callaghan, 1996)
ϕ No data given on total recovery or inlet recovery (ie ballistic fraction) in vitro.
(1) from (Biddiscombe *et al.*, 1993)
(2) from (Arppe and Vidgren, 1994)
(3) from (Newman *et al.*, 1989)

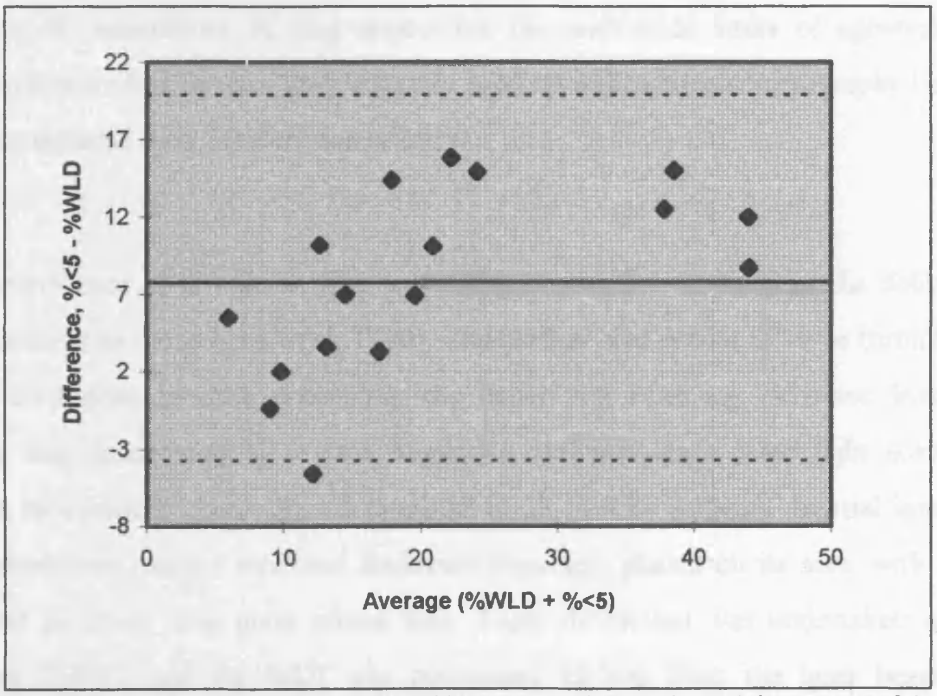
Study	%WLD	%S4 + F	%<5μm	%<3μm	Reference
MDI	11.0	9.5	18.0	9.0	(Newman *et al.*, 1989)
Syncroner	16.1	12.6	23	10.7	(Newman *et al.*, 1989)
MDI	8.8	6.3	10.8	–	(Newman, 1998)
Spacer	11.3	9.7	14.9	–	(Newman, 1998)
MDI	10.7	19.6	25.1		(Newman *et al.*, 1995)
Chamber	31.6	29.4	44.1		(Newman *et al.*, 1995)
MDI	15.8	18.9	25.9	–	(Newman, 1998)
MDI	3.2	6.7	8.7	–	(Newman, 1998)
MDI	7.5	13	17.7		(Pitcairn *et al.*, 1997)
MDI	16.7	23.4	31.6	–	(Newman, 1998)
MDI	15.3	13.2	18.6		(Newman *et al.*, 1996)
SMI	39.7	42.8	48.4		(Newman *et al.*, 1996)
MDI	14.3	27.2	30.1	–	(Newman, 1998)
SMI	31.1	42	46.1	–	(Newman, 1998)
MDI	38	–	50	–	(Ashworth *et al.*, 1991)
MDI	9.2	–	8.8	4.0	(Vidgren *et al.*, 1987) & (Barry and O'Callaghan, 1996)
Nebuhaler	14.4	–	9.8	4.7	– " –

Data used in folllowing discussion. 'Study' gives the type of drug delivery device assessed; '%WLD' the percentage whole lung deposition measured by gamma scintigraphy; '%S4+F' is the percentage of drug collected on stage 4 and filter of the MSLI where appropriate. In some studies the HPMLI or the Andersen impactor were used; '%<5μm' and '%<3μm' are the percentage of drug recovered from the impinger in particles smaller than 5μm or 3μm respectively, from a log-probability plot of the data. Unfortunately there is insufficient data in many of the studies to complete the table, particularly the % <3μm column. Further study details in the accompanying Table.

The relationship between gamma scintigraphy and the mass of drug collected in particles smaller than 5μm is shown in the following graph. The line is the line of identity:



The values are highly correlated (correlation coefficient 0.924). Again, levels of agreement may be calculated:



Difference between lung deposition measured by gamma scintigraphy and the amount of drug in particles smaller than 5μm measured by inertial impaction, plotted against the average. The solid line is the group average difference, and the dotted lines two standard deviations above and below the group average.

From this, it can be seen that on average inertial impaction, using 5μm as the cut off diameter, overestimates lung deposition measured by gamma scintigraphy by 8.1%. The 95% limits of agreement (± 2 standard deviations) are from -3.8% to 19.9%. Thus 95% of the time the two methods will give measurements that differ by less than 23.6%, a similar level of agreement to that found for scintigraphic and urinary excretion methods above.

Newman (1998) then investigated the utility of in vitro measurements to estimate the relationship of lung deposition of drug between different devices. Seven studies compared two devices directly. In the five studies comparing metered dose inhalers, there was a good level of agreement between the ratio of percentage of drug mass in particles smaller than 5μm, measured in vitro, and the ratio of whole lung deposition, measured by gamma scintigraphy, with the in vitro data accurately predicting the direction of the relationship between the two devices, underestimating the magnitude of the relationship in three of the five studies examined. In vitro data did not accurately predict the relationship between metered dose inhalers and dry powder inhalers in the two studies presented by Newman. Thus in vitro studies correlate well with scintigraphic estimations of lung deposition, but with wide limits of agreement. In comparative studies, in vitro data correctly predicts differences in scintigraphy between different metered dose inhalers and spacers.

Further evidence of in vitro/in vivo correlation comes from a study of the delivery of peptide drugs to the lungs (Adjei, 1990). The particle size output of three formulations of metered dose inhalers containing the leutenising releasing hormone leuprolide acetate was determined by inertial impaction and low angle laser light scattering. Human bioavailability was then determined in 23 healthy subjects. Inertial impaction was undertaken using a modified Andersen impactor, placed on its side, with a 5cm diameter by 25cm long glass intake tube. Laser diffraction was undertaken using a Malvern 2600C, and the MDI was positioned 12.5cm from the laser beam. The experimental set-up is described in detail in the paper. There was agreement between all three methods in the rank order of formulations, using particles smaller than 4.7μm aerodynamic diameter as the cut off for defining the 'respirable fraction' and the area

under the curve of the blood concentration - time profile (AUC), as shown in the table:

	Plasma levels	% of drug <4.7μm *	
Formulation	AUC ng /ml/hr	Inertial impaction	Laser Diffraction
A	7.80	9	7
B	33.14	36	53
C	25.95	27	33

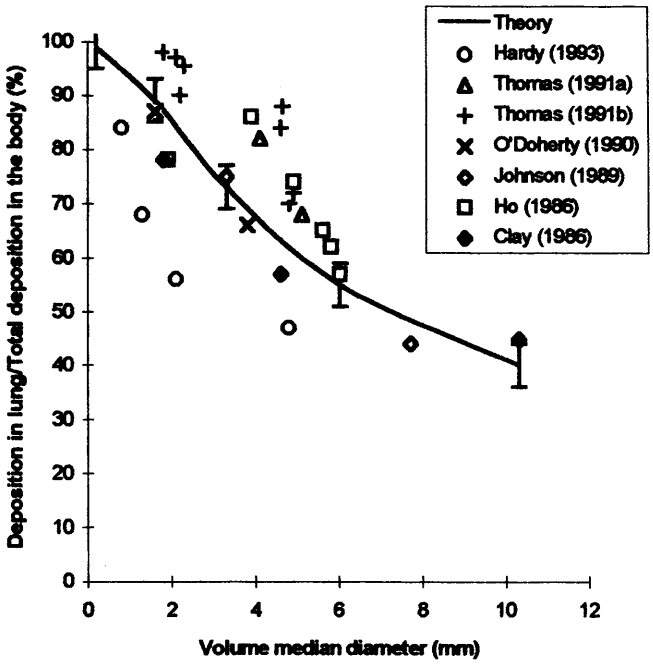
* Estimated from graphs in {3383}

Evidence of in vitro/in vivo correlation will also be demonstrated in Chapter 7, where it will be shown that the conclusions of in vitro studies performed as part of this thesis have subsequently been supported by clinical and pharmacokinetic studies by others.

Nebulisers

Studies using standard two dimension gamma scintigraphy have found the mean percentage of the dose deposited in the lung from a jet nebuliser to be between 2% (Asmundsson, 1973) and 12% (Lewis and Fleming, 1985). Studies of a number of jet nebulisers in terms of extrabronchial and intrabronchial deposition in normal subjects have shown marked differences, presumably reflecting variations in nebulisation rate and droplet size, as well as inter-individual differences.

Clarke (1995) addressed the question of whether measurement of particle diameter has relevance to the clinical situation by reviewing studies of radiolabelled deposition of drug in the lung and droplet particle size measured by laser diffraction. The figure plots percentage of aerosol deposition in the lung as a percentage of that deposited in the patient versus the mass median droplet diameter of the nebulised aerosol cloud. A reasonable correlation is found between the MMD as measured by laser diffraction and thoracic deposition. However, the data presented only represents the fractionation of the aerosol cloud between the oropharynx and the lung. The variable airways obstruction of the volunteers used in these studies would not have a major effect on oropharyngeal filtering.



The author (Clark, 1995) found insufficient data in the same papers to study the relationship between particle size and penetration of aerosol into the lung periphery.

Is particle size relevant to the clinical effect of aerosols?

A number of experimental and theoretical studies (Heyder *et al.*, 1986; Heyder, 1982; Heyder and Rudolf, 1984; Gerrity *et al.*, 1979) have suggested that particles of 1μm to 5μm aerodynamic diameter deposit optimally in the lungs, with minimal extrathoracic airways deposition. Rees (1982) administered 250μg of terbutaline in different particle size ranges to ten asthmatics and ten non-asthmatics. Changes in airways resistance and spirometry were greatest after inhaling drug particles smaller than 5μm, compared with those between 5 and 10μm, or between 10 and 15μm. Clay (1987) demonstrated greater bronchodilation after the inhalation of terbutaline aerosol with an MMAD of 1.8μm compared to 4.6μm and 10.3μm in a group of six stable asthmatics.

Nebulised bronchodilator aerosols of MMAD 1.5μm to 3.3μm produce greater bronchodilation than those with MMAD greater than 5μm (Johnson *et al.*, 1989; Ruffin *et al.*, 1981). In contrast, Mitchell (1987) failed to show a difference in either radio-labelled particle deposition or bronchodilatation in 8 asthmatics following

inhalation of salbutamol either in particles of MMAD 1.4μm or of MMAD 5.5μm. Dolovich (1981) demonstrated an equal bronchodilator response in six stable asthmatics with particles of 0.55μm and 2.4μm.

Douglas (1986) and Hadfield (1986) compared the spirometric response to nebulised salbutamol in stable asthmatics, altering the particle size by adjusting the nebuliser driving gas flow. They found no difference in spirometry between different particle sizes, although both studies failed to quantify the actual drug dose delivered to the lungs, using relatively large nominal doses which may have induced maximal bronchodilatation.

Godfrey (1974) demonstrated a protective effect of 2μm, but not 11 μm, monodisperse sodium cromoglycate particles in children with exercise induced asthma. Patel (1990) gave monodisperse aerosols of isoproterenol MMAD 2.8μm and 5.6μm to eight mild asthmatics, and found a two to four fold greater spirometric response with the smaller particles. Particles were generated by a spinning top generator, and dried prior to inhalation. The particles could therefore be expected to undergo hygroscopic growth in the airways to a greater degree than similar sized aqueous particles produced by a medical nebuliser, and the particle size presented to the lung in this study was probably larger than that measured in the drying chamber. In a series of experiments (Zanen *et al.*, 1994; Zanen *et al.*, 1995; Zanen *et al.*, 1996; Zanen *et al.*, 1998) monodisperse aerosols of salbutamol or ipratropium bromide were administered to patients with asthma. Particles were generated by a spinning top generator and dried in a holding chamber to give monodisperse aerosols with MMAD 1.5μm, 2.8μm or 5μm. The concentration of particles in the chamber was measured, and the volume of aerosol inhaled by the subject altered to give an equal dose of drug at each particle size. However, details of the patient inhalation are not given in the papers, and again dry drug particles may be expected to have different hygroscopic growth patterns to aqueous nebulised solutions in the respiratory tract. Zanen found that particles of both salbutamol and ipratropium with a dry MMAD of 2.8μm gave a greater degree of bronchodilation than 5μm particles. This result was found in patients with both mild and severe airflow obstruction (FEV_1 >70% and 38% respectively). Zanen then compared the effect of 8μg of monodisperse ipratropium bromide, MMAD 2.8μm,

with 40μg delivered by metered dose inhaler and Aerochamber spacer, demonstrating equal improvement in specific airways conductance and spirometry.

Unfortunately this study is flawed by the use of a five second pause between actuation of the metered dose inhaler into the Aerochamber and inhalation by the patient, effectively reducing the delivered dose. Also the authors state that 'care was taken to avoid electrostatic charging of the spacer', but give no details of what this means. In vitro measurements with a 'time of flight' particle sizer suggested that 11.6μg of drug was delivered to the patient from the aerosol with a MMAD of 1.8μm, although it is not clear if the five second pause was included in the in vitro measurements. The standard deviation of the in vitro measurements was higher than the mean value, suggesting poor reproducibility of the measurement in the authors laboratory. Time of flight analysis may not be comparable with other particle sizing methods (Chege *et al.*, 1997) making interpretation of this study difficult. .

The accompanying table summarises studies investigating the effect of particle size on bronchodilator response.

Many factors other than particle size affect lung deposition, and these are discussed in Chapter 4.1. The deposition patterns of inhaled aerosols vary greatly between individuals, particularly in the extrathoracic airways (Stahlhofen *et al.*, 1981; Heyder *et al.*, 1982; Svartengren *et al.*, 1994) making it particularly difficult to compare deposition studies using different subjects, especially where the studies have small subject numbers.

Svartengren and colleagues have produced a number of studies demonstrating improved lung deposition of 6μm particles using an extremely low inhalation flow rate (0.04l/s) (Camner *et al.*, 1997; Anderson *et al.*, 1995). In addition, pulmonary drug delivery has been enhanced experimentally by using low density gases (Anderson *et al.*, 1993; Svartengren *et al.*, 1989) or large, porous particles of low density (Edwards *et al.*, 1998)

Airway calibre also affects lung deposition. Melchor (1993) demonstrated significantly higher peripheral lung deposition in normal subjects compared to asthmatics, and Pavia

has suggested a relationship between peripheral lung deposition and FEV_1 in asthmatic subjects (Pavia *et al.*, 1977). A number of studies have demonstrated lower lung deposition following experimentally induced bronchoconstriction (Svartengren *et al.*, 1987; Svartengren *et al.*, 1984; Svartengren *et al.*, 1986). Peak plasma concentrations of fenoterol are lower in asthmatic subjects compared to normal subjects following identical inhalation protocols (Newnham *et al.*, 1993; Lipworth *et al.*, 1995), as are peak salbutamol concentrations in severe asthmatics compared to normal and mildly asthmatic subjects (Lipworth and Clark, 1997)

There has been much recent debate about whether a particular particle size range can define so called respirable particles (Newhouse, 1998). In the past particles in the range 1μm to 5μm have been called 'respirable' (Newman, 1985), implying a 1:1 relationship between the amount of drug in a particular size range, and the amount of drug depositing in the lungs. As discussed above, however, this is rarely so. To avoid misunderstanding, it is preferable to avoid the use of such terms, or to explicitly define them in terms of particles of a certain aerodynamic size range. In this thesis results are presented to give the MMAD, GSD and mass of drug contained in particles smaller than five and three micrometers aerodynamic diameter.

Table: Effect of aerosol particle size on bronchodilator response

Study	Patients	Aerosol Characteristics	Result
(Dolovich *et al.*, 1981)	6 stable asthmatics	MMAD 0.55μm vs. 2.4μm	Equal response
(Clay and Clarke, 1987)	6 stable asthmatics	MMAD 1.8μm vs. 4.6μm vs. 10.3μm	Slight increase with smaller particles
(Johnson *et al.*, 1989)	8 stable asthmatics	MMAD 3.3μm vs. 7.7μm	Increased FEV1 and increased radiolabelled deposition with smaller particles (3.3μm)
(Mitchell *et al.*, 1987)	8 stable asthmatics	MMAD 1.4μm vs. 5.5μm	Equal deposition (radio-labelled) and effect (FEV1)
(Hadfield *et al.*, 1986)	10 stable asthmatics	MMAD 11.3μm vs. 16μm	No difference
(Douglas *et al.*, 1986)	40 chronic asthmatics	MMAD 4μm vs. 11μm	No difference
(Persson and Wiren, 1988)	12 stable asthmatics. Dry powder inhaler	90, 40 or 5 μg of terbutaline in particles <5μm	Increased bronchodilation with larger dose (90μg dose)
(Rees *et al.*, 1982)	10 non-asthmatic and 10 asthmatic subjects	MDI terbutaline with particles <5μm, 5-10μm or 10-15μm	Greatest change in airways resistance and spirometry in both groups with particles <5μm
(Clay *et al.*, 1986)	6 stable asthmatics	MMAD 1.8μm vs. 4.6μm vs. 10.3μm	Increased lung deposition (radiolabelled) with smaller particles (1.8μm)
(Patel *et al.*, 1990)	8 mild asthmatics	MMAD 2.5μm vs. 5μm	Increased bronchodilatation with 2.5μm particles
(Zanen *et al.*, 1994)	8 stable asthmatics, FEV_1 >70% predicted	Monodisperse salbutamol aerosol, MMAD 1,5μm vs. 2.8μm vs. 5μm	Greater improvement in spirometry with 2.8μm particles
(Zanen *et al.*, 1995)	8 stable asthmatics, FEV_1 >70% predicted	Monodisperse ipratropium bromide aerosol, MMAD 1,5μm vs. 2.8μm vs. 5μm	Greater improvement in spirometry with 2.8μm particles
(Zanen *et al.*, 1996)	8 stable asthmatics, FEV_1 38% predicted	Monodisperse salbutamol and ipratropium bromide aerosol, MMAD 1,5μm vs. 2.8μm vs. 5μm	Greater improvement in spirometry with 2.8μm particles
(Zanen *et al.*, 1998)	10 stable asthmatics,	8μg of monodisperse ipratropium bromide aerosol, MMAD 2.8μm vs. 11.6μg from MDI + Aerochamber, MMAD 1.8μm	No difference in specific airways conductance or spirometry.

The Glass Multi-Stage Liquid Impinger

The Glass Multi-Stage Liquid Impinger (MSLI) is a four stage inertial impaction device (see figure) consisting of an inlet or 'throat' unit, a main section containing the four impaction stages, and an absolute filter to capture the finest particles. The impaction stages are each connected by a round jet. The top three stages have a sintered glass impaction plate, and in the fourth stage the jet curves to direct the airflow onto the wall of the stage. A filter housing unit connects to the fourth stage, and consists of two hemispheric glass pieces, between which the terminal filter and a supporting metal plate are inserted. The use of inertial impaction devices is discussed in Chapter 4.5.

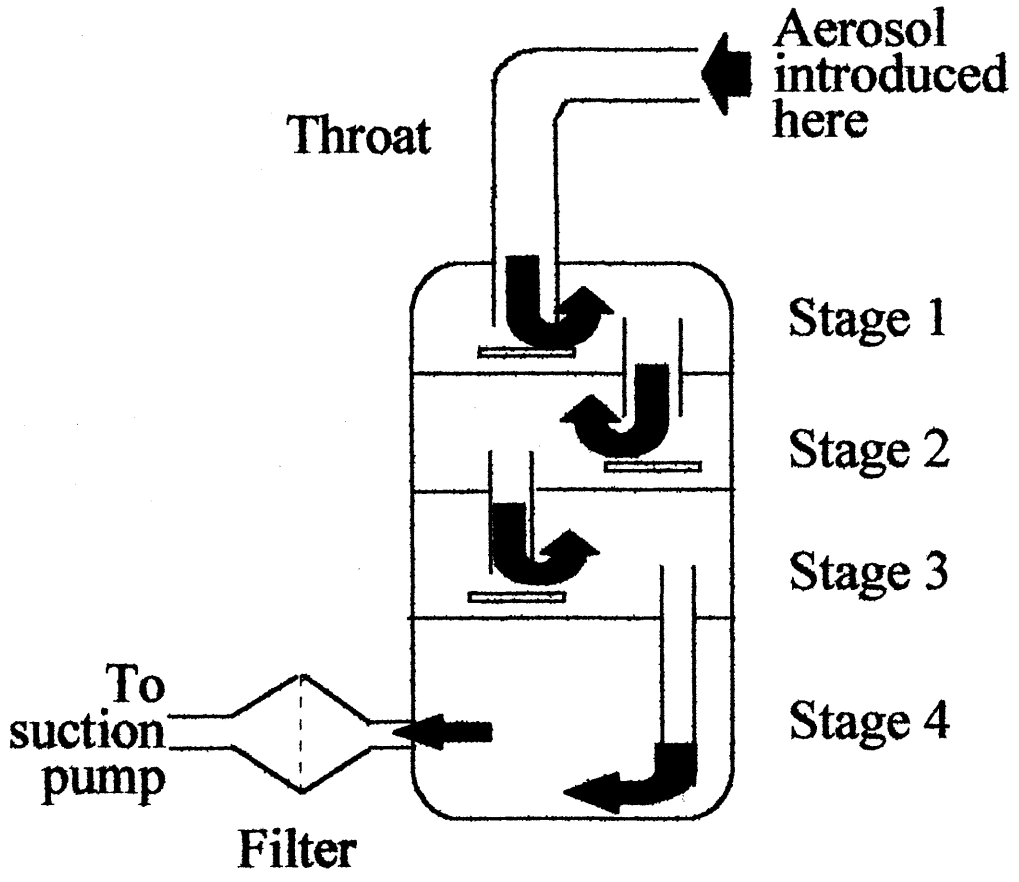


Figure. The glass multistage liquid impinger (MSLI)

Glass multistage liquid impingers were made by Scientific Glass, Nottingham, UK. Four MSLIs were used in total, although, except where otherwise noted, one MSLI was used consistently for each individual experiment described in this thesis.

Experimental apparatus

The MSLI throat unit was secured to the body of the MSLI by two metal clamps (Fisher Scientific Instruments, Loughborough, UK), and further supported by a retort stand and clamp. Silicone bungs were placed in stages one, two and three, after the addition of the appropriate solvent to each stage (see below), where appropriate. The filter unit was assembled using a 7cm glass fibre filter (Whatman GF/C filter). The unit was attached to the MSLI stage four, and held in place by a retort stand and clamp. The distal end of the filter unit was then attached to a vacuum pump by rubber tubing .

An Edwards ECB8 rotary vacuum pump was used (Edwards, Surrey, UK). Flow rate through the MSLI was adjusted by means of a gate clamp on the rubber tubing connecting the MSLI to the pump. Flow was measured at the MSLI throat by a screen pneumotachograph and Furness micromanometer (Furness Controls Ltd., Bexhill Surrey). Flow was measured before each MSLI run, and was adjusted to be 60l/min ± 2l/min. The pneumotachograph and micromanometer were calibrated by passing air through them at flows from 0 to 80l/min, measured on Fisher Controls Series 2000 Rotameters (KDG Mobrey Ltd, Crawley, UK), and noting the reading on the micromanometer scale. The reading on the micromanometer was linear across this range of flows.

Calibration of the MSLI

The MSLIs were calibrated by Fisons Research and Development (Loughborough, UK). The method used to calibrate the MSLI involved spraying an aerosol cloud across the analyser beam of a laser diffraction particle sizer (Malvern Instruments, Malvern, UK), and collecting the aerosol cloud in the MSLI. A comparison was then made between the data derived from the laser particle sizer and the MSLI. The stages of the MSLI have a large range of cut off diameters (approximately 1μm to 20μm). To collect sufficient aerosol on all stages of the MSLI, two different nebulisers were used. One, the Collinson, produced an aerosol with a mass median diameter of 3.0μm, the other, the Nippon, produced a coarser aerosol with a mass median diameter of 7.0μm.

Di-n-butyl phthalate was nebulised by one of the two nebulisers, and collected in the MSLI. Each stage of the MSLI was washed out with isopropyl alcohol, and the

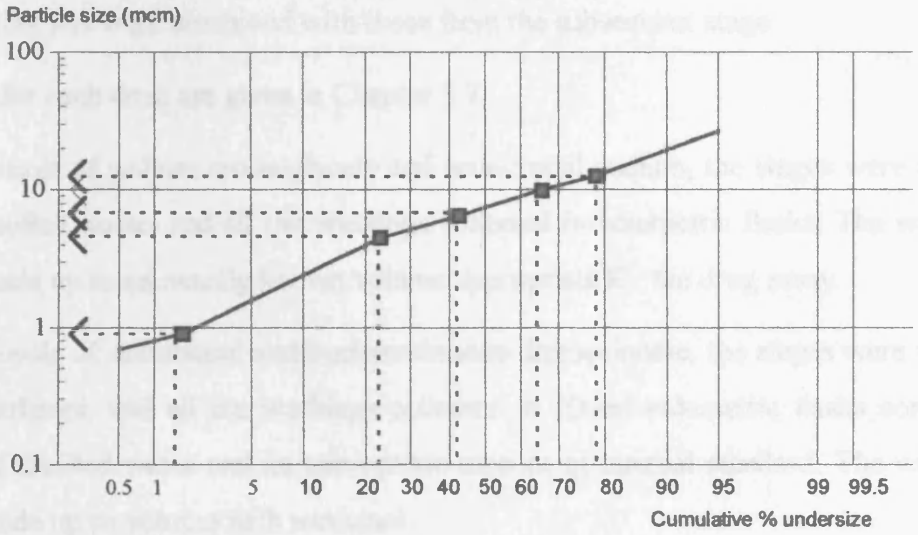
other, the Nippon, produced a coarser aerosol with a mass median diameter of 7.0μm.

Di-n-butyl phthalate was nebulised by one of the two nebulisers, and collected in the MSLI. Each stage of the MSLI was washed out with isopropyl alcohol, and the amount of di-n-butyl phthalate collected on each stage was determined by UV spectrophotometry. The procedure was carried out in triplicate with both nebulisers.

From data derived from the laser particle sizer, the cumulative percentage of particles below a certain size was plotted against particle diameter on logarithmic probability paper. As the percentage mass of drug collected on each stage of the MSLI was known, the cut off diameters could be read from the graph, as shown in the following hypothetical example:

Idealised graph from laser particle sizer.

Particle diameter vs. cumulative % undersize by weight.



Example MSLI Results		
Location	Cumulative % undersize	cut off diameter from graph (μm)
Throat	78.4	12.6
Stage 1	65.7	10
Stage 2	42.7	6.5
Stage 3	23.1	4.5
Stage 4	1.6	0.9

The blue line shows the results obtained from the laser particle sizer. As the percentage mass of the aerosol recovered from each stage of the MSLI is known, the cut off diameters for each stage can be interpolated from the graph, and are shown by the red dots. From this the stage cut offs can be interpolated (Table).

in the table:

MSLI No:	28	29	37	38
Throat	14.4	12.5	12.6	12.8
Stage 1	11.2	10.0	10.1	10.3
Stage 2	7.0	6.5	6.8	7.0
Stage 3	4.7	4.5	4.3	4.2
Stage 4	1.0	0.9	1.0	0.9

Sample preparation from the MSLI

After the aerosol had been collected in the MSLI, the throat, filter unit and each stage were washed with an appropriate solvent, and the amount of drug collected at each stage determined as outlined below. Care was taken to wash all surfaces of the MSLI, especially the impaction plates and connecting jets. Washings from the inside of the connecting jets were combined with those from the subsequent stage.

Details for each drug are given in Chapter 5.7.

For aerosols of sodium cromoglycate and nedocromil sodium, the stages were washed with distilled water, and all the washings collected in volumetric flasks. The washings were made up to an exactly known volume appropriate for the drug assay.

For aerosols of salbutamol and beclomethasone diproprionate, the stages were washed with methanol, and all the washings collected in 100ml volumetric flasks containing 30ml of distilled water and an appropriate amount of internal standard. The washings were made up to volume with methanol.

For aerosols of budesonide, each stage was washed with an accurately known volume of internal standard solution, and an aliquot of the washings retained for analysis.

Removal of surfactants

Aerosols of sodium cromoglycate and nedocromil sodium were analysed by UV spectrophotometry. Surfactants present in the drug formulation interfere with this assay, and were therefore removed by washing the experimental apparatus with trichlorofluoromethane (Isceon 11, Rhone Poulenc, Bristol) prior to the aqueous wash.

Washing efficiency

To determine the efficiency of the washing procedure, a sodium cromoglycate metered dose inhaler (5mg per actuation) was actuated into an MSLI, set up as above. Each stage of the MSLI was washed with 50ml of distilled water. The amount of sodium cromoglycate contained in the washings was determined by spectrophotometry (see 5.7). Each stage of the MSLI was then washed a second time, and the recovery of sodium cromoglycate expressed as a percentage of that recovered from the first wash. Four MSLI runs were undertaken. Mean recovery of sodium cromoglycate in the second wash was 1.05% of the first wash (95% confidence intervals 0.5-1.6%).

Analysis of MSLI results

The MMAD, GSD and mass of drug contained in particles of a certain size can be derived from the MSLI data according to the method of May (Fuchs, 1978). The mass of drug collected at each stage is measured, and from this the cumulative percentage of the total collected at each stage and below calculated (the 'cumulative percentage undersize'), in a similar manner to that described above for calibrating the MSLI.

Plotting the cumulative percentage undersize against the $D_{50\%}$ for that stage on logarithmic probability paper gives a straight line when the aerosol particle size is log-normally distributed. From this line the MMAD, GSD and other parameters may be determined. This method determines values of MMAD correctly, but overestimates GSD, especially for monodisperse aerosols (GSD approximating to 1). Aerosols from metered dose inhalers and nebulisers used in this thesis were typically heterodisperse in character, and this problem does not arise.

In practice this process was automated in a Microsoft Excel worksheet. A line of best fit was fitted to the experimental data using the method of least squares, and visually compared to the experimental data. When the data and fitted line were closely approximated, data was derived using the least squares function. However, especially when devices with a large ballistic component such as metered dose inhalers were used, the experimental data lay not on a straight line, but had a small curvature at either end.

In this case, the line of best fit, and the subsequent values for MMAD etc., were derived from the mid points of the graph, as suggested by Fuchs (1978).

Airflow through the MSLI

The MSLI operates at a constant airflow, and was calibrated at a flow of 60l/min. Lower flows may be used with the MSLI (May, 1966), but the cut off diameters (d_{50}) for each stage are altered, according to the equation:

$$\text{New } d_{50} = \text{Old } d_{50} \times \sqrt{\frac{\text{Old flow (l/min)}}{\text{New flow (l/min)}}}$$

This topic is discussed further in Chapter 4.5.

The addition of fluid to the MSLI stages

In experiments using pressurised metered dose inhalers, the stages of the MSLI were dry. For experiments using nebulisers, the appropriate solvent for the drug analysis was added to each stage of the MSLI. Enough fluid was added to moisten the glass impaction plate, taking care not to cover it, or to add too much fluid to stage four, allowing it to spill over into the filter unit (May, 1966).

The addition of solvent was intended to reduce particle bounce and the chances of stage overload. It also increased the relative humidity in the MSLI (see below).

Temperature and Relative Humidity

The effect of fluid in the MSLI stages, and its effect on the measured particle size, was evaluated. Temperature in stages one to four, and relative humidity in stages one to three and the filter unit was measured using previously calibrated thermocouples (K type, Fluke Corporation, USA) and humidity probes (VH-L model, Vaisala, Finland). The MSLI was operated for five minutes dry, and with either distilled water or ethanol in the stages. Temperature and relative humidity were noted every thirty seconds. For each condition, triplicate runs were performed.

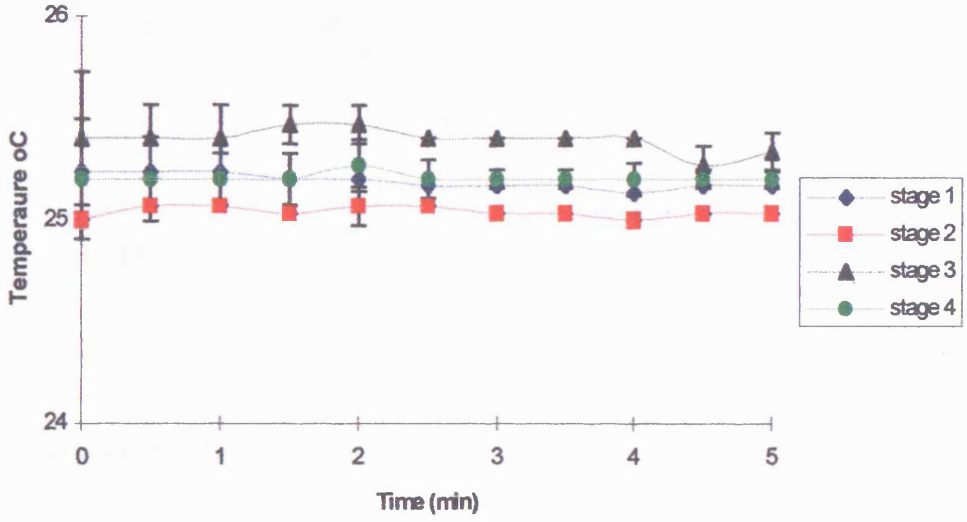
Laboratory temperature was 25°C, relative humidity 30%.

to three and the filter unit was measured using previously calibrated thermocouples (K type, Fluke Corporation, USA) and humidity probes (VH-L model, Vaisala, Finland). The MSLI was operated for five minutes dry, and with either distilled water or ethanol in the stages. Temperature and relative humidity were noted every thirty seconds. For each condition, triplicate runs were performed.

Laboratory temperature was 25°C, relative humidity 30%.

With the stages dry, temperature was constant (°C, mean and SD):

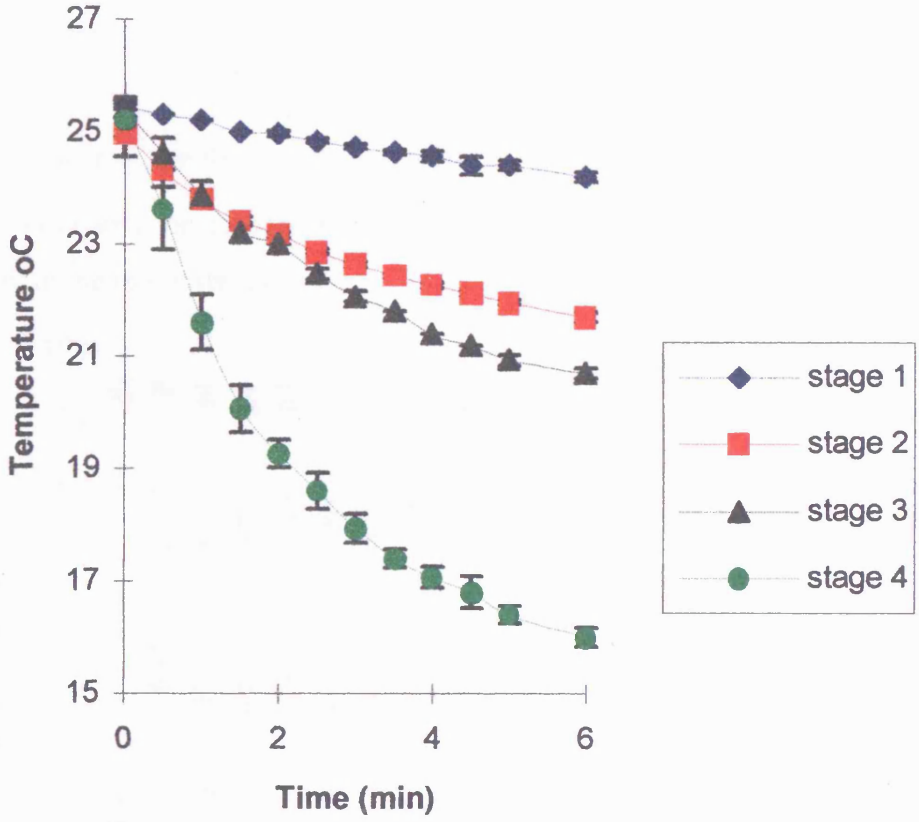
Time (min)	stage 1	stage 2	stage 3	stage 4
0	25.2 (0.3)	25.0 (0.3)	25.4 (0.3)	25.2 (0)
0.5	25.2 (0.2)	25. 1 (0.2)	25.4 (0.2)	25.2 (0)
1	25.2 (0.1)	25.1 (0.1)	25.4 (0.2)	25.2 (0)
1.5	25.2 (0.2)	25.0 (0.1)	25.5 (0.1)	25.2 (0)
2	25.2 (0.1)	25.1 (0.1)	25.5 (0.1)	25.3 (0.1)
2.5	25.2 (0.1)	25.1 (0.1)	25.4 (0)	25.2 (0)
3	25.2 (0.1)	25.0 (0)	25.4 (0)	25.2 (0)
3.5	25.2 (0.1)	25.0 (0)	25.4 (0)	25.2 (0)
4	25.1 (0.1)	25.0 (0.1)	25.4 (0)	25.2 (0)
4.5	25.2 (0.1)	25.0 (0)	25.3 (0.1)	25.2 (0)
5	25.2 (0.1)	25.0 (0)	25.3 (0.1)	25.2 (0)



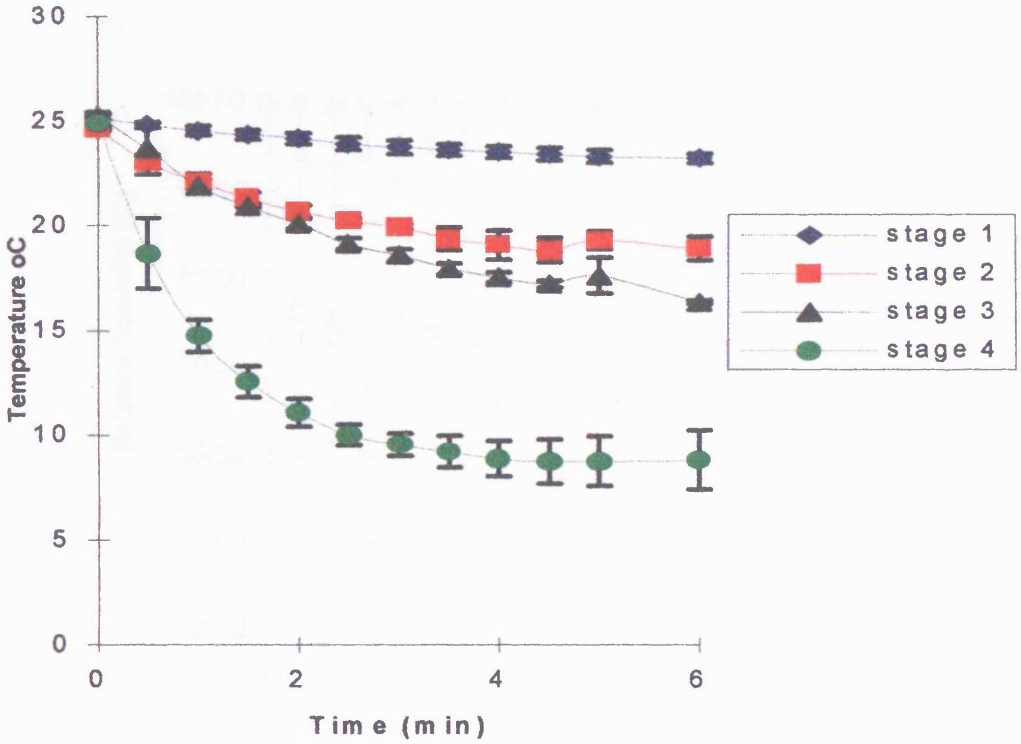
As was humidity after the first 30 seconds (% relative humidity, mean and SD):

Air 30 L/min	stage 1	stage 2	stage 3	stage 4
0	31.5 (0.5)	28.7 (1.1)	28.3 (1.5)	29.0 (1.5)
0.5	30.4 (0.1)	27.0 (0.2)	25.4 (1.1)	24.7 (1.1)
1	30.4 (0.1)	27.0 (0.2)	25.7 (1.0)	24.7 (1.0)
1.5	30.5 (0.2)	27.0 (0.2)	25.6 (1.0)	24.8 (1.0)
2	30.5 (0.2)	27.2 (0.1)	25.7 (0.9)	24.7 (0.9)
2.5	30.5 (0.2)	26.9 (0.2)	25.3 (0.6)	24.7 (0.6)
3	30.6 (0.2)	27.3 (0.2)	25.8 (0.8)	24.7 (0.8)
3.5	30.6 (0.1)	27.1 (0.2)	25.6 (0.9)	24.7 (0.9)
4	30.6 (0.2)	27.1 (0.3)	25.4 (1.0)	24.5 (1.0)
4.5	30.6 (0.3)	27.2 (0.1)	25.6 (1.0)	24.5 (1.0)
5	30.8 (0.2)	25.0 (0.0)	25.8 (0.9)	24.8 (0.9)

However, with the stages wet with either water or ethanol, temperature fell:

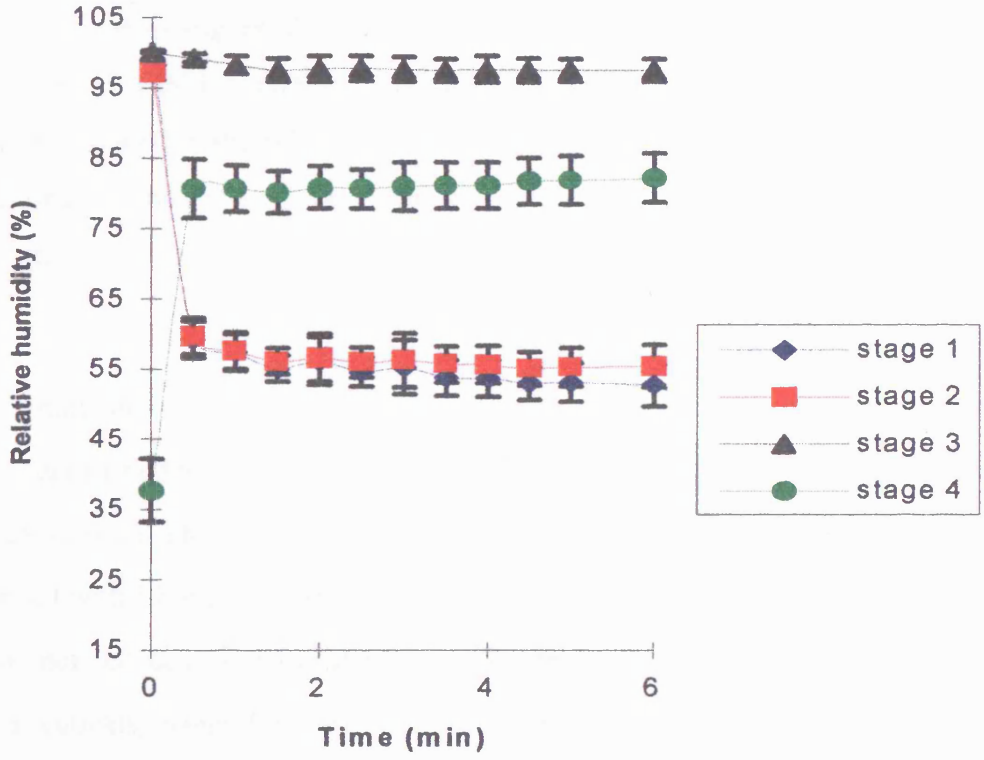


Effect of ambient air flow through wet (20ml water per stage) MSLI on temperature in each stage.

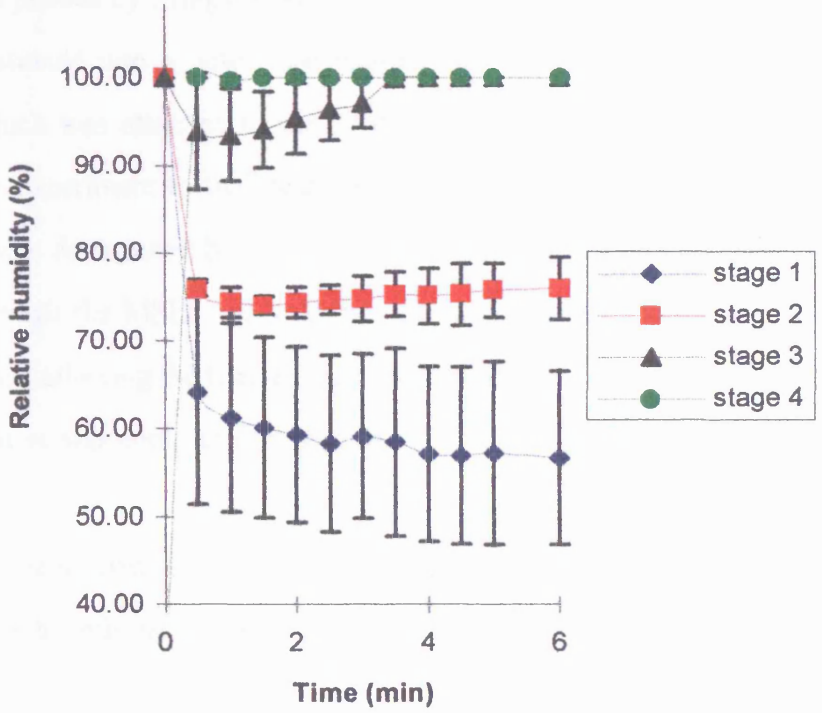


Effect of ambient air flow through wet (20ml ethanol per stage) MSLI on temperature in each stage

And humidity was increased compared to operating the MSLI with the stages dry, especially in the lower stages.



Effect of ambient air flow through wet (20ml water per stage) MSLI on humidity in each stage.



Effect of ambient air flow through wet (20ml ethanol per stage) MSLI on temperature in each stage

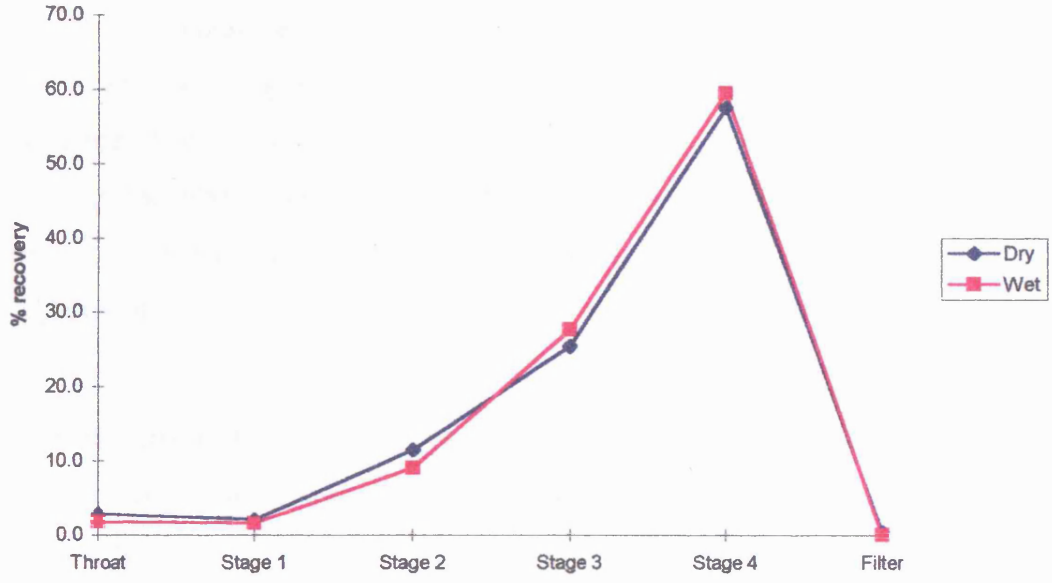
These experiments suggest that there is little change in the temperature and humidity of the air in the MSLI when used without solvent in the stages. In contrast, both water and aerosol placed in the MSLI stages lead to rapid falls in temperature as the device is operated. In most cases, these changes are almost complete by two minutes of operation.

To determine the effect of wetting the MSLI stages on measured aerosol particle size, the amount of budesonide depositing in the MSLI from a metered dose inhaler used with an Aerochamber spacer was measured with the MSLI stages either dry or moistened with 10 mls of ethanol.

A new metered dose inhaler of budesonide 200μg per actuation (Pulmicort, Astra Pharmaceuticals, Kings Langley, UK) was obtained. The first ten actuations were fired to waste. Immediately prior to each experiment, the MDI was shaken for thirty

seconds and primed by firing one actuation. The MDI was then shaken for ten seconds and then actuated into a new Aerochamber MV spacer (Trudell Medical, London, Canada) which was attached to the MSLI. This procedure was repeated five times during each experiment to facilitate the drug assay. The amount of drug collected at each stage was determined by high pressure liquid chromatography (see Chapter 5.7). Airflow through the MSLI was switched on two minutes prior to attachment of the Aerochamber, allowing the temperature and humidity changes to come to steady state. The experiment was completed in triplicate.

The percentage of drug recovered from each stage is shown in the following graph for the experiments with the MSLI stages dry and wet:



This experiment suggests that the measured particle size of drug from a metered dose inhaler is not affected by wetting the MSLI stages, and by the humidity and temperature changes that occur during operation. In contrast, high environmental humidities have been shown in some studies to affect particle size measured with a cascade impactor (but not a liquid impinger) (Martin *et al.*, 1988), although not in all

studies confirm this (Miller and Purrington, 1996). Particles from MDIs may have reduced hygroscopic growth as they contain hydrophobic additives (Hickey and Martonen, 1993). Particles also travel through the MSLI quickly, and may not have time to reach equilibrium with the conditions in each stage before impacting.

Comparative experiments in this thesis were undertaken under controlled conditions, and the MSLI operating procedure, particularly with respect to the addition of solvent to the MSLI stages, was strictly controlled. In general, MDIs were tested with the MSLI stages dry, and nebulisers were tested with the stages wet.

Connecting the spacer device to the MSLI

In the experiments described in this thesis, the spacer device was held to one side of the MSLI throat at the same level ('off line'), and immediately after metered dose inhaler actuation the MSLI was moved to attach the throat to the spacer. Others have assessed spacers by connecting them to the inertial impaction device before metered dose inhaler actuation, and switching on the vacuum pump either just before or just after actuating the metered dose inhaler. To determine the effect of these different methods of assessment, the MSLI was used to determine the amount and particle size of sodium cromoglycate available from a metered dose inhaler (Intal, 5mg per actuation, Fisons, Loughborough, UK) and Fisonair spacer when attached to the MSLI in one of three ways:

'Continually attached' - the spacer is attached to the MSLI throat by a short plastic sock. The MSLI pump is switched on. The metered dose inhaler is actuated into the spacer device.

'In line' - the spacer is held in a retort stand with the mouthpiece 1cm from the MSLI throat, in line with the throat opening. The MSLI pump is switched on. The metered dose inhaler is actuated into the spacer device and the spacer is immediately attached to the MSLI throat by a short plastic sock.

'Off line' - the spacer is held in a retort stand with the mouthpiece to one side of the MSLI throat. The MSLI pump is switched on. The metered dose inhaler is actuated into the spacer device and the spacer is attached to the MSLI throat by a short plastic

sock. The movement of the spacer causes a very short delay between metered dose inhaler actuation and attaching the spacer to the MSLI throat.

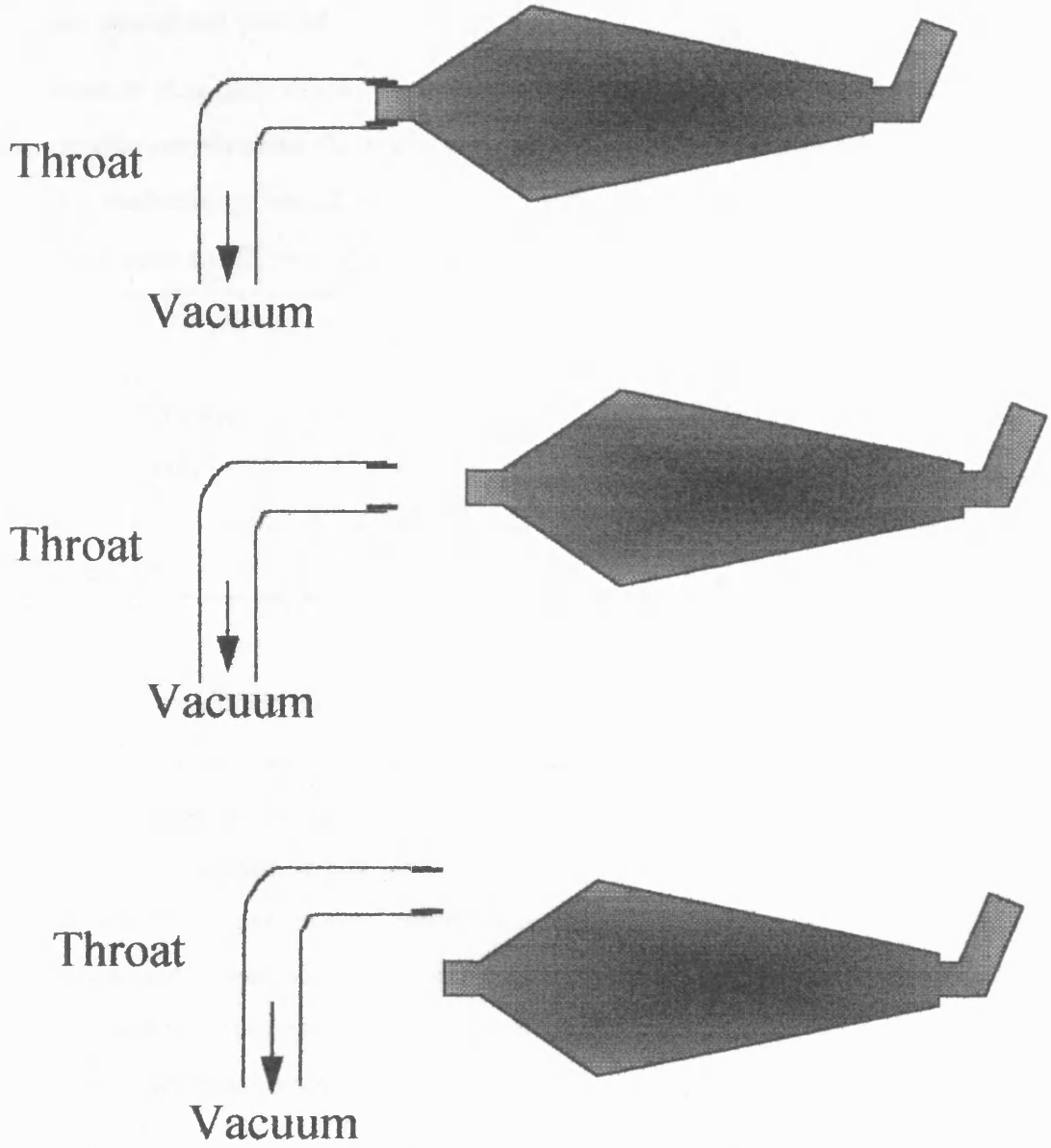


Figure: Position of the spacer and MSLI relative to each other at the time of MDI actuation: Continually attached (top), In line (middle), Off line (Bottom). At the time of MDI actuation, the spacer was positioned 'off line' for the experiments described in this thesis, and the MSLI moved to connect it with the spacer.

The process of actuating the metered dose inhaler into the spacer and attaching the spacer to the MSLI throat, where necessary, was repeated ten times to aid the drug assay. The different stages of the MSLI and parts of the experimental apparatus were then washed with trichlorofluoromethane to remove the propellants and then with

distilled water. The amount of sodium cromoglycate in the washings was determined by UV spectrophotometry (see Chapter 5.7). The experiment was repeated six times for each attachment method.

The amount of sodium cromoglycate recovered from the experimental apparatus, the mass median aerodynamic diameter (MMAD), the geometric standard deviation (GSD) and the calculated amount of drug contained in particles smaller than 5μm aerodynamic diameter (mean and 95% confidence intervals), per 5mg actuation, is given in the table:

	Continually attached	In line	Off line
Total recovery (mg)	**4.66** (4.6-4.7)	**4.62** (4.5-4.7)	**4.49** (4.4-4.6)
MMAD (μm)	**7.65** (7.0-8.3)	**6.14** (5.9-6.4)	**5.8** (5.5-6.1)
GSD	**2.24** (2.1-2.3)	**2.14** (2.0-2.2)	**2.15** (2.1-2.2)
mg <5μm	**0.52** (0.44-0.60)	**0.50** (0.46-0.53)	**0.29** (0.27-0.31)

Clearly there are differences in the drug recovery and measured particle size dependent upon the actuation and attachment procedure used. To overcome these and to standardise the connection procedure between the MSLI and spacers used in this study, the MSLI was mounted on a movable platform and the MSLI was moved to the spacer immediately after metered dose inhaler actuation. The spacer was held in place in a retort stand and clamp, and the spacer attached to the MSLI for at least ten seconds to ensure complete emptying. Any variations from this method are noted in the individual experiments described below.

Total output/filter studies

The total output of drug from spacers and nebulisers under constant flow or breathing simulation was assessed by collecting the drug onto electrostatic filter pads ('Filtrete' 3M, Minnesota, sold as Pari Electrostatic Pads, Pari Medical, West Byfleet, UK).

Filters were held in plastic filter holders (Pari Medical, West Byfleet, UK). The filter holders screwed together to allow placement of the filter, A plastic insert was used to reduce the dead space to eleven millilitres.

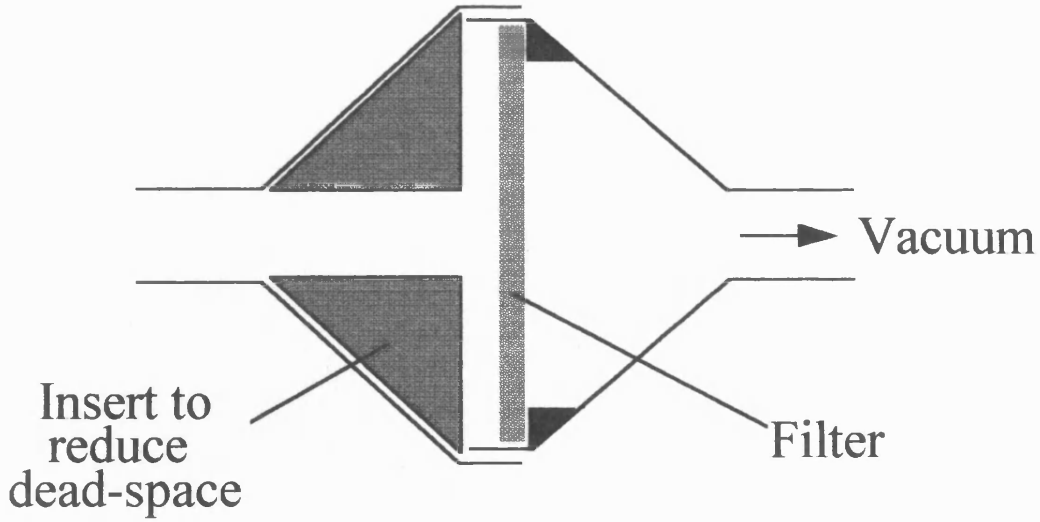


Figure. Filters and Filter Housing.

To measure the recovery of drug from the filters, filters were primed with a known amount of drug solution, allowed to dry and then washed with an appropriate solvent. The amount of drug collected was determined by high performance liquid chromatography (Chapter 5.7). This was repeated six times with each drug used in the filter studies. The recovery of budesonide from primed filters was 100.4% (95% confidence intervals 97.5% to 103.3%) of 200μg of drug. The recovery of salbutamol from primed filters was 99.3% (95% confidence intervals 97.8% to 100.8%) of 90μg of drug.

To ensure that the filters collected all drug particles, the recovery of drug from two filter assembles positioned in line was determined, with an air flow of 60l/min through the filters. The recovery of drug from the second of the two filters was less than the limit of detection of the drug assay.

Breathing simulation

To simulate the respiratory patterns of patients using inhalational drug delivery devices, spacers and nebulisers were tested using a breathing simulator, (Pari Sinus Breathing Simulator, Pari GmbH, Starnberg, Germany). This comprises a piston pump (volume 250mls or 1,000mls), connected to a drive wheel which rotates at a variable rate. The piston may be connected to different positions on the drive wheel, producing a different excursion of the piston with each rotation, and hence altering the volume of air pumped, analogous to the tidal volume. The frequency of the rotations, analagous to the respiratory rate, could also be adjusted between nought and sixty revolutions per minute. Finally the ratio between 'inspiratory' and 'expiratory' fraction could be varied.

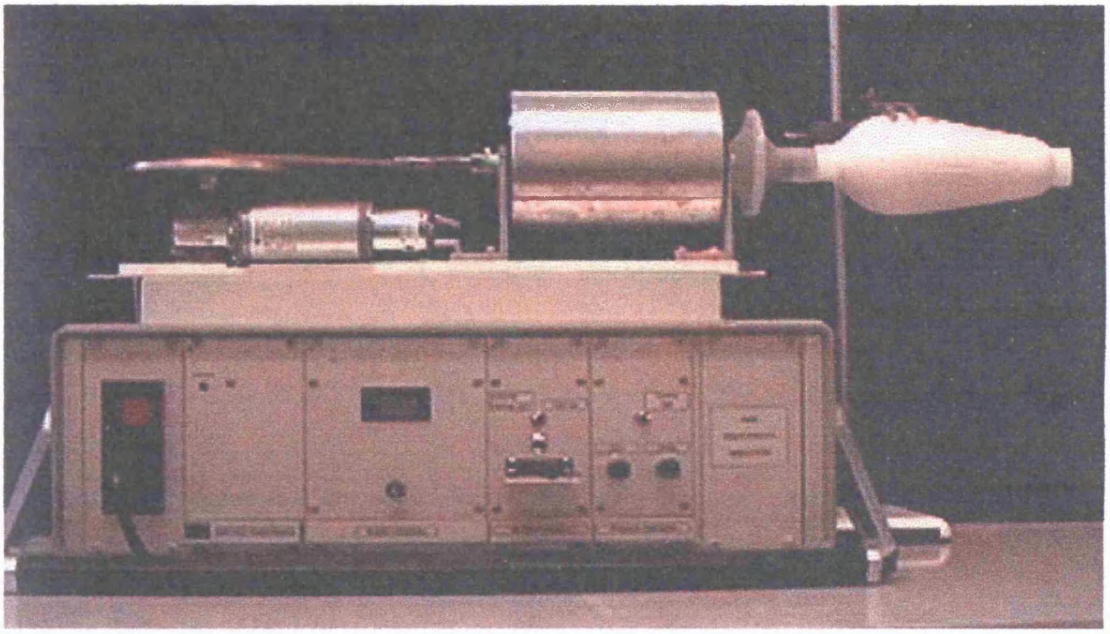


Figure: The Pari Sinus Breathing Simulator.

Filters placed between the breathing simulator and the inhalational drug delivery device under test (Pari electrostatic filter pads, Pari GmbH, Starnberg, Germany) collected drug released from the device. The filters were held in a plastic filter holder (dead space 11 ml). Spacers assessed with a face mask were held against a face plate

which the filter holder fitted (figure). Spacers without a face mask were connected directly to the filter holder. Nebulisers were connected to the filter holder by the T-piece or mouth-piece supplied by the nebuliser manufacturer. Waste aerosol released during 'expiration' was scavenged using a household vacuum cleaner or collected onto an 'expiratory' filter (figure).

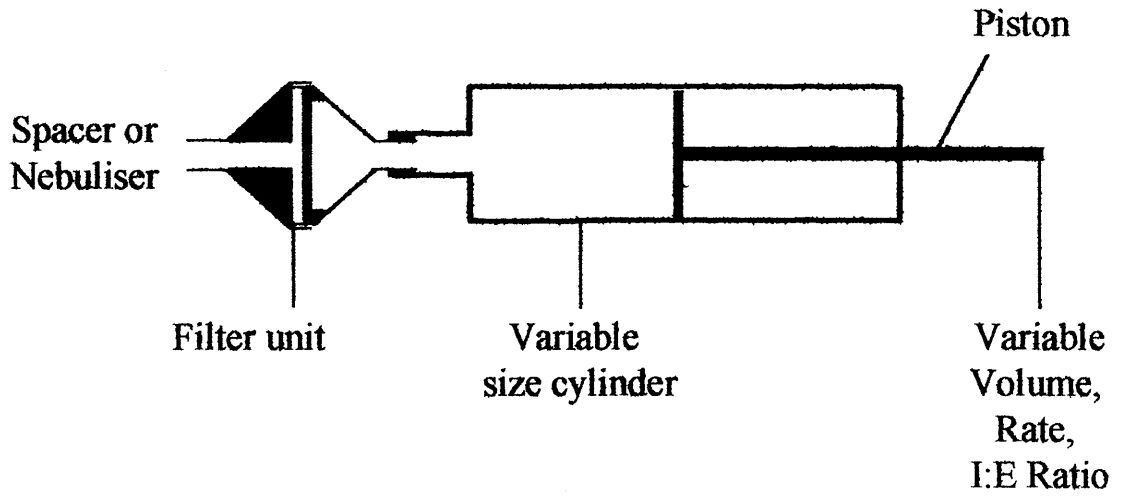


Figure: Schematic of the breathing simulator.

The breathing simulator was calibrated by measuring flow with a screen pneumotachograph linked to a personal computer running Respiratory Analysis Software Program (Physiologic, UK).

Eight runs of up to 25 breaths were repeated with the breathing simulator set at two different breathing patterns; 150ml tidal volume, 20 breaths per minute, inspiratory fraction 40%; and 600ml tidal volume, 12 breaths per minute, inspiratory fraction 40%.

Mean tidal volume was 140ml (SD 0.9) and 576ml (SD 1.8); Mean respiratory rate 19 breaths per minute (SD 0.1) and 11.4 breaths per minute (SD 0.02). Mean inspiratory fraction was 43.6%. These results suggest that the breathing simulator delivers slightly less volume than set at a slightly lower rate, but that the pattern is stable with low variability between breaths.

Measurement Aerosol Speed and Volume by High Speed Video

High speed video recording was undertaken using the Kodak EKTAPRO HS 4540 motion analysis system (Kodak Ltd, Hemel Hempstead, UK), sampling images at 4500 or 9000 frames per second, and recording them on a VHS video tape.

The camera was positioned to allow the fully developed aerosol plume to be captured in the frame. Exact details of camera positioning relative to the MDI were noted, and were reproduced exactly for all recordings in each experiment. Each video frame recorded displayed a unique identification number, date of recording and the elapsed time.

The experimental area was lit by a series of tungsten filament lamps arranged sequentially above and below the area of interest in the focal plane of the aerosol plume, and to the sides. The lights were positioned just outside the video frame and at a distance from each other to provide continuous and even lighting throughout the length of the fully developed plume. A black velvet background was used for maximum contrast.

Live images are recorded by the camera in solid state memory in a 'ring buffer'. This means that the camera saves a continuous series of images until the buffer is full, and then sequentially overwrites the oldest image with a new one until an event is triggered. Triggering may be configured to save a series of frames from the time of triggering, from before triggering, or from around the time of triggering. The video was triggered manually at the onset of MDI actuation. Images were then recorded onto a VHS video tape.

A video player (Sharp VC 8381H, Sharp Corp., Japan) and monitor (Hitachi VM 910E/K, Hitachi Denshi, Japan) were used to play back the video at a much slower rate, and measurements of the aerosol cloud were made with callipers on the monitor screen, starting from when the cloud could first be discerned appearing at the MDI adapter exit. A 30cm rule was recorded on video held horizontally and vertically to calibrate the monitor measurements.

Measurements were made from the MDI adapter to the leading edge of the cloud, allowing aerosol speed to be computed. Volume of the aerosol cloud was estimated by measuring the maximum vertical dimension of the cloud, the horizontal distance from the MDI adapter to the maximum vertical dimension, and the distance from there to the leading edge of the cloud. The cloud was assumed to be conical in shape from the MDI adapter to its maximum vertical dimension, and cylindrical from there on to the leading edge. Measurements of volume and speed were made until the leading edge of the aerosol cloud left the video frame or could not be discerned.

Video images of the aerosol cloud were imported onto a personal computer (Asymetrix Learning Systems, USA) and further enhanced using Aldus Photostyler v1.1 (Aldus Europe Ltd, Edinburgh, UK).

Measurement of Electrostatic Charge

The evaluation of static charge was made indirectly, by measuring the electrical field that the charge produced in its surroundings. The electrical field at a certain point represents the force exerted on a unit positive charge at the point where the measurement is made. Electric field is expressed in units of volts/meter.

Electrostatic field meters are of two main types, 'induction probe' and 'field mill' instruments (British Standards Institute, 1995 and 1996). Induction probe instruments consist of a sensing surface with a high capacitance to earth connected to a high input impedance amplifier (Taylor and Secker, 1994). The signal V (in volts) observed when a sensing surface of area A (in m^2) with an input capacitance C (in Farads), is exposed to an electric field E (in volts/m) is given by the equation: $V = \varepsilon_0 EA/C$, where ε_0 is the electric constant (8.85×10^{-12} F/m). Induction probe instruments have a finite input time constant because of input leakage resistance, and are therefore used for relatively short measurement times, and require zeroing prior to each measurement. Field mill type instruments use a rotating or oscillating screen (chopper) to modulate the coupling of the electrostatic field with the sensing surface {Chubb, 1990}. So long as the timescale of the modulation is substantially shorter than the product of the input capacitance and the effective input resistance to earth, an alternating signal is generated which is independent of the rate of modulation and of the value of the input resistor. Phase sensitive detection of this alternating signal, after amplification, generates and output signal whose strength and polarity relate proportionally and directly to the observed electrostatic field.

Two electrostatic meters were used in this project. Initially the Simco Electrostatic Locator type SS-2 (loaned by Static Safe Environments Ltd., Birmingham, UK), an induction probe meter, was used. In order to make measurements inside assembled spacer devices, the Simco SS-2 was later replaced by the smaller JC-211 Electrostatic Mini-Probe (John Chubb Instrumentation Ltd., Cheltenham, UK), a field mill instrument.

Description of the Simco SS-2 electrostatic locator

This is an induction probe meter consisting of a meter and probe housing (figure), and a remote probe connected to the instrument by a three foot coaxial cable. The cylindrical probe is some 35mm in diameter and 20mm high. Observations are displayed on an analogue needle in either volts or kV/m. The meter allows a range of potential to be measured by a combination of a x1 and x2 multiplier switch, and a variable aperture size for the remote probe. These allow readings to be modified x1 (no aperture fitted) x10 and x100 with decreasing aperture size. Readings from the meter are corrected by the appropriate multiplier, dependent upon the aperture used. A control on the meter allows the zero point to be adjusted with the aperture closed and the meter connected to earth by a grounding wire (selected by using the probe grounding switch). Operation is by internal batteries, and a function switch on the meter allows the battery condition to be checked.

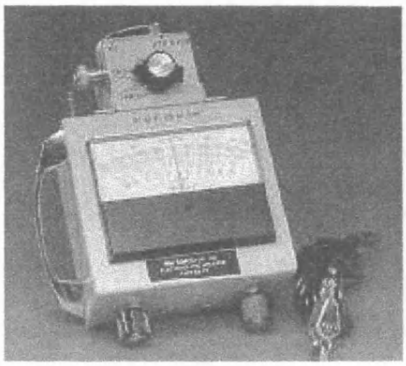


Figure: Simco Electrostatic Locator SS-2

Calibration of the Simco SS-2

The Electrostatic Locator was calibrated by the manufacturer to measure the potential of large flat sheets, by placing the locator 2 inches from a flat metal sheet 16 inches square. A known electrical potential was then applied to the sheet, and the locator meter adjusted to read this potential. Thus if the metal sheet were replaced by a

With this calibration method, flat sheets larger than 16 inches square with the same surface charge density as the calibration sheet will give essentially the same potential reading. However, smaller sheets, with the same charge density, can give much lower readings on the locator. For this reason it may be impossible to make accurate measurements of electrical potential for comparison between small or curved charged objects of slightly different size or shape. However, comparisons between different objects of the same size and shape would be valid, although the factory calibration, and hence the absolute value of the units of measurement, would not be.

Use of the Simco SS-2

The SS-2 was used to measure the electrostatic field within spacer devices. As the remote probe head was too large to pass though either the mouthpiece or actuator ends of spacer devices used in this study, these were disassembled prior to measurement.

The meter was grounded prior to use by connection to earth, and the meter battery condition checked prior to each use.

The probe aperture was closed by covering with the palm of the hand, and the meter reading adjusted to zero with the probe grounding switch set to 'Ground'. The lowest sensitivity aperture for the remote probe was selected. Each part of the spacer was held in a retort stand and, after setting the probe grounding switch to 'Operate', the remote probe of the SS-2 was placed within the spacer 5cm from the edge and 5cm from the spacer wall. The field strength at that point was noted. If the measured field strength was less than 10% of full scale deflection, the measurement was repeated with the next most sensitive aperture for the remote probe.

Description of the JC 211 Electrostatic Mini-Probe

This is a field mill type instrument consisting of a probe some 2.4mm in diameter and 275mm long {Chubb, 1990}. The probe contains a drive and pre-amplifier unit, and is cable connected to a signal processing and power supply unit. Observations are displayed on a 3 digit liquid crystal display with polarity and low battery indication.

This is a field mill type instrument consisting of a probe some 2.4mm in diameter and 275mm long {Chubb, 1990}. The probe contains a drive and pre-amplifier unit, and is cable connected to a signal processing and power supply unit. Observations are displayed on a 3 digit liquid crystal display with polarity and low battery indication. Sensitivity is adjusted by selecting a sensitivity range manually or by an auto-ranging option. Sensitivity ranges from 200-20,000 volts FSD. In air the maximum potential is limited to about 7kV by corona discharging from the probe tip. Operation is either by the internal batteries or mains power. Analogue signal outputs (± 2V FSD) may be made to a chart recorder (Servoscribe 1S RE54120, Smith Industries Ltd, London, UK) or computer (IBM 80286 Personal Computer).

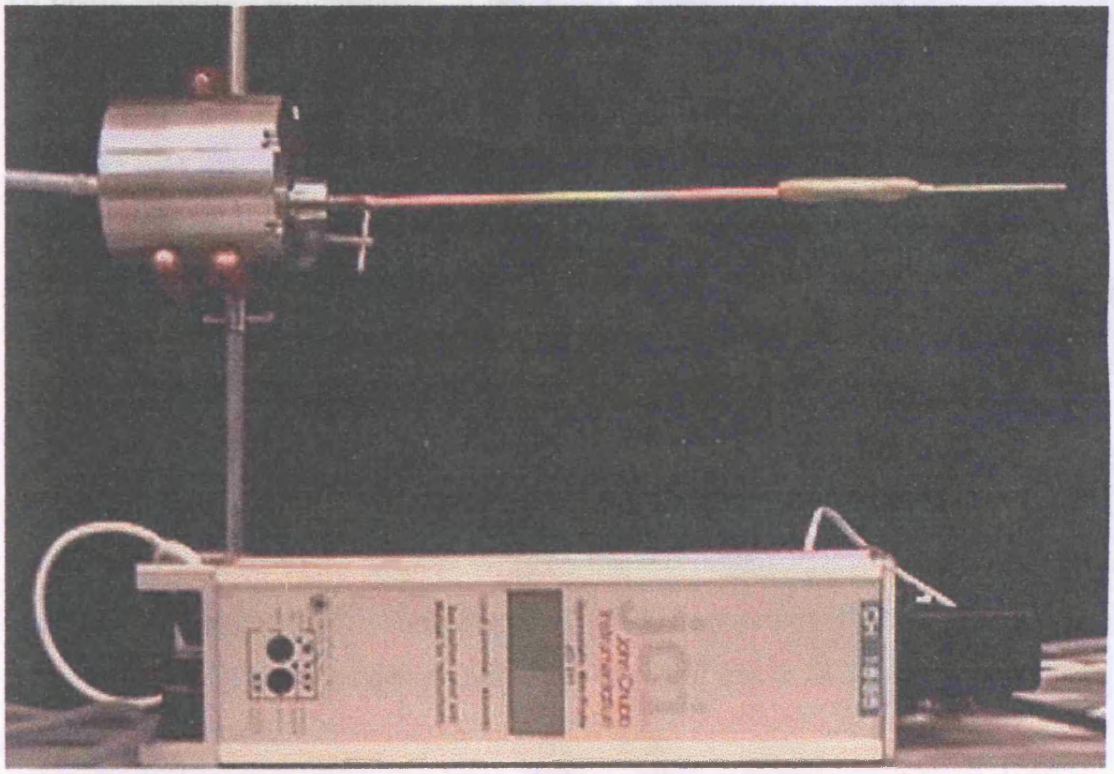


Figure: JC-211 Electrostatic Mini-Probe.

Calibration of the JC 211

The probe was calibrated prior to first use and subsequently at intervals during the project by the manufacturer. Sensitivity and linearity of the machine were checked by placing the earthed probe in a 'calibration chamber', a clean metal container of at least 150mm diameter and 200mm length, raised to a precisely known voltage. Meter

Use of the JC 211

The JC 211 probe was held horizontally in a retort stand and switched on. The spacer was held by another retort stand placed on two wheeled carriages which ran along to short pieces of track. Field strength inside both assembled and disassembled spacers was measured by smoothly moving the spacer towards the probe, so that the probe passed through the middle of the spacer. The measured field strength was recorded on a chart recorder (Servoscribe 1S RE54120, Smith Industries Ltd, London, UK), and the minimum and maximum filed strength noted on the chart paper.

To better regulate the movement of the spacer relative to the probe, an XY plotter (26000 A3 Plotter, Bryans Ltd., UK) was modified to carry the spacer and software written to control the movement of the spacer and to record spacer position and measured field strength. Mounted on a wooden holder, the spacer moved towards the probe so that the probe passed through the middle of the spacer, and the field strength was measured continuously. The spacer position (from the plotter) and the output voltage (± 2V from the JC 211) at each point were recorded as an array on an IBM personal computer (80286, IBM Corp., USA) via an analogue to digital conversion board (DT2801A, Data Translation). To convert the output field strength from the mini probe to the observed potential, after the recording, the spacer moved to the point of maximum field strength, and the value on the JC211 was entered manually into the computer. The output values from the JC 211 in the array were then corrected to this maximum value. The array was exported to Microsoft Excel for further manipulation and graphical output.

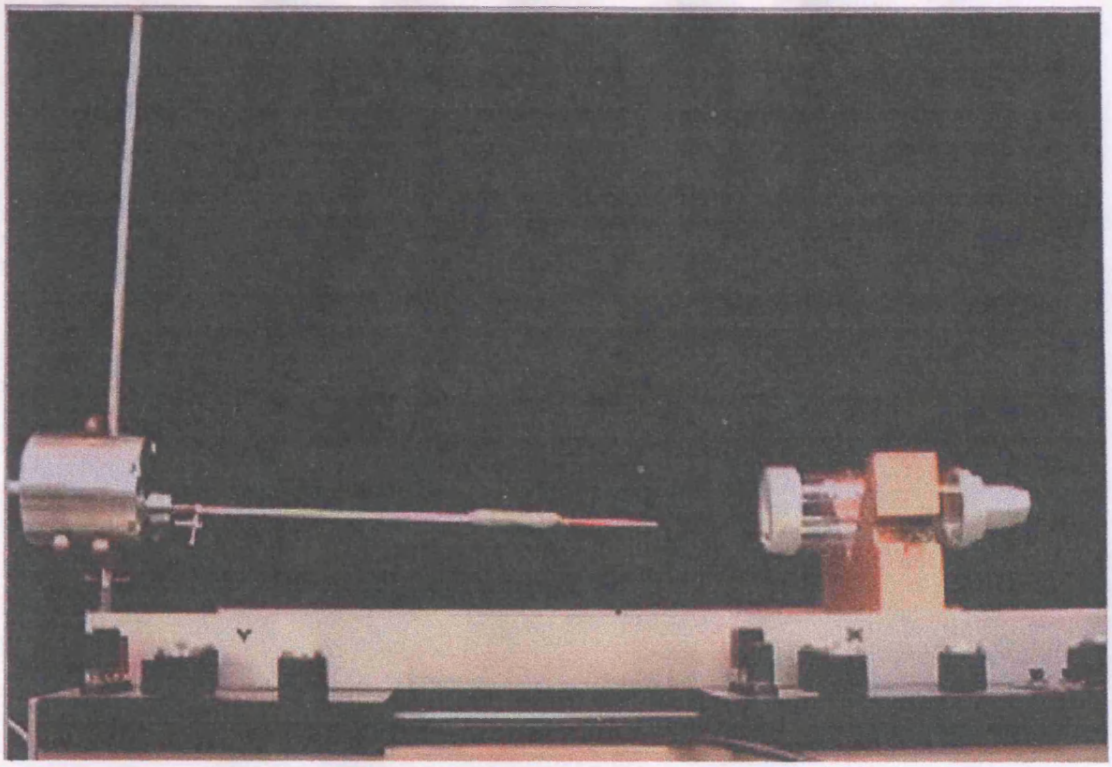


Figure. The JC 211 in operation - linear motion regulators

Units of field strength

Both the SS-2 and JC211 instruments give a measurement of field strength in units of Volts. However, due to the complex shape of spacers and the interaction of charge from different surfaces, the actual voltage recorded will not be equivalent to that produced by the same surface charge on a flat surface, or inside a different shaped spacer. Comparisons of field strength between different types of spacer may not therefore be valid, and for this reason field strength is quoted in arbitrary units, and comparisons are made only between different spacers of the same type.

Effect of local objects and laboratory conditions.

When making measurements, it was noted that the electrical potential could vary depending on the presence of other charged objects in the vicinity. For this reason, objects such as highly charged items of clothing or rubber gloves were not worn

static charge measurements, and the external conditions in the laboratory were kept constant.

Electrostatic charge is also affected by the humidity of the environment. While it was not possible to control humidity in the laboratory, this was recorded for each observation made.

Drug Assays

Throughout this project, drugs were assayed by spectrophotometry (sodium cromoglycate and nedocromil sodium), high performance liquid chromatography (salbutamol, beclomethasone dipropionate and budesonide) or fluorescence polarisation (gentamicin). The latter was undertaken by Dr R Swann, Consultant Microbiologist, Leicester Royal Infirmary, and is not considered further in the methods section of this thesis.

Weighing

Substances were weighed on a Mettler ME30 balance, (range 0-1000mg, 0.001mg graduations) or a Mettler H72 balance, (range 0-160gm, 0.1mg graduations), both MSE Scientific Instruments, Crawley, UK.

Glassware

Glassware was obtained from Fisher Scientific Ltd, Loughborough, UK.

Source of reagents

Sodium cromoglycate and nedocromil sodium were a gift from Fisons Research and Development, Loughborough, UK.

Salbutamol, beclomethasone dipropionate, testosterone and benzyl biphenyl were a gift from Glaxo Group Research, Ware, UK.

Budesonide and fluocinolone acetonide were a gift from Astra Draco, Lund, Sweden.

Helium was obtained from BOC Ltd.

All other reagents were obtained from Fisher Chemicals PLC, Loughborough, UK

Water was prepared by double distillation and deionised (Elgastat, UK).

Spectrophotometric assay - Sodium cromoglycate.

Washings from the experimental apparatus were collected in volumetric flasks and diluted to volume with distilled water. A Philips SP6-500 spectrophotometer was used to determine the absorbance of the solution at 326nm. The amount of sodium cromoglycate (mg) in the washings is given by the equation:

$$\frac{\text{Absorbance} \times \text{Volume of solution} \times 10}{E_1^1}$$

where the E_1^1 is the absorbance of a 1% solution of sodium cromoglycate in water, measured in a 1cm cell at 326nm (see below)

Spectrophotometric assay - Nedocromil sodium.

Washings from the experimental apparatus were collected in volumetric flasks and diluted to volume with distilled water. A Philips SP6-500 spectrophotometer was used to determine the absorbance of the solution at 253nm. The amount of nedocromil sodium (mg) in the washings is given by the equation:

$$\frac{\text{Absorbance} \times \text{Volume of solution} \times 10}{E_1^1}$$

where the E_1^1 is the absorbance of a 1% solution of nedocromil sodium in water, measured in a 1cm cell at 326nm (see below)

Determination of E (1%, 1cm)

The E (1%, 1cm) is the absorbance exhibited by a 1% solution of a compound in a given solvent measured in a 1cm cell at a given wavelength. In practice it is determined by accurately measuring the absorbance at a lower concentration and calculating the theoretical absorbance a 1% solution would produce. The following criteria need to be

considered when determining the E_1^1 of a standard batch of material:

- Allowance for the presence of any impurity should be made. The moisture content is of prime importance, and was measured by Fisons plc, who supplied the sodium cromoglycate and nedocromil sodium standards, by the Karl Fischer method.
- The linear range of absorbance for the test compound in solution should be determined, and measurements, both to determine the E_1^1 and to measure samples, should only be made within this range.
- Samples and standards must be analysed at the same wavelength and on the same instrument, as different instruments give slightly different absorbance readings for a given sample. The E_1^1 was determined at the start of each experiment described in the thesis that used this method of drug analysis, and after any maintenance (such as a bulb change) to the spectrophotometer.
- Standards and samples must be dissolved in the same solvents and should ideally be of similar concentrations. Complete dissolution and mixing of the compound is essential.

Procedure - Sodium cromoglycate:

Approximately 0.1gm of sodium cromoglycate of known moisture content was accurately weighed and dissolved in distilled water in a 250ml volumetric flask. This was diluted to volume and mixed well. A 10ml aliquot was transferred to a 100ml volumetric flask and this was diluted to volume and mixed well. The absorbance of the solution at 326nm was measured five times. The E_1^1 is given by the equation:

$$E_1^1 = \frac{\text{Absorbance} \times \text{original dilution (ml)} \times \text{second dilution (ml)}}{\text{weight (mg)} \times \text{aliquot (ml)} \times (100 - \text{moisture content})}$$

The E_1^1 was determined for a total of five solutions, and the mean calculated. The E_1^1 for sodium cromoglycate was approximately 155. Multiple dilution's of the test solutions were made. The E_1^1 was determined at different absorbences from these

solutions, and found to be linear for absorbances between 0.06 and 1.0.

Procedure - Nedocromil sodium:

Approximately 0.1gm of nedocromil sodium of known moisture content was accurately weighed and dissolved in distilled water in a 500ml volumetric flask. This was diluted to volume and mixed well. A 5ml aliquot was transferred to a 250ml volumetric flask and this was diluted to volume and mixed well. The absorbance of the solution at 253nm was measured five times. The E_1^1 was calculated as above.

The E_1^1 was determined for a total of five solutions, and the mean calculated. The E_1^1 for nedocromil sodium was approximately 850. Multiple dilutions of the test solutions were made. The E_1^1 was determined at different absorbances from these solutions, and found to be linear for absorbances between 0.06 and 1.0.

<u>High performance liquid chromatography (HPLC).</u>

The chromatography systems used for HPLC during this project were varied from the following equipment, depending on availability and breakdowns. One set of equipment was used for each experiment described in the subsequent chapters:

Pumps:

Milton Roy Constametric 3000 (Milton Roy, Florida, USA).

GBC LC1110 (GBC Scientific equipment PTY. Dandenong, Australia).

Spectraphysics G19 Gradient pump (Spectraphysics, San Jose, California, USA).

Spectraphysics P25 Isocratic pump (Spectraphysics, San Jose, California, USA).

Autosamplers:

Gilson 231 Autosampler & 401 Dilutor (Gilson Medical Electronics Inc., Winsconsin, USA).

Spectraphysics SP8780 Autosampler (Spectraphysics, San Jose, California, USA).

Shimadzu SIL5A Autosampler & SCL6A Controller (Shimadzu Corp, Kyoto, Japan).

Column heaters:

Block heater 7970, (Jones Chromatography, Barry, Wales).

Detectors:

Spectromonitor D, UV Detector (Milton Roy, Florida, USA).

Spectromonitor III, UV Detector (Milton Roy, Florida, USA).

GBC LC1200 UV Detector (GBC Scientific equipment PTY. Dandenong, Australia).

Pye Unicam LC UV Detector (Pye Unicam, Cambridge, UK).

HPLC columns and fittings were obtained from Fisher Scientific, Loughborough, UK. Peek or stainless steel tubings and fittings were used throughout. The columns and solvents used are noted in the individual methods below.

Solution measurements were made and dispensed with Gilson Pipettes (Gilson Medical Electronics Inc., Winsconsin, USA) or Zippette Dispensers (Jencons Scientific, Bedfordshire, UK).

Computation of results.

Chromatograms were integrated using a Philips PU4811 integrator (Pye Unicam, Cambridge, UK), or were collected on a personal computer (Dell dimension, 486/25s) and integrated using specialised HPLC integration software (SUMMIT, version 1.2., Comus, Newcastle, UK).

Internal Standard Methodology.

This method of determining how much of the compound of interest is in a sample relies on accurately adding a known amount of another compound (an *internal standard*) to the samples, and using that as a marker for the compound of interest.

Firstly solutions are made up containing a known amount of the compound of interest, and a known amount of the internal standard (Reference solutions). From chromatograms of these solutions, the relative detector response of the two compounds can be computed under the chromatographic conditions.

For instance, assume that a reference solution containing, say, 10mg of the compound of interest, and 10mg of internal standard has a chromatogram with the peak due to the compound of interest being 500 units of area, and that due to the internal standard 1000 units of area. In this case the relative response of the two compounds is 2, as the internal standard produces a peak twice as large as the same mass of the compound of interest.

An accurately known amount of internal standard is added to the sample, most commonly in these experiments MSLI stage washings. Assume that 10mg of internal standard is added to the MSLI stage, which is then washed out and an aliquot taken for analysis. Also assume that in the resulting chromatogram the peak due to the compound of interest is 1.5 times the area of the peak due to the internal standard. We know from the reference solution that the relative response is, in this case, 2. The amount of compound of interest present is therefore 2 times 1.5 times the amount of internal standard present, or 30mg.

It is important to note that the size of the peaks is not important, but the relationship between the two is.

The advantages of the internal standard method are that as long as the internal standard and compound of interest are freely mixed in the sample, once the internal standard is added, spillages and other losses from the experimental apparatus and differences in sample handling and injection volumes between sample are unimportant. The internal standard method also removes the need to volumetrically wash out each stage of the MSLI, as long as an accurately known amount of internal standard has been added. The use of an accurate dispenser to give the same amount of internal standard to the samples and reference solutions also improves accuracy.

The ideal internal standard should be stable under the experimental conditions, should have a peak that does not interfere with the peak of the compound of interest, and the relative response between the compound of interest and the internal standard should be constant across the range of expected concentrations.

HPLC system suitability checks

Before each batch of samples were assayed, the HPLC system was checked by injecting a series of standard or reference solutions. The following parameters were calculated for these injections, and found to be within the limits given in the methods below. If the chromatograms were unsatisfactory, the HPLC system was adjusted until the sample injections were acceptable.

Resolution factor between two peaks (Rs)

$$Rs = \frac{2(t_2 - t_1)}{w_1 + w_2}$$

Where

t_1 = the retention time, in minutes, of the first peak.

t_2 = the retention time, in minutes, of the second peak.

w_1 = the width, in minutes, of the first peak at its base.

w_2 = the width, in minutes, of the second peak at its base.

Number of theoretical plates (N)

$$N = 5.54 \times \left(\frac{t_1}{w_{1\,1/2}} \right)^2$$

Where

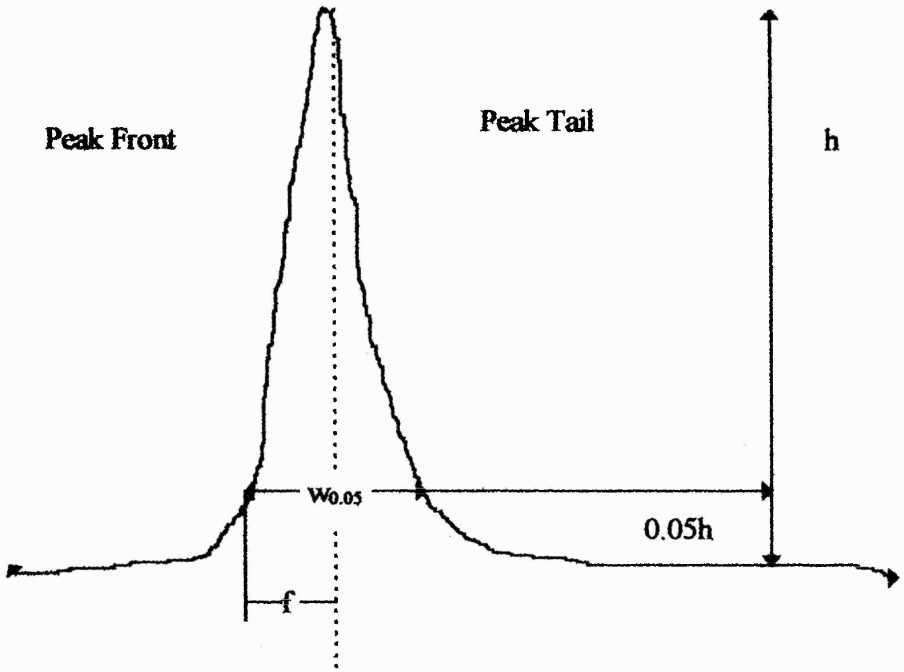
t_1 = the retention time, in minutes, of the first peak.

$w_{1½}$ = the width, in minutes, of the first peak at half its height.

Tailing factor (T)

The tailing factor is the width of the peak at 5% height, divided by twice the width between the start of the peak and the peak maximum at 5% height:

$$T = \frac{W_{0.05}}{2f}$$



HPLC Determination of Salbutamol

Typical chromatographic conditions.

Column:	10cmx4.5mm id. 5 µm Spherisorb ODS1 column or equivalent.
Column Temperature:	40°C
Mobile Phase:	75% methanol and 25% 0.1% w/v ammonium acetate solution. Filtered and degassed
Flow rate:	2ml/min
Detection:	UV at 276nm.
Injection volume:	200µl
Measurement:	Integration of peak area, relationship with internal standard.
Retention times:	Salbutamol 1.28min.
	Benzyl Biphenyl 3.00min.

Reagents.

Salbutamol	Working Standard
Benzyl Biphenyl	Internal Standard
Methanol	HPLC grade
Water	Distilled and purified.
Ammonium Acetate	HPLC grade

Standard Solutions

Mobile phase.

500mg of ammonium acetate (HPLC grade) was weighed into a 500ml volumetric flask. It was dissolved in distilled water and made up to volume.

1500ml of methanol (HPLC grade) was added to 500ml of 0.1% w/v ammonium acetate solution. The resultant solution was mixed, filtered through a Whatman GF/A

glass fibre filter and degassed for ten minutes by helium sparging. The ratio of the mobile phase components was altered to give acceptable retention times and resolutions.

Salbutamol standard solutions.

Duplicate standard solutions containing 10mg ± 1mg of salbutamol in 100ml of methanol were prepared.

Internal standard solution.

A solution containing 10mg ± 1mg of benzyl biphenyl in 100ml of methanol was prepared.

Reference Solutions.

A solution containing 30 ml of distilled water, 5ml of internal standard solution and 5ml of salbutamol standard solution was prepared in 100ml volumetric flasks, and made up to volume with methanol. This was repeated with the second salbutamol standard solution

HPLC system checks and validation.

Prior to each experiment, the performance of the HPLC system was checked by the following procedure:

The HPLC system was switched on, and a stable baseline established.

A blank solution of mobile phase was injected. The resulting chromatogram was observed to ensure that there were no relevant contaminating peaks.

The reference solution was injected.

Retention times for the two peaks was observed to be similar to those given above.

The resolution factor (Rs) between the two peaks of interest was greater than 1.5.

The number of theoretical plates (N) was greater than 300.

The tailing factor (T) was less than 2.

The method used for calculating the resolution factor, theoretical plates and tailing factor are given above.

The first reference solution was injected six times.

The coefficient of variation of the area of the standard peak was calculated to be less than 3%.

Duplicate injections of the two reference solutions were made and the repeatability of the standards calculated, such that:

$$\frac{A_{STD1}}{W_{STD1}} \times \frac{W_{STD2}}{A_{STD2}} = 1 \pm 0.03$$

Where:

A_{STD1} = Mean salbutamol peak area of the first reference solution

A_{STD2} = Mean salbutamol peak area of the second reference solution

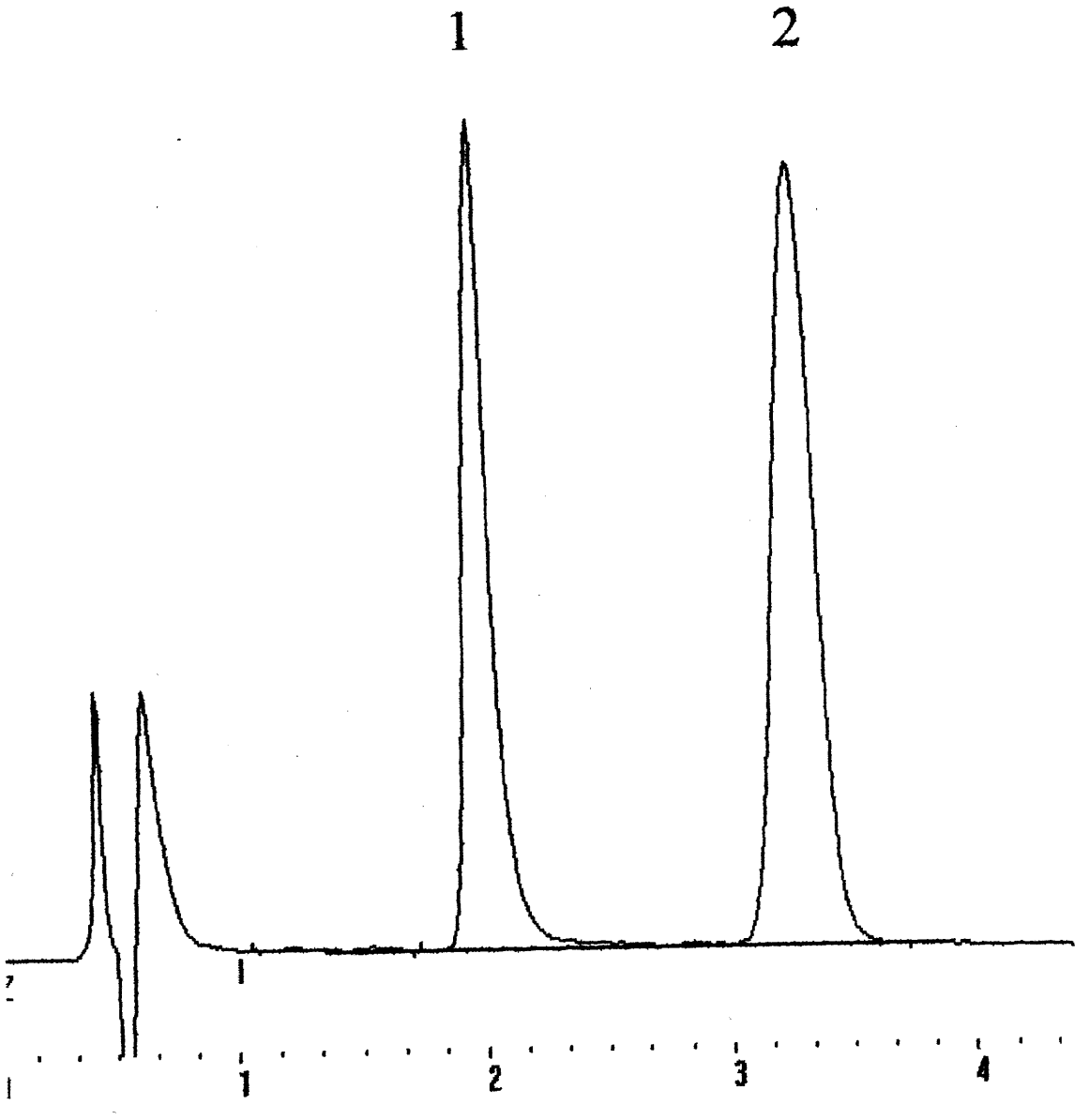
W_{STD21} = Weight mg of salbutamol from the first reference solution

W_{STD1} = Weight mg of salbutamol from the second reference solution

Where these criteria were not satisfied, the HPLC equipment was inspected for any faults, new solutions were made up or the HPLC column replaced as necessary, and the system checks repeated.

A typical chromatogram is shown in the figure.

Typical salbutamol chromatogram



1 Salbutamol
2 Benzyl biphenyl (internal standard)

Calculations

The response factor R for each injection of the reference solutions was calculated, where R is:

$$\frac{\text{Area Internal Standard Peak} \times \text{Weight drug in Reference Solution} \times \text{\% Purity drug}}{\text{Area drug Peak} \times \text{Weight Internal Standard in Reference Solution} \times 100}$$

The mean response factor, MRF, was then calculated.

For each sample, the amount of drug recovered per actuation is:

$$\frac{\text{Area drug Peak} \times \text{Weight Internal Standard in Sample} \times \text{MRF}}{\text{Area Internal Standard Peak} \times \text{No of Actuations.}}$$

Linearity, limit of detection and repeatability of the HPLC assay,

A stable baseline was established, and a single injection of reference solution was analysed. Resolution between salbutamol and internal standard peaks and theoretical plates were calculated.

Salbutamol solutions of concentration from 30μg/ml to 0.05μg/ml were made by serial dilution. Each solution was injected five times and integrated using Summit software.

The assay was linear across the range of concentrations tested (correlation coefficient > 0.99).

The limit of detection of the salbutamol peak was taken as that where no peak could be discerned at the expected retention time. The injection with the lowest concentration of salbutamol (0.05μg/ml) was still identified and integrated. The limit of detection of the assay was therefore less than 0.05μg/ml.

The coefficient of variation for the injection was less than 2% for concentrations of salbutamol greater than 0.5μg/ml. The variability of the assay rose significantly at lower concentrations, to 12.8% at 0.05μg/ml.

Detection of carry over between injections.

Injection of a solution containing 10μg salbutamol/ml was then made, followed by four injections of mobile phase to which internal standard had been added. The resulting chromatograms were examined to detect any carry over between injections. Where peaks were integrated at the same retention time as salbutamol would have been expected, the amount of salbutamol represented by these peaks was calculated, and expressed in absolute terms and in terms of a percentage of the amount of salbutamol in the first injection.

Peaks were integrated at 1min 37 seconds (the retention time of the salbutamol peak) on the first three of the four blank injections. These peaks, which appeared to be integration of 'noise', represented less than 0.01μg of salbutamol/ml, giving a percentage carryover of less than 0.1%.

It is not clear if the carryover detected is real, or whether it represents integration of noise. If it is present, any carryover is small, but may be significant for injections where a very low recovery (i.e. the throat) follows a very high one (i.e. the spacer). To avoid this blank solutions were run after the highest concentration samples in subsequent experiments.

HPLC Determination of Beclomethasone Dipropionate

Typical chromatographic conditions.

Column:	10cmx4.5mm id. 5 µm Spherisorb ODS1 column.
Column Temperature:	50°C
Mobile Phase:	75% methanol and 25% distilled water. Filtered and degassed
Flow rate:	2ml/min
Detection:	UV at 239nm.
Injection volume:	100µl
Measurement:	Integration of peak area, relationship with internal standard.
Retention times:	Beclomethasone dipropionate 1 min 30 Sec.
	Testosterone 3 min.

Reagents.

Beclomethasone dipropionate	Working Standard
Testosterone propionate	Internal Standard
Methanol	HPLC grade
Water	Distilled and purified.

Standard Solutions

Mobile phase.

1500ml of methanol (HPLC grade) was added to 500ml of distilled water. This was mixed, filtered through a Whatman GF/A glass fibre filter and degassed for ten minutes by helium sparging. The ratio of the mobile phase components was altered to give acceptable retention times and resolutions.

Beclomethasone standard solutions.

Duplicate standard solutions containing 3.6mg ± 0.3mg of beclomethasone dipropionate in 100ml of methanol were prepared.

Internal standard solution.

A solution containing 30mg ± 3mg of testosterone propionate in 100ml of methanol was prepared.

Reference Solutions.

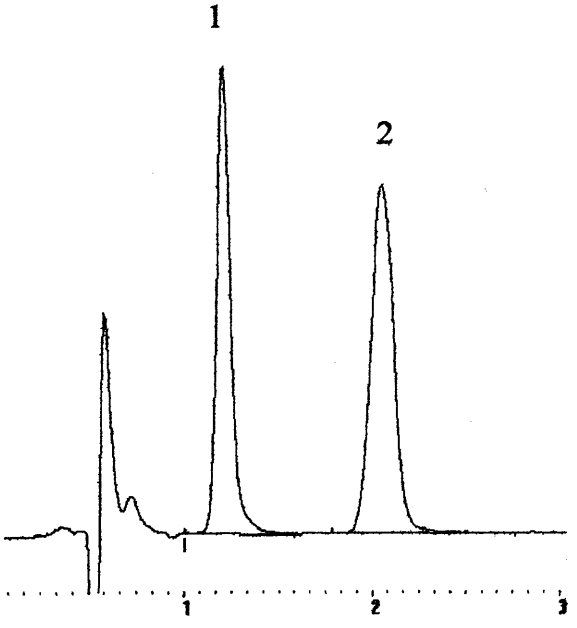
A solution containing 30 ml of distilled water, 5ml of internal standard solution and 5ml of beclomethasone standard solution was prepared in 100ml volumetric flask, and made up to volume with methanol. This was repeated with the second beclomethasone standard solution.

HPLC system checks and validation.

Prior to each experiment, the performance of the HPLC system was checked by the following the same procedure as outlined above for salbutamol.

A typical chromatogram is shown in the figure.

Typical beclomethasone chromatogram



1 Beclomethasone diproprionate
2 Testosterone proprionate (internal standard)

Calculations

The response factor R was calculated for each injection of the reference solutions, where R is:

$$\frac{\text{Area Internal Standard Peak} \times \text{Weight drug in Reference Solution} \times \%\text{ Purity drug}}{\text{Area drug Peak} \times \text{Weight Internal Standard in Reference Solution} \times 100}$$

The mean response factor, MRF, was calculated.

For each sample, the amount of drug recovered per actuation is:

$$\frac{\text{Area drug Peak} \times \text{Weight Internal Standard in Sample} \times \text{MRF}}{\text{Area Internal Standard Peak} \times \text{No of Actuations.}}$$

Linearity, limit of detection and repeatability of the HPLC assay,

This was estimated in the same way as described above for salbutamol.

The assay was linear across the range of concentrations tested, from 0-30μg/ml (correlation coefficient > 0.99).

The limit of detection of the assay was less than 0.05μg/ml.

The coefficient of variation for the injection was less than 2% for concentrations of beclomethasone greater than 0.2μg/ml. The variability of the assay rose significantly at lower concentrations.

HPLC Determination of Budesonide (First method)

This method was used for experiments described in chapter 6.1, 6.2 and some of the experiments in chapter 6.11. It was succeeded by the second, more robust method, described later in this chapter.

Typical chromatographic conditions.

Column:	25cmx4.5mm id. 5 µm CN column.	
Column Temperature:	Ambient	
Mobile Phase:	80% heptane and 20% ethanol. Filtered and degassed	
Flow rate:	1ml/min	
Detection:	UV at 254nm.	
Injection volume:	10µl	
Measurement:	Integration of peak area, relationship with internal standard.	
Retention times:	Fluocinolone acetonide	0 min 55 sec.
	Budesonide epimer B	2 min 00 sec.
	Budesonide epimer A	2 min 30 sec.

Reagents.

Budesonide	Working Standard
Fluocinolone acetonide	Internal Standard
Heptane	HPLC grade
Ethanol	Analytical grade

Standard Solutions

Mobile phase.

800ml of heptane (HPLC grade) was added to 200ml of ethanol (analytical grade). This was mixed, filtered through a Whatman GF/A glass fibre filter and degassed for ten minutes by helium sparging. The ratio of the mobile phase components was altered to give acceptable retention times and resolutions.

Budesonide standard solutions.

Duplicate standard solutions containing 5mg ± 0.5mg of budesonide in 100ml of ethanol were prepared.

Internal standard solution.

A solution containing 5mg ± 0.5mg of fluocinolone acetonide in 500ml of ethanol was prepared.

Reference Solutions.

A solutions containing 20 ml of internal standard solution and 5ml of budesonide standard solution was prepared. This was repeated with the second budesonide standard solution.

HPLC system checks and validation.

Prior to each experiment, the performance of the HPLC system was checked by the following the same procedure as outlined above for salbutamol.

HPLC Determination of Budesonide (Second method)

Due to problems with the resolution using the first budesonide HPLC method, and safety concerns about the use of large volumes of heptane, a second method was used for experiments described in chapters 6.3, 6.4, 6.6, 6.8, 6.13 and some of the experiments in chapter 6.11. This method is described here.

Typical chromatographic conditions.

Column:	5cmx4.5mm id. 5 µm Spherisorb ODS1 column
Column Temperature:	Ambient
Mobile Phase:	43% ethanol and 57% distilled water. Filtered and degassed
Flow rate:	2ml/min
Detection:	UV at 254nm.
Injection volume:	100µl
Measurement:	Integration of peak area, relationship with internal standard.
Retention times:	Fluocinolone acetonide 2 min 10 sec.
	Budesonide epimer B 4 min 30 sec.
	Budesonide epimer A 5 min 30 sec.

Note that the budesonide results in a fused peak, caused by epimers A and B. Epimer B elutes before epimer A.

Reagents.

Budesonide	Working Standard
Fluocinolone acetonide	Internal Standard
Water	Distilled and purified.
Ethanol	Analytical grade
Sodium dihydrogen phosphate	Analytical grade
Orthophosphoric acid	Analytical grade

Standard Solutions

Mobile phase.

430ml of ethanol (analytical grade) was added to 570ml of distilled water. This was mixed, filtered through a Whatman GF/A glass fibre filter and degassed for ten minutes by helium sparging. The ratio of the mobile phase components was altered to give acceptable retention times and resolutions.

Budesonide standard solutions.

Duplicate standard solutions containing 10mg ± 1mg of budesonide in 100ml of ethanol were prepared.

Internal standard solution.

A solution containing 10mg ± 1mg of fluocinolone acetonide in 500ml of ethanol was prepared.

Reference Solutions.

A solutions containing 20 ml of internal standard solution and 5ml of budesonide standard solution was prepared. This was repeated with the second budesonide standard solution.

0.025M Phosphate buffer

This was prepared by dissolving 15.84g of sodium dihydrogen phosphate in distilled water, adding 1.14g of orthophosphoric acid and diluting to 5,000ml with distilled water.

Sample preparation

Reference solutions and samples were prepared in ethanol. 0.5mls of reference or

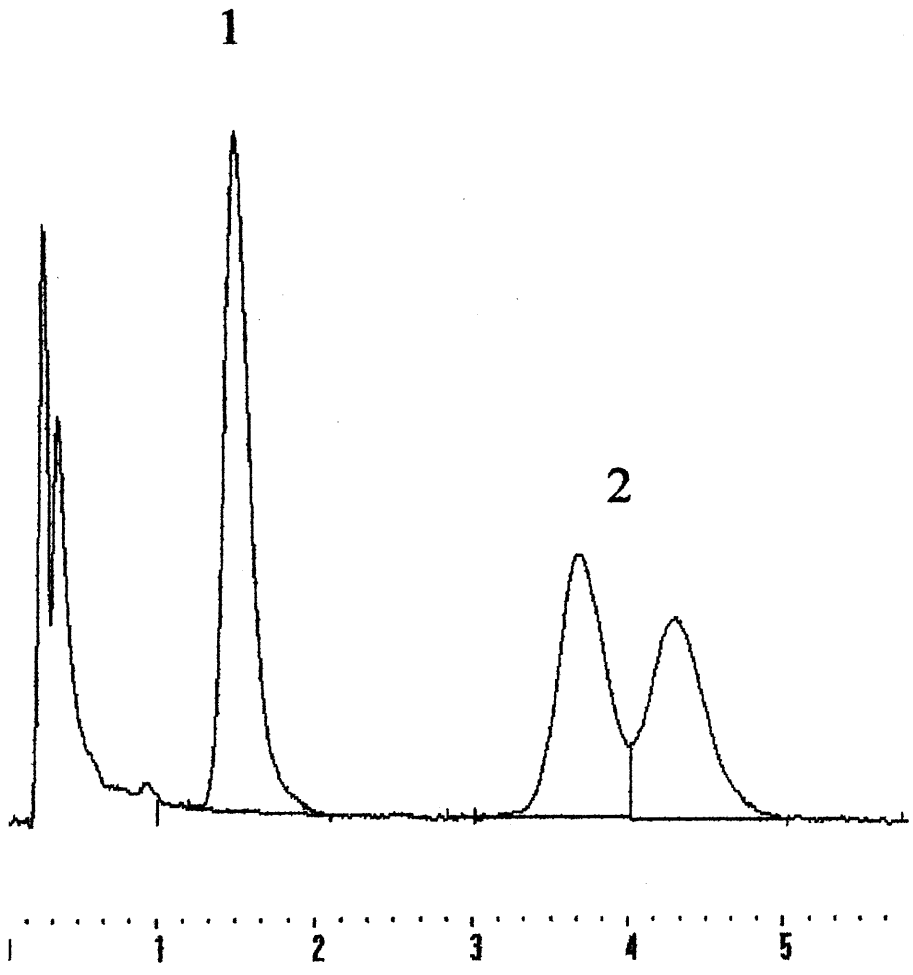
sample were mixed with 0.8mls of phosphate buffer prior to the HPLC assay.

HPLC system checks and validation.

Prior to each experiment, the performance of the HPLC system was checked by the following the same procedure as outlined above for salbutamol.

A typical chromatogram is shown in the figure.

Typical budesonide chromatogram



1 Fluocinolone acetonide (internal standard)
2 Budesonide (epimers B and A)

Calculations

Calculate the response factor F for each injection of the reference solutions, where F is:

$$\frac{\left(\frac{\text{Area budesonide peak}}{\text{Area internal standard peak}}\right) \times 50 \times 100}{m \times a \times 5}$$

where m is the amount of budesonide in the standard solutions (mg)
and a is the purity of budesonide in percentage

Calculate the mean response factor, MRF.

For each sample, the amount of drug recovered per actuation is:

$$\frac{\left(\frac{\text{Area budesonide peak}}{\text{Area internal standard peak}}\right) \times 1000}{\text{mean F} \times \text{number of actuations}}$$

Linearity, limit of detection and repeatability of the HPLC assay,

This was estimated in the same way as described above for salbutamol.

The system response was linear across the range of concentrations tested, from 0.1μg/ml to 200μg/ml (correlation coefficient > 0.99).

The limit of detection of the assay was 0.1μg/ml.

The coefficient of variation for the injection was 2.6% for concentrations of beclomethasone greater than 1μg/ml. The variability of the assay rose significantly at lower concentrations.

The effect of delay between metered dose inhaler actuation and sampling from spacer devices on the amount of drug recovered.

Aim

To determine the effect of delay between metered dose inhaler actuation and sampling from spacer devices on the amount of drug recovered.

Methods

The glass multistage liquid impinger was used to determine the MDI output under different conditions. The experiments were undertaken using nedocromil sodium, 2mg per actuation (Tilade, Fisons plc, Loughborough, UK) with the Fisonair™ (Fisons plc, Loughborough, UK); salbutamol, 100μg per actuation (Ventolin, Allen & Hanburys Ltd, Uxbridge, UK) and beclomethasone dipropionate, 100μg per actuation (Becotide, Allen & Hanburys Ltd, Uxbridge, UK) with the Volumatic™ (Allen & Hanburys Ltd, Uxbridge, UK); budesonide 200μg per actuation (Pulmicort, Astra Pharmaceuticals, Kings Langley, UK) with the Nebuhaler™ (Astra Pharmaceuticals, Kings Langley, UK); salbutamol and beclomethasone with the Aerochamber™ (Trudell Medical, London, Canada).

New metered dose inhalers of each drug were obtained. The first ten actuations from each MDI was fired to waste. Immediately prior to each experiment, the MDI was shaken for thirty seconds and primed by firing one actuation. The MDI was then shaken for ten seconds and actuated into the spacer, which was attached to the MSLI after a delay of one, five, ten or twenty seconds. This procedure was repeated ten times during each experiment to facilitate the drug assay.

For nedocromil sodium, each stage of the MSLI was washed quantitatively with distilled water. The amount of drug collected in each stage was assayed by UV spectrophotometry (see Chapter 5.7). For salbutamol, beclomethasone dipropionate and budesonide, each stage was washed with methanol or ethanol and the amount of drug collected at each stage determined by high pressure liquid chromatography.

Different Fisonair, Nebuhaler, Volumatic and Aerochamber spacers were used for each experiment, repeated four times with each spacer type. Except where otherwise noted, spacers were removed from their original packaging immediately before each experiment.

Results

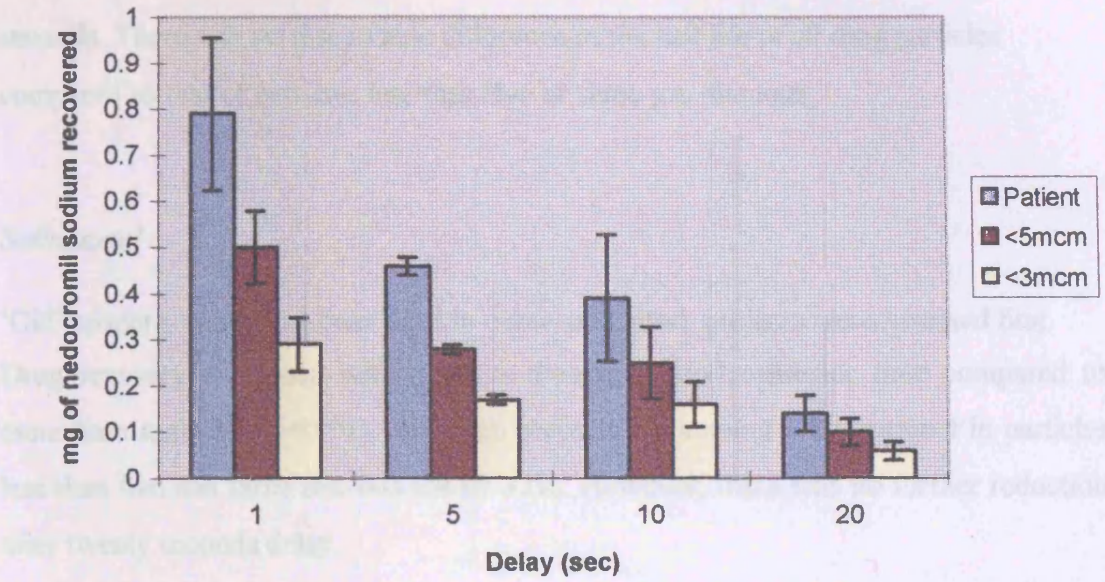
Nedocromil sodium

Drug recovery decreased with increasing residence time. After five seconds the amount of nedocromil sodium in particles less than five and three microns fell by 43%, after ten seconds by 50% and 47% respectively and after twenty seconds by 81% and 79% respectively.

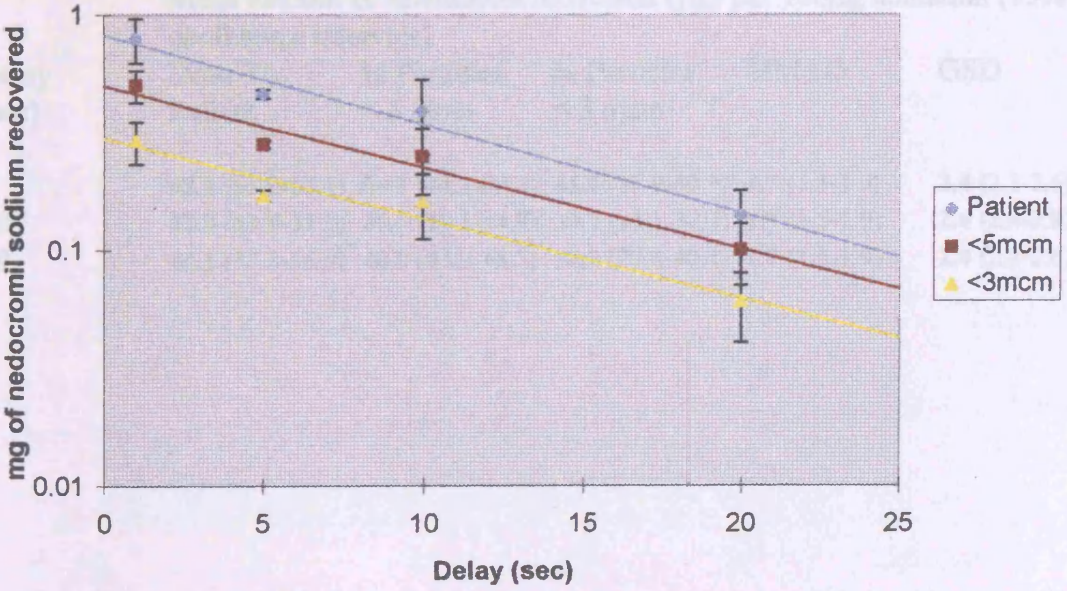
The amount of drug recovered (mean and 95% confidence intervals), the MMAD and GSD for the different experiments are given in the table.

The effect of delay on the delivery of Nedocromil Sodium from the Fisonair.

Delay (seconds)	Mean amount of Nedocromil Sodium recovered (mg) per 2mg actuation (95% confidence intervals).				
	Dose To Patient	In Particles < 5 mcm	In Particles < 3 mcm	MMAD	GSD
1	**0.79** (0.62-0.96)	**0.50** (0.42-0.58)	**0.29** (0.23-0.35)	**3.93** (3.41-4.45)	**2.00** (1.90-2.10)
5	**0.46** (0.44-0.48)	**0.28** (0.27-0.29)	**0.17** (0.16-0.18)	**3.95** (3.89-4.01)	**2.00** (1.95-2.05)
10	**0.39** (0.25-0.53)	**0.25** (0.17-0.33)	**0.16** (0.11-0.21)	**3.60** (3.29-3.91)	**2.10** (2.00-2.20)
20	**0.14** (0.10-0.18)	**0.10** (0.07-0.13)	**0.06** (0.04-0.08)	**3.50** (3.23-3.77)	**2.50** (1.98-3.02)



Plotted on a semi-log scale, the decrease in drug recovery is shown in the next graph, with a line of best fit also plotted.



The decrease in the recovery of nedocromil sodium (in particles smaller than 5μm) with time is best described by the equation:

Log[NS] = -0.302 - 0.0346(time)

R-squared = 0.96, $p<0.01$.

The 'half life' of nedocromil sodium in the Fisonair spacer was approximately 8 seconds. There was no discernable difference in the half life of all drug particles compared to that of particles less than five or three μm diameter.

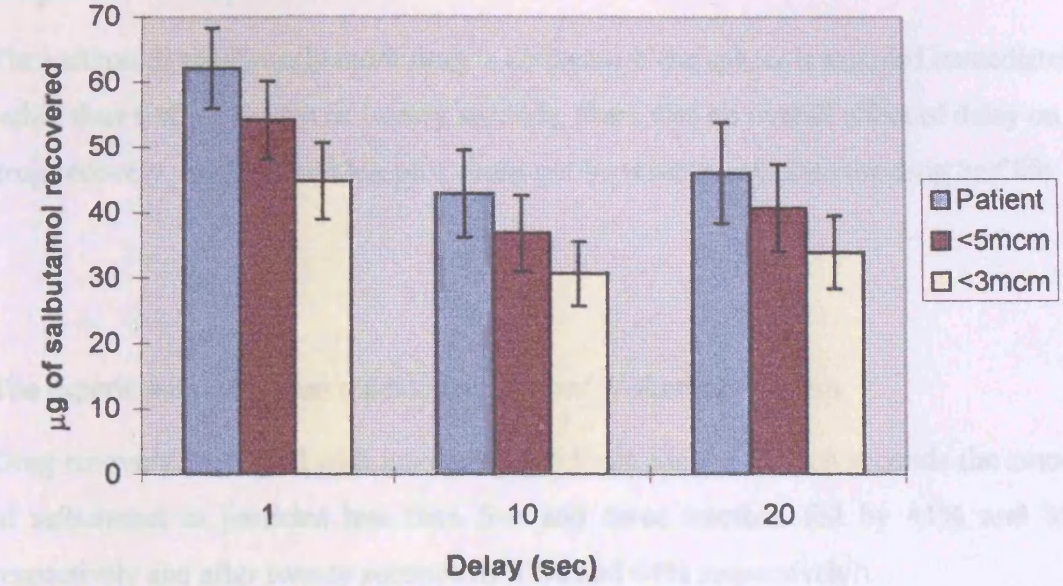
Salbutamol

'Old' spacers, which had been used in other, unrelated, projects were assessed first. Drug recovery decreased with a ten or twenty second residence time compared to immediate sampling ($p<0.01$). After ten seconds the amount of salbutamol in particles less than five and three microns fell by 32%. However, there was no further reduction after twenty seconds delay.

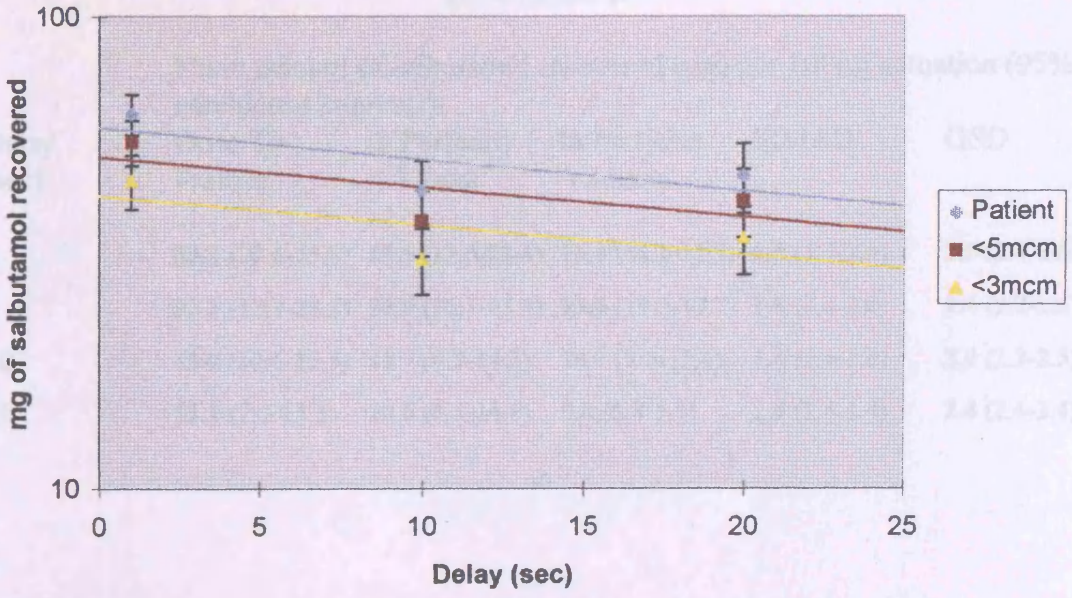
The amount of drug recovered (mean and 95% confidence intervals), the MMAD and GSD for the different experiments are given in the table.

The effect of delay on the delivery of salbutamol from the Volumatic.
Mean amount of salbutamol recovered (μg) per 100μg actuation (95% confidence intervals).

Delay (sec)	Dose To Patient	In Particles < 5 mcm	In Particles < 3 mcm	MMAD	GSD
1	**62.1** (55.8-68.4)	**54.2** (48.2-60.2)	**44.9** (38.9-50.9)	**1.7** (1.5-1.9)	**2.4** (2.2-2.6)
10	**42.9** (33.9-51.9)	**36.9** (29.1-44.7)	**30.7** (24.1-37.3)	**1.7** (1.5-1.9)	**2.6** (2.4-2.8)
20	**46.1** (37.3-54.9)	**40.8** (33.2-48.5)	**34.0** (27.6-40.4)	**1.7** (1.5-1.9)	**2.4** (2.2-2.6)



Plotted on a semi-log scale, the decrease in drug recovery is shown in the next graph, with a line of best fit also plotted.



The decrease in the recovery of salbutamol (in particles smaller than 5µm) with time may be described by the equation:

Log[Salb] = 1.7 - 0.006(time)

R-squared = 0.16, p=0.125.

Thus although significantly more drug is delivered if the spacer is sampled immediately, rather than waiting for ten or twenty seconds, there was no overall effect of delay on drug recovery, and the semi log plot could not be used to calculate the drug half life.

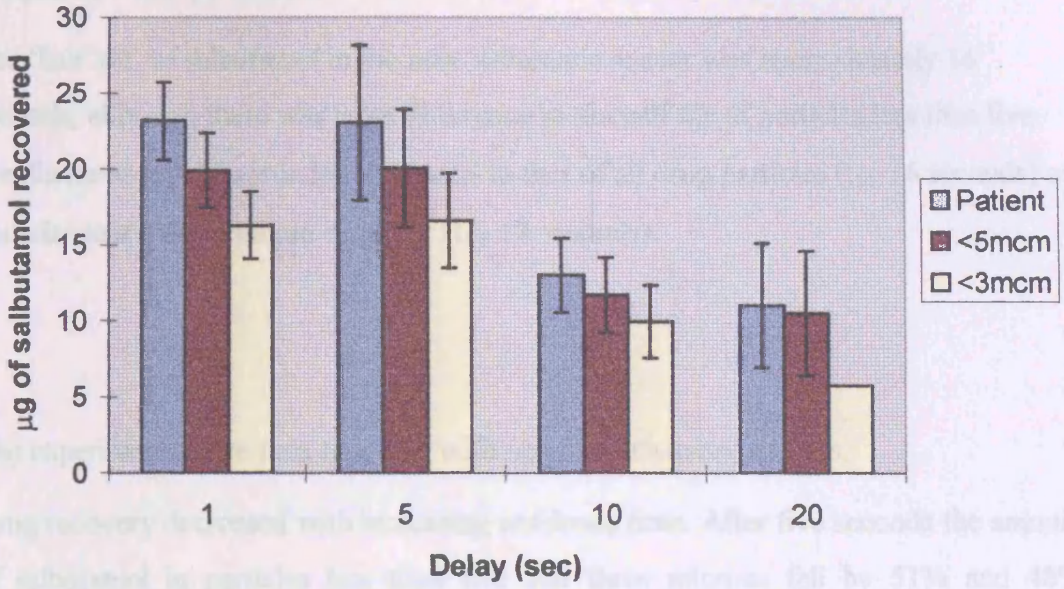
The experiments were then repeated with 'new' Volumatic spacers.

Drug recovery decreased with increasing residence time. After ten seconds the amount of salbutamol in particles less than five and three microns fell by 41% and 39% respectively and after twenty seconds by 47% and 64% respectively.

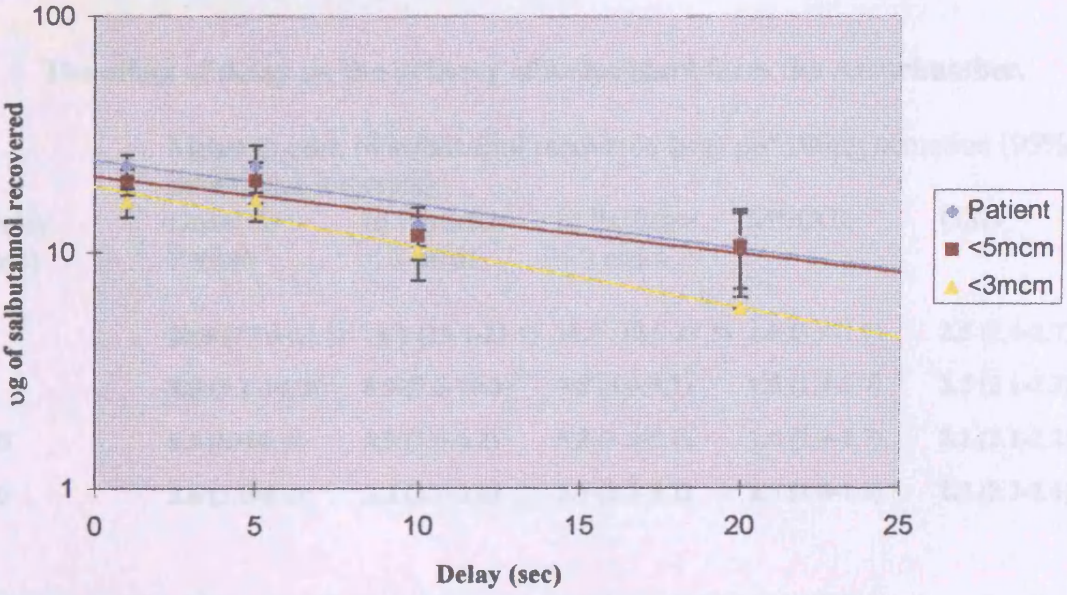
The amount of drug recovered (mean and 95% confidence intervals), the MMAD and GSD for the different experiments are given in the table.

The effect of delay on the delivery of salbutamol from the Volumatic (new spacer).

	Mean amount of salbutamol recovered (μg) per 100μg actuation (95% confidence intervals).				
Delay (sec)	Dose To Patient	In Particles < 5 mcm	In Particles < 3 mcm	MMAD	GSD
1	**23.2** (20.6-25.8)	**19.9** (17.5-22.4)	**16.3** (14.1-18.5)	**1.9** (1.7-2.0)	**2.5** (2.4-2.6)
5	**23.1** (17.9-28.2)	**20.0** (16.1-23.9)	**16.6** (13.5-19.7)	**1.8** (1.6-2.0)	**2.4** (2.2-2.6)
10	**13.0** (10.6-15.5)	**11.7** (9.2-14.2)	**10.0** (7.6-12.4)	**1.6** (1.4-1.8)	**2.4** (2.3-2.5)
20	**11.1** (7.0-15.1)	**10.6** (6.5-14.6)	**5.8** (5.8-5.8)	**1.4** (1.4-1.4)	**2.4** (2.4-2.4)



Plotted on a semi-log scale, the decrease in drug recovery is shown in the next graph, with a line of best fit also plotted.



The decrease in the recovery of salbutamol (in particles smaller than 5µm) with time is best described by the equation:

Log[salb] = 1.33 - 0.0178(time)

R-squared = 0.54, $p<0.001$.

The 'half life' of salbutamol in the new Volumatic spacer was approximately 16 seconds, although there was large difference in the half life of particles less than five μm diameter ($t_{1/2}$ 19 seconds) compared to that of all drug particles ($t_{1/2}$ 16 seconds) or particles less than three μm diameter ($t_{1/2}$ 12 seconds).

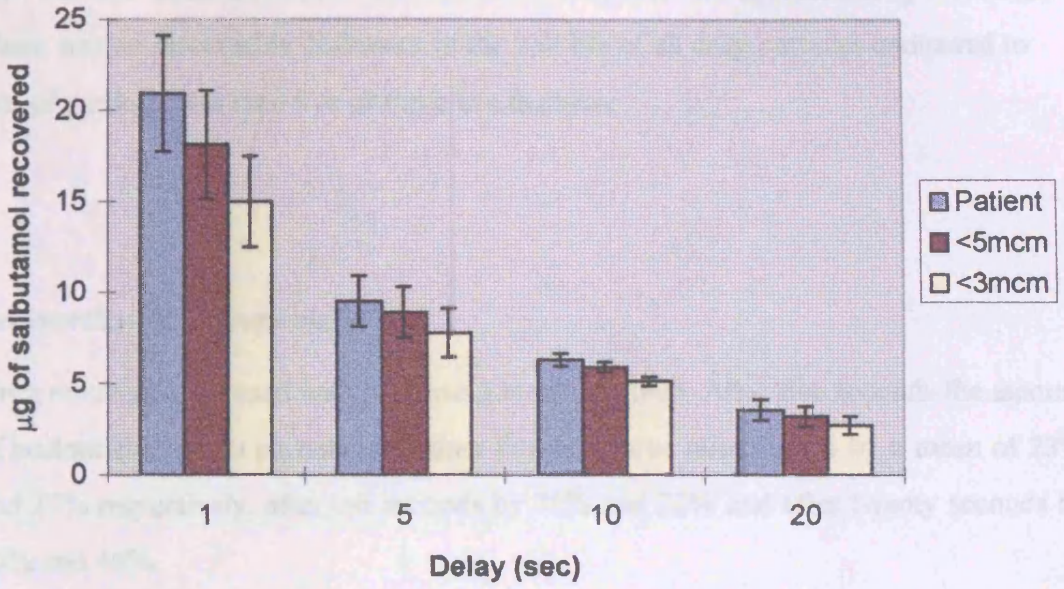
The experiments were then repeated with new Aerochamber spacers.

Drug recovery decreased with increasing residence time. After five seconds the amount of salbutamol in particles less than five and three microns fell by 51% and 48% respectively, after ten seconds by 67% and 65% respectively and after twenty seconds by 82%.

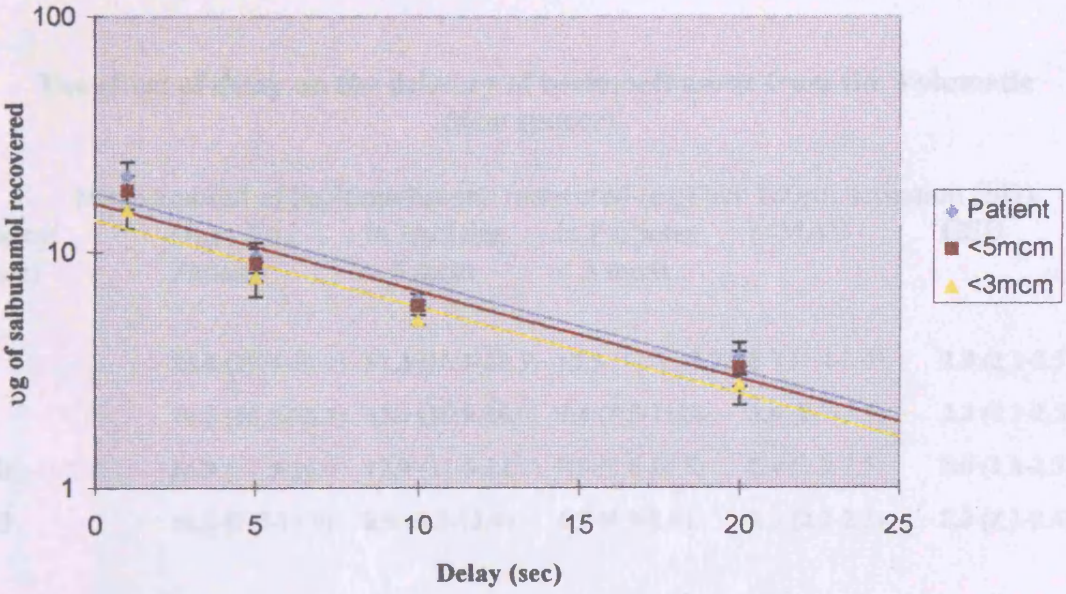
The amount of drug recovered (mean and 95% confidence intervals), the MMAD and GSD for the different experiments are given in the table.

The effect of delay on the delivery of salbutamol from the Aerochamber.

	Mean amount of salbutamol recovered (μg) per 100μg actuation (95% confidence intervals).				
Delay (sec)	Dose To Patient	In Particles < 5 mcm	In Particles < 3 mcm	MMAD	GSD
1	20.9 (17.8-24.1)	18.1 (15.1-21.1)	15.0 (12.5-17.5)	1.8 (1.8-1.9)	2.5 (2.4-2.7)
5	9.5 (8.1-10.9)	8.9 (7.5-10.3)	7.8 (6.4-9.1)	1.5 (1.4-1.7)	2.2 (2.1-2.2)
10	6.3 (6.0-6.6)	5.9 (5.6-6.2)	5.2 (4.9-5.4)	1.5 (1.4-1.7)	2.2 (2.1-2.2)
20	3.6 (3.0-4.1)	3.2 (2.7-3.8)	2.7 (2.2-3.2)	1.7 (1.6-1.8)	2.3 (2.3-2.4)



Plotted on a semi-log scale, the decrease in drug recovery is shown in the next graph, with a line of best fit also plotted.



The decrease in the recovery of salbutamol (in particles smaller than 5µm) with time is best described by the equation:

Log[salb] = 1.20 - 0.037(time)

R-squared = 0.90, $p<0.001$.

The 'half life' of salbutamol in the Aerochamber spacer was approximately 8 seconds. There was no discernable difference in the half life of all drug particles compared to that of particles less than five or three μm diameter.

Beclomethasone dipropionate

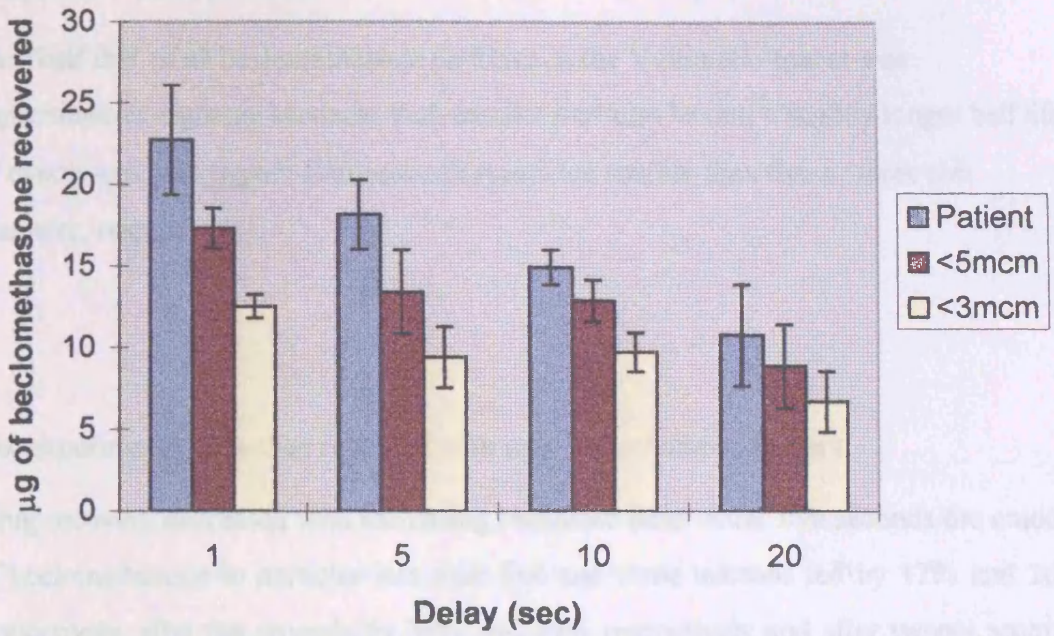
Drug recovery decreased with increasing residence time. After five seconds the amount of beclomethasone in particles less than five and three microns fell by a mean of 23% and 27% respectively, after ten seconds by 25% and 22% and after twenty seconds by 49% and 46%.

The amount of drug recovered (mean and 95% confidence intervals), the MMAD and GSD for the different experiments are given in the table.

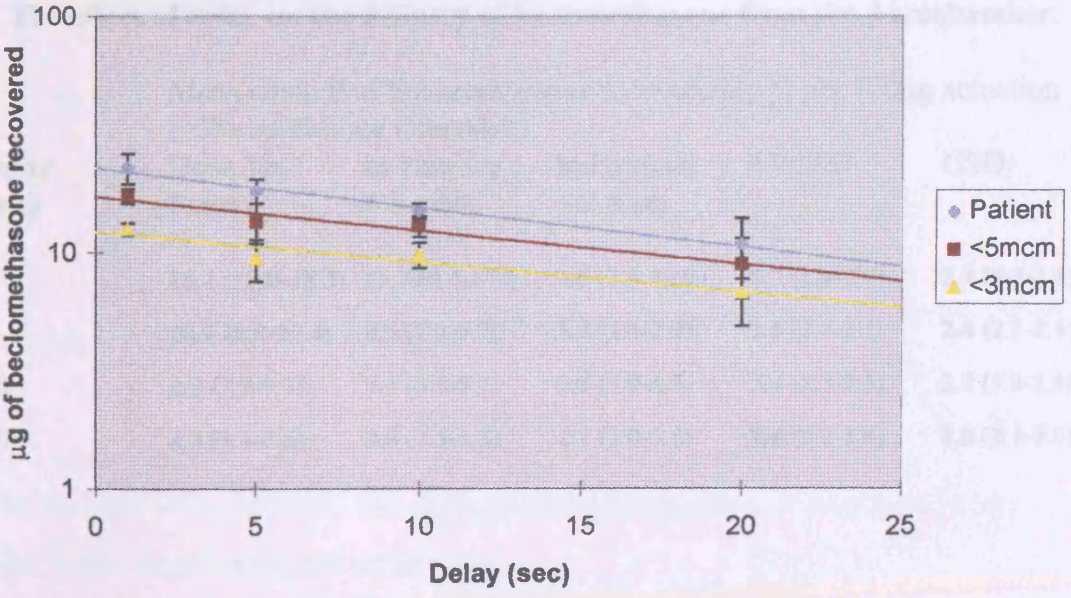
The effect of delay on the delivery of beclomethasone from the Volumatic (new spacer).

Mean amount of beclomethasone recovered (μg) per 100μg actuation (SD).

Delay (sec)	Dose To Patient	In Particles < 5 mcm	In Particles < 3 mcm	MMAD	GSD
1	**22.8** (19.4-26.1)	**17.3** (16.1-18.5)	**12.5** (11.8-13.2)	**2.7** (2.4-3.0)	**2.3** (2.1-2.5)
5	**18.2** (16.0-20.3)	**13.4** (10.9-16.0)	**9.4** (7.5-11.3)	**3.0** (2.7-3.3)	**2.3** (2.1-2.5)
10	**14.9** (13.9-16.0)	**12.9** (11.6-14.2)	**9.8** (8.6-10.9)	**2.4** (2.3-2.5)	**2.0** (1.8-2.3)
20	**10.8** (7.7-13.9)	**8.9** (6.3-11.4)	**6.7** (4.9-8.6)	**2.3** (2.2-2.5)	**2.3** (2.1-2.4)



Plotted on a semi-log scale, the decrease in drug recovery is shown in the next graph, with a line of best fit also plotted.



The decrease in the recovery of beclomethasone (in particles smaller than 5µm) with time is best described by the equation:

Log[BDP] = 1.23 - 0.0148(time)

R-squared = 0.67, $p<0.001$.

The 'half life' of all beclomethasone particles in the Volumatic spacer was approximately eighteen seconds, with smaller particles having a slightly longer half life of twenty-one and twenty-four seconds (particles smaller than five or three μm diameter, respectively).

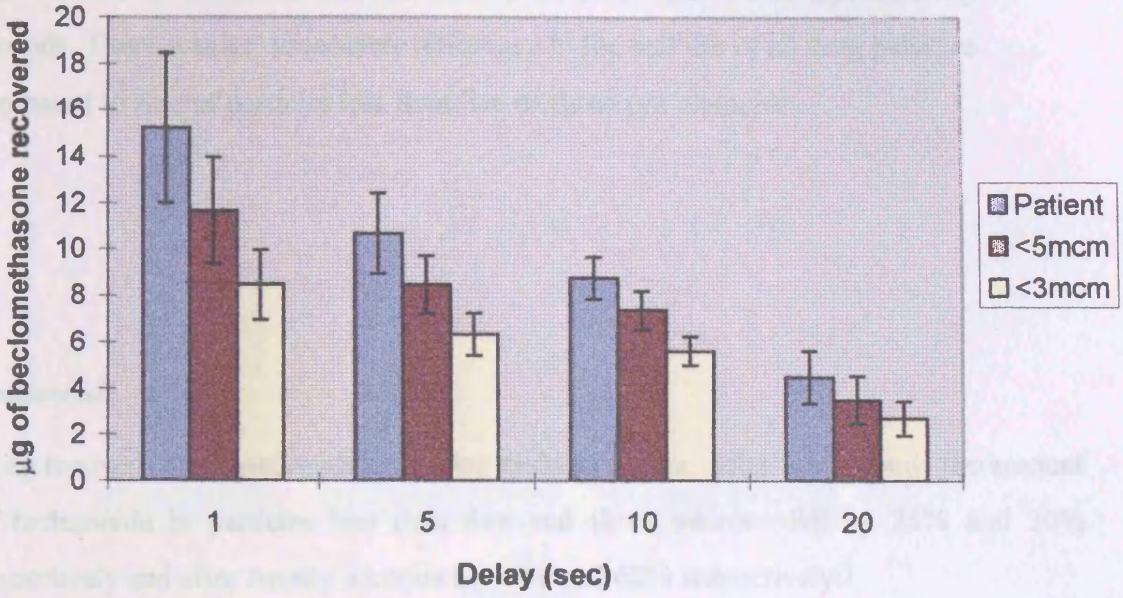
The experiments were then repeated with new Aerochamber spacers.

Drug recovery decreased with increasing residence time. After five seconds the amount of beclomethasone in particles less than five and three microns fell by 17% and 26% respectively, after ten seconds by 39% and 34% respectively and after twenty seconds by 70% and 68% respectively.

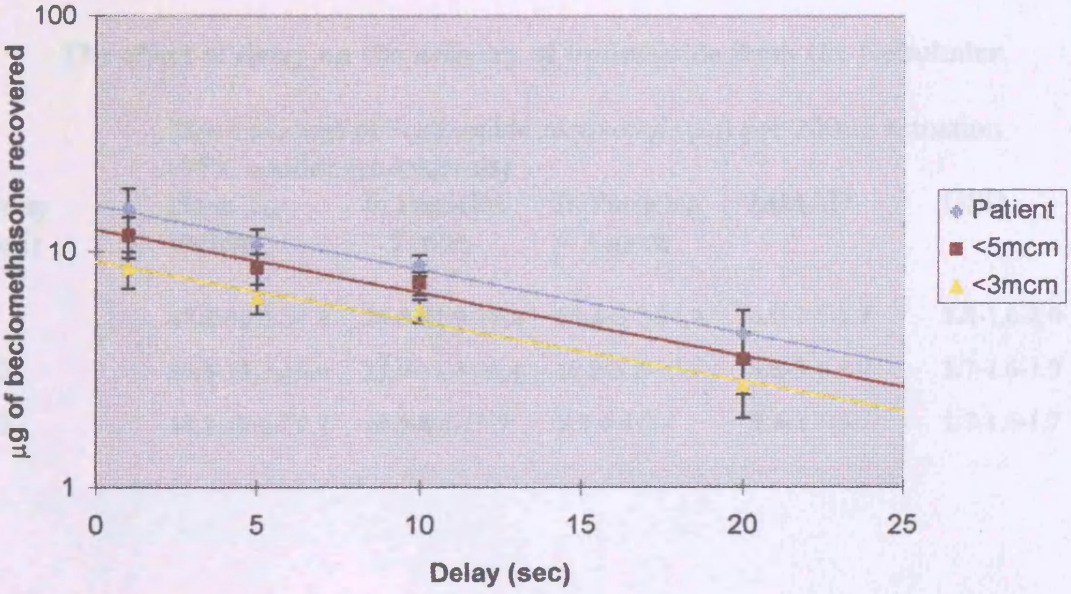
The amount of drug recovered (mean and 95% confidence intervals), the MMAD and GSD for the different experiments are given in the table.

The effect of delay on the delivery of beclomethasone from the Aerochamber.

	Mean amount of beclomethasone recovered (μg) per 100μg actuation (95% confidence intervals).				
Delay (sec)	Dose To Patient	In Particles < 5 mcm	In Particles < 3 mcm	MMAD	GSD
1	15.2 (12.0-18.5)	11.7 (9.4-14.0)	8.5 (7.0-10.0)	2.7 (2.4-2.9)	2.4 (2.3-2.4)
5	10.7 (8.9-12.4)	8.5 (7.2-9.7)	6.3 (5.4-7.2)	2.5 (2.4-2.5)	2.4 (2.3-2.4)
10	8.8 (7.9-9.7)	7.4 (6.5-8.2)	5.6 (5.0-6.3)	2.4 (2.3-2.5)	2.2 (1.8-2.6)
20	4.5 (3.3-5.6)	3.5 (2.5-4.5)	2.7 (2.0-3.5)	2.4 (2.2-2.6)	2.8 (2.1-3.6)



Plotted on a semi-log scale, the decrease in drug recovery is shown in the next graph, with a line of best fit also plotted.



The decrease in the recovery of beclomethasone (in particles smaller than 5µm) with time is best described by the equation:

Log[BDP] = 1.09 - 0.0274(time)

R-squared = 0.82, $p<0.001$.

The 'half life' of beclomethasone in the Aerochamber spacer was approximately 12 seconds. There was no discernable difference in the half life of all drug particles compared to that of particles less than five or three μm diameter.

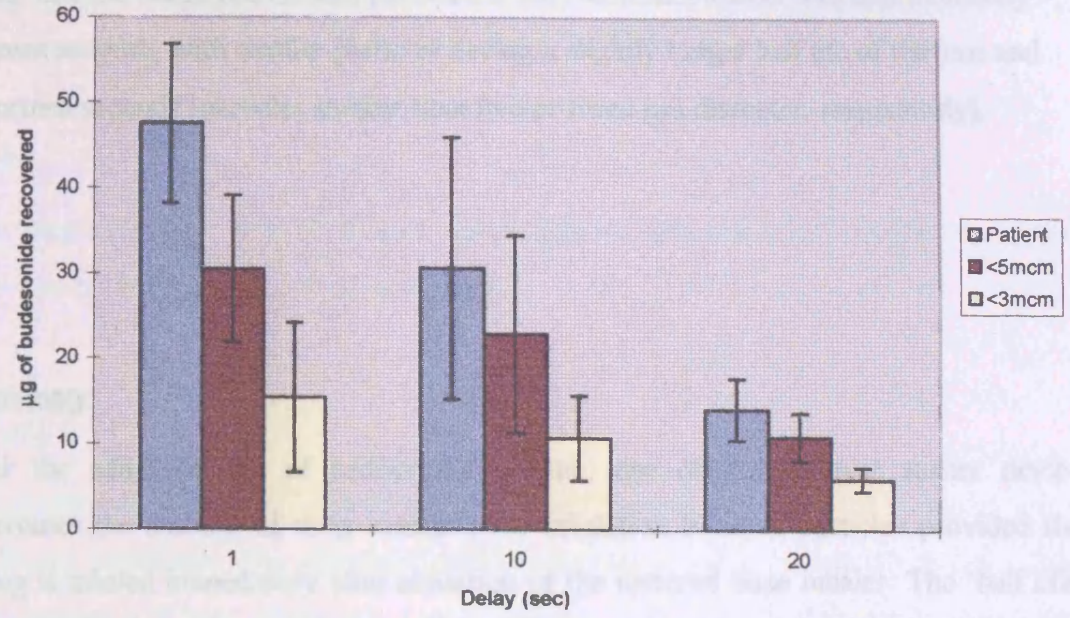
Budesonide

Drug recovery decreased with increasing residence time. After ten seconds the amount of budesonide in particles less than five and three microns fell by 25% and 30% respectively and after twenty seconds by 64% and 62% respectively.

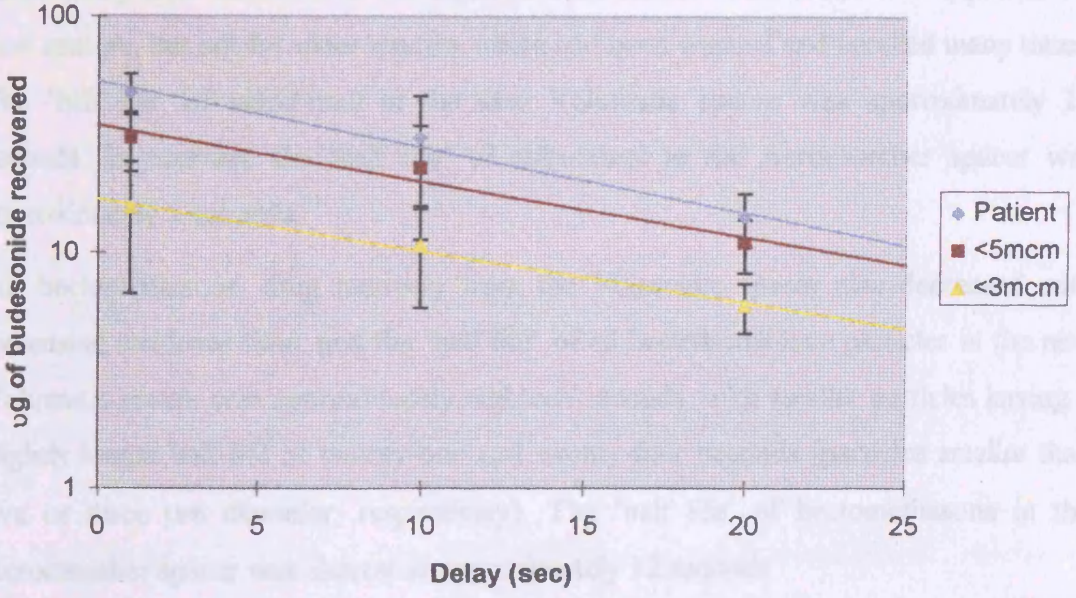
The amount of drug recovered (mean and 95% confidence intervals), the MMAD and GSD for the different experiments are given in the table.

The effect of delay on the delivery of budesonide from the Nebuhaler.

Delay (sec)	Mean amount of budesonide recovered (μg) per 200μg actuation (95% confidence intervals). Dose To Patient	In Particles < 5 mcm	In Particles < 3 mcm	MMAD	GSD
1	47.5-38.2-56.8	30.5-21.9-39.2	15.4-6.7-24.2	4.0-3.4-4.7	1.8-1.6-2.0
10	30.6-15.3-46.0	22.9-11.3-34.4	10.8-5.8-15.7	3.5-3.1-3.9	1.7-1.6-1.9
20	14.2-10.6-17.7	10.9-8.1-13.7	5.9-4.5-7.4	3.4-3.3-3.5	1.7-1.6-1.7



Plotted on a semi-log scale, the decrease in drug recovery is shown in the next graph, with a line of best fit also plotted.



The decrease in the recovery of budesonide (in particles smaller than 5µm) with time is best described by the equation:

Log[Bud] = 1.48 - 0.0234(time)

R-squared = 0.33, p=0.04.

The 'half life' of all budesonide particles in the Nebuhaler spacer was approximately eleven seconds, with smaller particles having a slightly longer half life of thirteen and fourteen seconds (particles smaller than five or three μm diameter, respectively).

Summary

For the administration of nedocromil sodium, use of the Fisonair spacer device increases the amount of drug available for inhalation in small particles provided the drug is inhaled immediately after actuation of the metered dose inhaler. The 'half life' of nedocromil sodium in the Fisonair spacer was approximately 8 seconds.

For the administration of salbutamol, use of the Volumatic spacer device increases the amount of drug available for inhalation in small particles provided the drug is inhaled immediately after actuation of the metered dose inhaler. This effect was apparent for new spacers, but not for older spacers which had been washed and handled many times. The 'half life' of salbutamol in the new Volumatic spacer was approximately 16 seconds. In contrast, the 'half life' of salbutamol in the Aerochamber spacer was approximately 8 seconds.

For beclomethasone, drug recovery from the Volumatic spacer also decreased with increasing residence time, and the 'half life' of all beclomethasone particles in the new Volumatic spacer was approximately eighteen seconds, with smaller particles having a slightly longer half life of twenty-one and twenty-four seconds (particles smaller than five or three μm diameter, respectively). The 'half life' of beclomethasone in the Aerochamber spacer was shorter at approximately 12 seconds.

Similar results were found with budesonide from the Nebuhaler, 'half life' approximately eleven seconds (all particles), with smaller particles having a slightly longer half life of fourteen seconds

The effect of multiple actuations of metered dose inhalers into spacer devices on the amount of drug recovered.

Aim

To determine the effect of multiple actuations of metered dose inhalers into spacer devices on the amount of drug recovered.

Methods

The glass multistage liquid impinger was used to determine the MDI output under different conditions. The experiments were undertaken using nedocromil sodium, 2mg per actuation (Tilade, Fisons plc, Loughborough, UK) with the Fisonair™ (Fisons plc, Loughborough, UK); salbutamol, 100µg per actuation (Ventolin, Allen & Hanburys Ltd, Uxbridge, UK) with the Volumatic™ (Allen & Hanburys Ltd, Uxbridge, UK); budesonide 200µg per actuation (Pulmicort, Astra Pharmaceuticals, Kings Langley, UK) with the Nebuhaler™ (Astra Pharmaceuticals, Kings Langley, UK).

New metered dose inhalers of each drug were obtained. The first ten actuations from each MDI was fired to waste. Immediately prior to each experiment, the MDI was shaken for thirty seconds and primed by firing one actuation. The MDI was then shaken for ten seconds and actuated into the spacer, which was immediately attached to the MSLI. To assess the effect of multiple actuations into the spacer, the MDI was actuated into the spacer one, two or five times prior to attaching it to the MSLI. This procedure was repeated until a total of ten actuations had been administered to the MSLI during each experiment to facilitate the drug assay.

For nedocromil sodium, each stage of the MSLI was washed quantitatively with water. The amount of drug collected in each stage was assayed by UV spectrophotometry. For salbutamol and budesonide, each stage was washed with methanol or ethanol and the amount of drug collected at each stage determined by high pressure liquid chromatography.

Different Fisonair, Nebuhaler, and Volumatic spacers were used for each experiment, repeated four times with each spacer type. Except where otherwise noted, spacers were removed from their original packaging immediately before each experiment.

Results

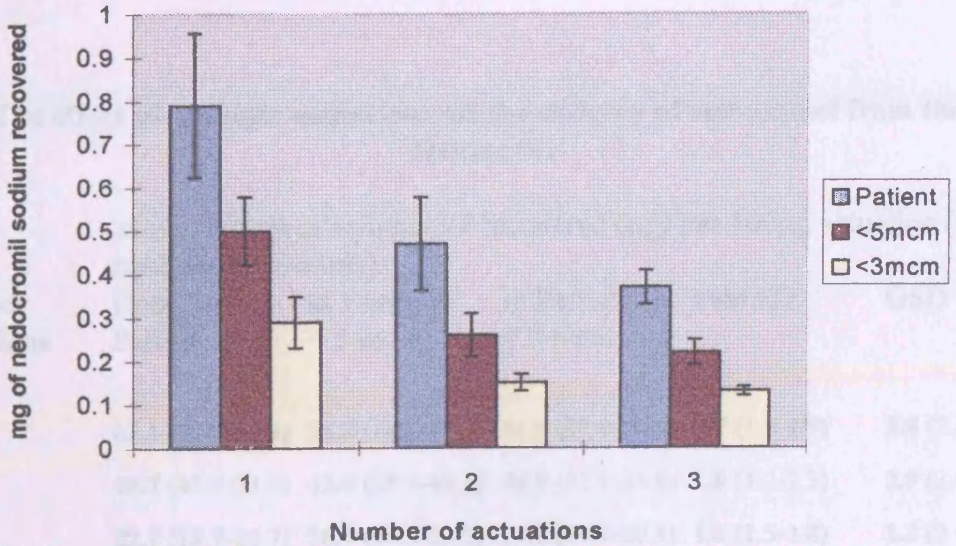
Nedocromil sodium

Multiple actuations decreased drug recovery in particles less than five microns by 47% (2 actuations) and 57% (3 actuations). For particles less than three microns, the reduction was 48% (2 actuations) and 57% (3 actuations).

The effect of multiple actuations of the MDI into the Fisonair on the delivery of nedocromil sodium.

Number of actuations — Mean amount of Nedocromil Sodium recovered (mg) per 2mg actuation (95% confidence intervals).

	Dose To Patient	In Particles < 5 mcm	In Particles < 3 mcm	MMAD	GSD
1	**0.79** (0.62-0.96)	**0.50** (0.42-0.58)	**0.29** (0.23-0.35)	**3.93** (3.41-4.45)	**2.00** (1.90-2.10)
2	**0.47** (0.36-0.58)	**0.26** (0.21-0.31)	**0.15** (0.13-0.17)	**4.43** (4.00-4.86)	**2.00** (1.57-2.43)
3	**0.37** (0.33-0.41)	**0.22** (0.19-0.25)	**0.13** (0.12-0.14)	**4.25** (4.15-4.35)	**2.10** (2.04-2.16)



Multiple actuations of nedocromil sodium into the Fisonair spacer prior to sampling reduced both the total amount of drug recovered, and that contained in particles smaller than 5μm and 3μm diameter (ANOVA, $p<0.01$). The change in MMAD between experiments was not significant.

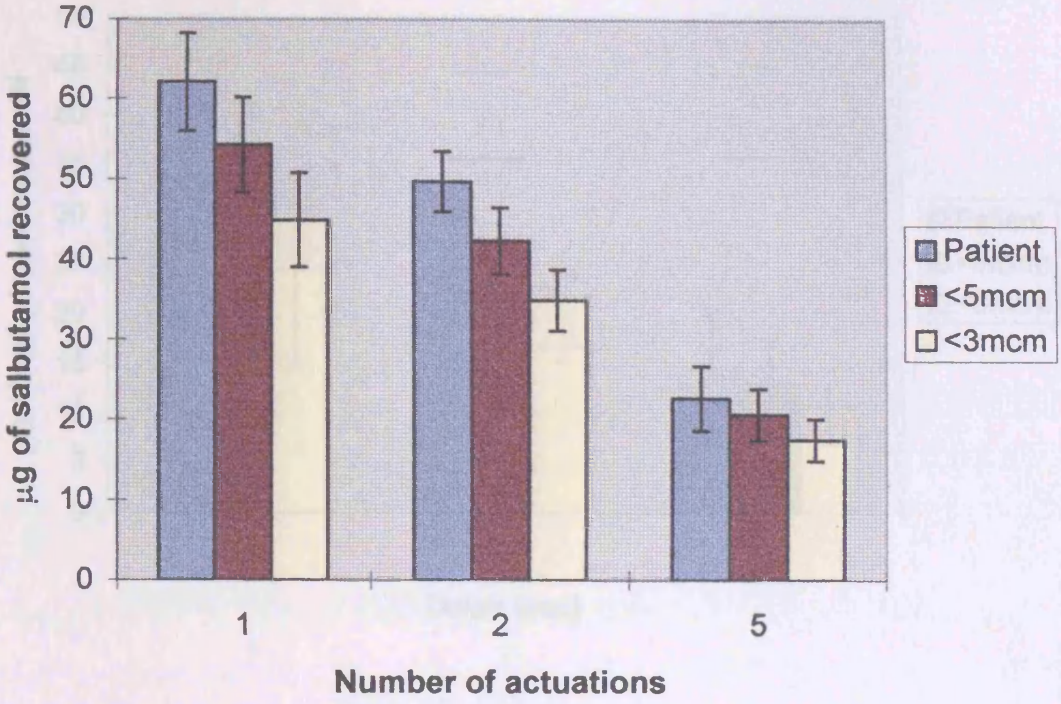
Salbutamol

As in chapter 6.1, salbutamol was assessed with 'old' Volumatic spacers, which had been used in other, unrelated, projects, and 'new' spacers, removed from their packaging immediately prior to the experiment.

With the 'old' spacers, multiple actuations decreased drug recovery per 100μg actuation by 22% (2 actuations) and 62% (5 actuations) for particles less than five microns compared with single actuations (ANOVA, $p<0.001$). Thus, when five 100μg actuations were released into the spacer before inhalation, only 103μg were recovered in particles smaller than five microns. If the same number of actuations had been administered with an 'inhalation' between each one, 270μg would have been recovered. The change in MMAD between experiments was not significant.

The effect of multiple actuations on the delivery of salbutamol from the Volumatic.

	Mean amount of salbutamol recovered (μg) per 100μg actuation (95% confidence intervals).				
Number actuations	Dose To Patient	In Particles < 5 mcm	In Particles < 3 mcm	MMAD	GSD
1	**62.1** (55.8-68.4)	**54.2** (48.2-60.2)	**44.9** (38.9-50.9)	**1.7** (1.5-1.9)	**2.4** (2.2-2.6)
2	**49.7** (45.9-53.4)	**42.4** (38.3-46.5)	**34.9** (31.1-38.8)	**1.8** (1.2-2.3)	**2.9** (2.0-3.9)
5	**22.7** (18.7-26.7)	**20.7** (17.5-23.9)	**17.5** (14.9-20.1)	**1.6** (1.5-1.8)	**2.3** (2.1-2.5)

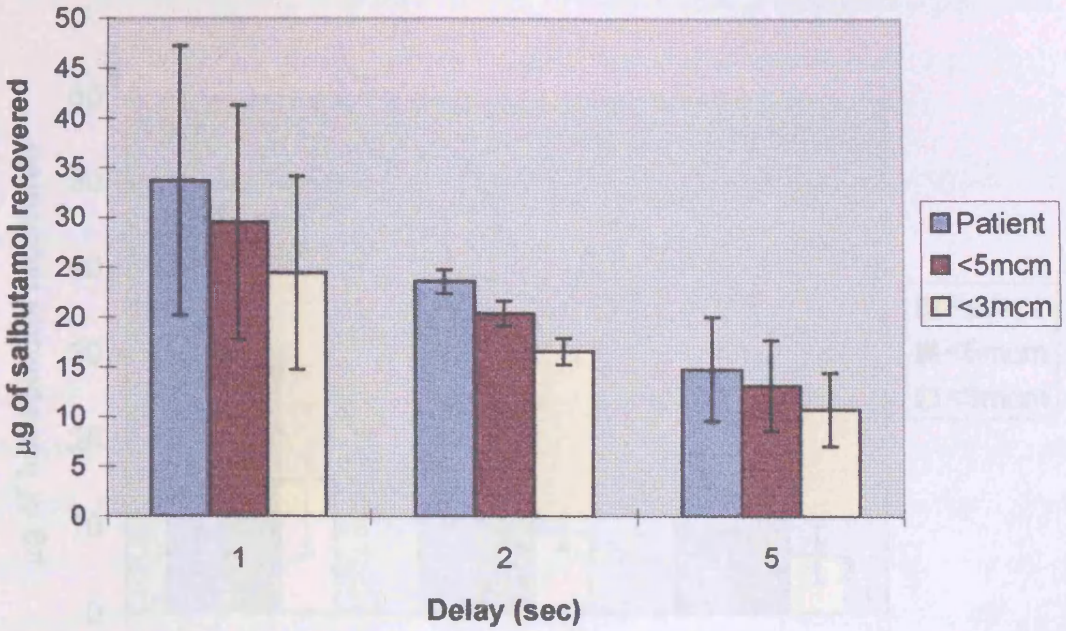


New Volumatic spacers were also assessed.

Multiple actuations decreased drug recovery per 100µg actuation by 31% (2 actuations) and 55% (5 actuations) for particles less than three or five microns compared with single actuations (ANOVA, p=0.075). The change in MMAD between experiments was not significant.

The effect of multiple actuations on the delivery of salbutamol from the Volumatic (new spacer)

Number actuations	Mean amount of salbutamol recovered (µg) per 100µg actuation (95% confidence intervals). Dose To Patient	In Particles < 5 mcm	In Particles < 3 mcm	MMAD	GSD
1	**33.7** (18.6-48.9)	**29.6** (16.5-42.7)	**24.5** (13.6-35.3)	**1.8** (1.7-1.9)	**2.4** (2.4-2.5)
2	**23.6** (22.2-25.1)	**20.4** (18.9-22.0)	**16.6** (15.0-18.2)	**1.9** (1.7-2.1)	**2.4** (2.2-2.7)
5	**14.8** (9.6-20.1)	**13.2** (8.6-17.7)	**10.8** (7.1-14.5)	**1.9** (1.8-2.0)	**2.3** (2.1-2.4)

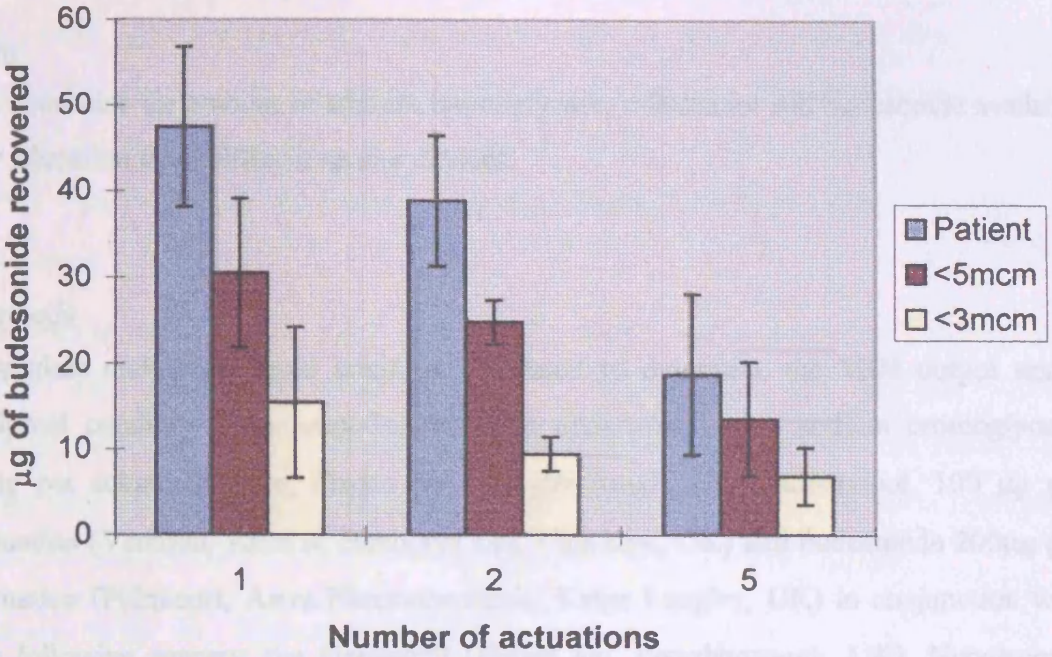


Budesonide

Multiple actuations decreased drug recovery per 200µg actuation by 39% and 19% (2 actuations) and by 55% (5 actuations) for particles less than three or five microns compared with single actuations (ANOVA, p=0.022). The change in MMAD between experiments was not significant.

The effect of multiple actuations on the delivery of budesonide from the Nebuhaler.

Number of actuations	Mean amount of budesonide recovered (µg) per 200µg actuation (standard deviation).				
	Dose To Patient	In Particles < 5 mcm	In Particles < 3 mcm	MMAD	GSD
1	**47.5** (38.2-56.8)	**30.5** (21.9-39.2)	**15.4** (6.7-24.2)	**4.0** (3.4-689.0)	**1.8** (1.6-2.0)
2	**38.9** (31.3-46.5)	**24.8** (22.3-27.4)	**9.4** (7.5-11.4)	**4.1** (3.6-4.7)	**1.6** (1.5-1.7)
5	**18.7** (9.4-28.1)	**13.6** (6.9-20.2)	**6.9** (3.5-10.2)	**3.6** (3.4-3.8)	**1.7** (1.6-1.8)



Summary

For the administration of nedocromil sodium via the Fisonair spacer, salbutamol via the Volumatic, and budesonide via the Nebuhaler, multiple actuations of the metered dose inhaler into the spacer prior to sampling reduces the recovery of drug in both small and large particles.

Inhalational Drug Delivery From Seven Different Spacer Devices.

Aim

To determine the amount of sodium cromoglycate, salbutamol and budesonide available for inhalation from different spacer devices.

Methods

The glass multistage liquid impinger was used to determine the MDI output under different conditions. The experiments were undertaken using sodium cromoglycate, 5mg per actuation (Intal, Fisons plc, Loughborough, UK), salbutamol, 100 μg per actuation (Ventolin, Allen & Hanburys Ltd, Uxbridge, UK) and budesonide 200μg per actuation (Pulmicort, Astra Pharmaceuticals, Kings Langley, UK) in conjunction with the following spacers; the Fisonair™ (Fisons plc, Loughborough, UK), Nebuhaler™ (Astra Pharmaceuticals, Kings Langley, UK), Volumatic™ (Allen & Hanburys Ltd, Uxbridge, UK), Inspirease™ (Key Pharmaceuticals Inc., Miami, Florida), Child Aerochamber™ (Trudell Medical, London, Canada), Aerosol Cloud Enhancer™ (DHD Corp, Canestota NY) and Dynahaler™ (Healthscan Products, Cedar Grove NJ). The combinations of drugs and spacers evaluated are given below:

Drug-Spacer combinations evaluated:

Sodium cromoglycate with the	Fisonair Nebuhaler Inspirease Aerochamber Aerosol Cloud Enhancer Dynahaler
Salbutamol with the	Volumatic Inspirease Aerochamber Dynahaler
Budesonide with the	Nebuhaler Inspirease Aerochamber.

New metered dose inhalers of each drug were obtained. The first ten actuations from each MDI was fired to waste. Immediately prior to each experiment, the MDI was shaken for thirty seconds and primed by firing one actuation. The MDI was then shaken for ten seconds and actuated into the spacer, which was immediately attached to the MSLI. This procedure was repeated ten times during each experiment to facilitate the drug assay.

For sodium cromoglycate, each stage of the MSLI was washed quantitatively with water. The amount of drug collected in each stage was assayed by UV spectrophotometry at a wavelength of 326nm. For salbutamol and budesonide, each stage was washed with methanol or ethanol respectively and the amount of drug collected at each stage determined by high pressure liquid chromatography.

Patients using the Aerosol Cloud Enhancer and the Dynahaler are instructed to commence inhalation prior to MDI actuation. These spacers, and for comparison the Fisonair, were also assessed by connecting the spacer to the MSLI *prior* to MDI actuation ('continually attached', see Chapter 5.1), mimicking an inspiratory flow through the spacer during actuation of the MDI. These experiments were undertaken with sodium cromoglycate only.

Four different Fisonairs, Nebuhalers, Aerosol Cloud Enhancers, Dynahalers and Volumatic spacers, three Aerochambers and one Inspirease were used. Experiments were repeated four times with each spacer type. Spacers were all cleaned with water and allowed to dry in air on the laboratory bench before each experiment. The laboratory temperature and relative humidity were recorded for each experiment.

Results

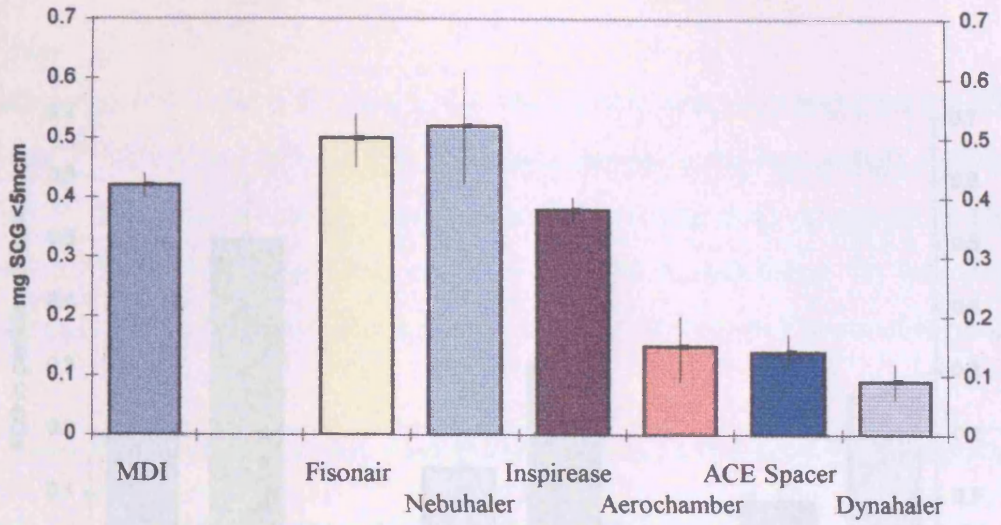
The amount of drug recovered in particles smaller than five and three microns, the MMAD and GSD, expressed as the mean and 95% confidence intervals for each group of experiments, are given in the tables below and as a percentage of the nominal dose from the MDI in the figures.

Sodium cromoglycate

There was a significant difference in the fine particle dose delivered from the different devices (ANOVA, $p<0.00001$). The Fisonair and Nebuhaler increased the fine particle dose by 18% and 24% of the MDI dose respectively (T Test, $p<0.01$ and $p=0.13$). The Inspirease delivered 90% of the MDI fine particle dose, whereas the smaller spacers all reduced the fine particle dose to 36% of that from the MDI with the Aerochamber, 33% with the Aerosol Cloud Enhancer and 21% with the Dynahaler. There was no difference between the fine particle dose delivered from either of the two larger volume spacers, the Nebuhaler and the Fisonair ($p=0.7$), or between the smaller volume spacers, the Aerochamber, Aerosol Cloud Enhancer and the Dynahaler ($p>0.05$)

The effect of the use of different spacer devices on the delivery of sodium cromoglycate

Method of delivery.	Mean amount of sodium cromoglycate recovered (mg) per 5mg actuation (95% Confidence Intervals).				
	Dose To Patient	In Particles < 5 μm	In Particles < 3 μm	MMAD μm	GSD
Direct from MDI:	**4.14** (3.99-4.29)	**0.42** (0.40-0.44)	**0.18** (0.15-0.20)	**13.8** (13.2-14.4)	**2.0** (1.8-2.2)
Via Fisonair	**1.34** (1.25-1.44)	**0.50** (0.45-0.54)	**0.24** (0.20-0.27)	**6.1** (5.9-6.4)	**2.1** (2.0-2.2)
Via Nebuhaler	**1.2** (1.0-1.3)	**0.52** (0.42-0.61)	**0.25** (0.21-0.28)	**4.9** (4.6-5.3)	**2.0** (1.98-2.05)
Via Inspirease	**0.83** ((0.75-0.92)	**0.38** (0.36-0.40)	**0.2** (0.19-0.21)	**5.5** (5.1-5.8)	**2.3** (2.2-2.3)
Via Aerochamber	**0.35** (0.22-0.48)	**0.15** (0.09-0.20)	**0.07** (0.05-0.10)	**5.9** (5.6-6.2)	**2.4** (2.2-2.6)
Via ACE Spacer	**0.46** (0.40-0.53)	**0.14** (0.11-0.17)	**0.07** (0.05-0.08)	**10.1** (8.3-11.9)	**3.1** (2.7-3.5)
Via Dynahaler	**0.32** (0.21-0.42)	**0.09** (0.06-0.12)	**0.04** (0.03-0.06)	**8.3** (7.7-8.8)	**2.4** (2.3-2.4)



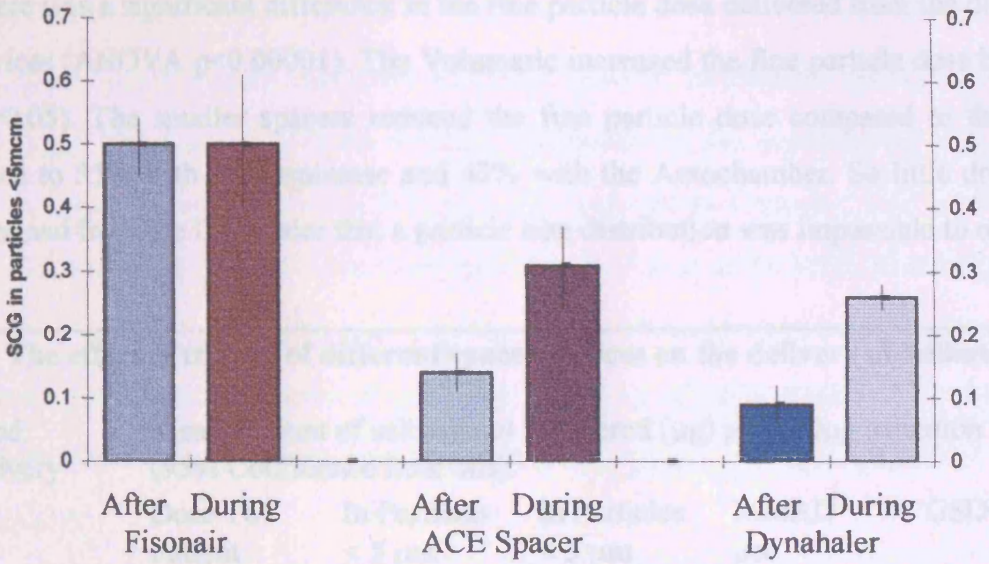
The amount of sodium cromoglycate (mg per 5mg dose) recovered in particles smaller than five μm aerodynamic diameter for six different spacer devices. T-bars represent one standard deviation.

Co-ordinated sampling and actuation

Sampling during MDI actuation, to represent co-ordination of inhalation and MDI actuation into the spacer, increased recovery of sodium cromoglycate from the Aerosol Cloud Enhancer (0.14 to 0.31mg) and Dynahaler (0.09 to 0.26mg). However, these spacers delivered less drug in small particles than the MDI or Fisonair assessed under the same conditions (ANOVA, p=0.0006).

The effect of the use of different spacer devices on the delivery of sodium cromoglycate, Sampling during MDI actuation.

Method. of delivery	Mean amount of sodium cromoglycate recovered (mg) per 5mg actuation (95% Confidence Intervals).				
	Dose To Patient	In Particles < 5 μm	In Particles < 3 μm	MMAD μm	GSD
Via Fisonair	**1.7** (1.50-1.89)	**0.50** (0.40-0.61)	**0.25** (0.17-0.33)	**7.5** (6.8-8.2)	**2.2** (2.1-2.3)
Via ACE	**2.6** (2.48-2.62)	**0.31** (0.24-0.37)	**0.14** (0.11-0.17)	**16.6** (12.5-20.6)	**2.7** (2.1-3.2)
Via Dynahaler	**1.0** (0.73-1.27)	**0.26** (0.24-0.28)	**0.13** (0.12-0.14)	**9.1** (7.4-10.7)	**2.5** (2.4-2.7)



Spacer type and time of sampling.

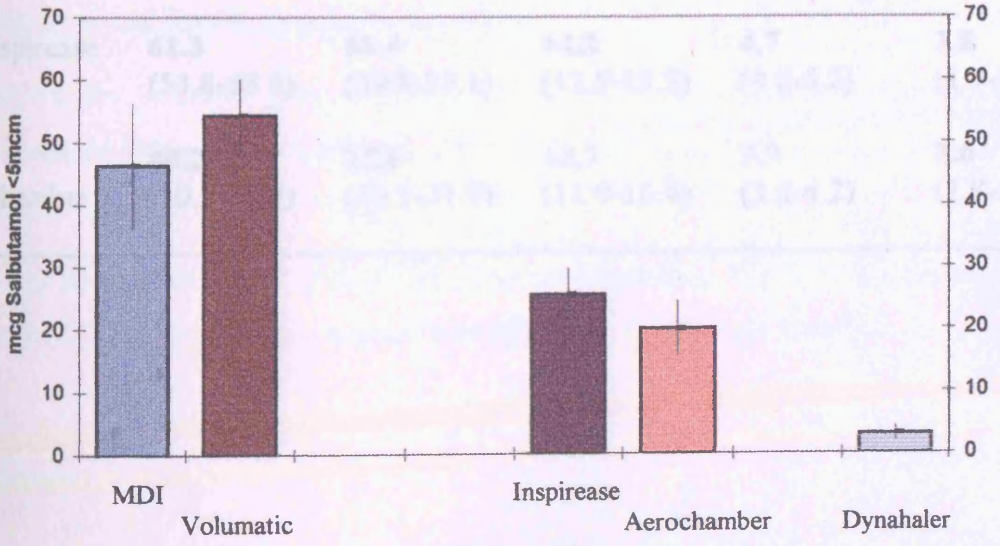
The amount of sodium cromoglycate (mg per 5mg dose) recovered in particles smaller than five μm aerodynamic diameter for three spacer devices sampled either one second after or during metered dose inhaler actuation

Salbutamol

There was a significant difference in the fine particle dose delivered from the different devices (ANOVA $p<0.00001$). The Volumatic increased the fine particle dose by 17% ($p=0.05$). The smaller spacers reduced the fine particle dose compared to the MDI alone to 55% with the Inspirease and 43% with the Aerochamber. So little drug was obtained from the Dynahaler that a particle size distribution was impossible to obtain.

The effect of the use of different spacer devices on the delivery of Salbutamol.

Method. of delivery	Mean amount of salbutamol recovered (µg) per 100µg actuation (95% Confidence Intervals).				
	Dose To Patient	In Particles < 5 µm	In Particles < 3 µm	MMAD µm	GSD
Direct from MDI:	**90.5** (81.4-99.6)	**46.3** (36.3-56.3)	**34.4** (27.5-41.3)	**5.2** (3.9-6.5)	**4.8** (3.2-6.4)
Via Volumatic:	**62.1** (56.0-68.2)	**54.3** (48.3-60.1)	**44.9** (39.0-50.8)	**1.7** (1.6-1.9)	**2.4** (2.3-2.5)
Via Inspirease	**30.1** (25.2-34.9)	**25.6** (21.5-29.7)	**21.0** (17.6-24.4)	**1.9** (1.7-2.0)	**2.6** (2.5-2.6)
Via Aerochamber	**23.4** (16.3-30.6)	**20.0** (15.6-24.5)	**16.2** (12.7-19.7)	**2.0** (1.2-2.8)	**1.9** (1.9-2.3)
Via Dynahaler	**3.1** (1.9-4.2)	**3.1** (1.9-4.2)	---	---	---



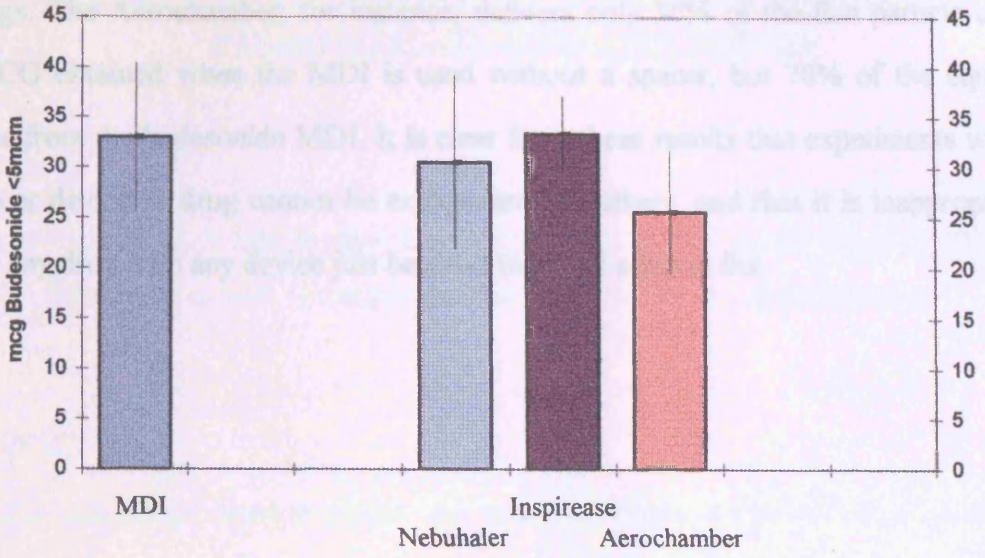
The amount of salbutamol (µg per 100µg dose) recovered in particles smaller than five µm aerodynamic diameter for four different spacer devices.

Budesonide

There was no statistically significant difference between the different spacers in the delivery of budesonide (ANOVA p=0.22). Nebuhaler and the Aerochamber both reduced the fine particle dose to 92% and 78% respectively compared with the MDI, whereas the Inspirease delivered the same amount of drug as the MDI. Reducing extrathoracic drug deposition is an important effect of spacer devices used with inhaled steroids, and the three spacers all reduced the delivery of larger particles. The total drug delivery was a quarter of that from the MDI when a spacer was used. (ANOVA p<0.0005).

The effect of the use of different spacer devices on the delivery of budesonide.

Method. of delivery	Mean amount of budesonide recovered (mcg) per 200µg actuation (95% Confidence Intervals).				
	Dose To Patient	In Particles < 5 µm	In Particles < 3 µm	MMAD µm	GSD
Direct from MDI:	**136.5** (131.6-141.5)	**33.0** (25.5-40.4)	**15.1** (12.2-18.0)	**10.2** (7.4-13.0)	**2.6** (2.3-2.9)
Via Nebuhaler	**47.5** (38.2-56.8)	**30.5** (21.9-39.2)	**15.4** (6.7-24.2)	**4.0** (3.4-4.7)	**1.8** (1.6-2.0)
Via Inspirease	**61.3** (53.8-68.8)	**33.4** (29.8-37.1)	**14.2** (12.5-15.9)	**4.7** (4.2-5.2)	**1.8** (1.7-1.8)
Via Aerochamber	**40.2** (30.2-50.4)	**25.6** (19.5-31.7)	**13.7** (11.0-16.4)	**3.9** (3.6-4.2)	**2.0** (1.8-2.2)



The amount of budesonide (μg per 200μg dose) recovered in particles smaller than five μm aerodynamic diameter for three different spacer devices.

To ensure that the metered dose inhalers were delivering the expected dose, prior to each experiment, they were actuated into a flask containing the appropriate solvent, and the amount of drug collected was determined by the appropriate assay, as described in chapter 5.7. The mean (SD) amount of drug recovered after a single actuation into a flask was 4.36 (0.28)mg for sodium cromoglycate, 101 (13)μg for salbutamol and 184 (9.5)μg for budesonide.

Metered dose inhalers were weighed before and after each experiment. Mean (SD) MDI weight loss per actuation was: sodium cromoglycate 138 (4.5)mg: Salbutamol 86 (1.2)mg: Budesonide 73 (2.6)mg.

During the experimental period the mean temperature was 23 degrees centigrade (range 21-25) and the mean relative humidity was 55% (range 51-63).

Summary

This study demonstrates large variations in the amount of drug delivered from different spacer devices, and variations in the relative efficacy of a spacer to deliver

drugs. The Aerochamber, for instance, delivers only 36% of the fine particle dose of DSCG obtained when the MDI is used without a spacer, but 78% of the equivalent dose from the budesonide MDI. It is clear from these results that experiments with one spacer device or drug cannot be extrapolated to others, and that it is inappropriate to use any drug with any device just because the MDI adapter fits.

The Effect of Spacer Length and Volume on the Amount of Drug Available for Inhalation from Spacers

Aim

The aim of this study was to determine the effect of altered spacer length and diameter on the delivery of sodium cromoglycate and budesonide from spacer devices.

Methods

A glass multistage liquid impinger was used to determine the amount of medication available from specially constructed spacers. These consisted of cylinders of plastic tubing of length 5cm, 10cm, 20cm, and 50cm and, for each length, of diameters 3cm, 5cm, 8cm and 10cm. One end of the cylinder was adapted to house the MDI actuator, the other to attach to the MSLI throat.

Sodium cromoglycate 5mg per actuation (Intal; Fisons PLC, Loughborough, UK) or budesonide 200μg per actuation (Pulmicort, Astra, Kings Langley, UK) were used. The MDI was shaken for ten seconds and one actuation was fired to waste. The MDI was then weighed, shaken again for ten seconds, and actuated into the spacer. The spacer was immediately attached to the MSLI throat for 30 seconds to ensure complete emptying of the spacer. The procedure was repeated ten times to facilitate the drug assay. Each spacer was evaluated on four occasions with each drug.

For sodium cromoglycate, each stage of the MSLI was washed out quantitatively with distilled water, and the washings assayed by spectrophotometry at a wavelength of 326nm. For budesonide each stage of the MSLI was washed out with 20ml of internal standard solution, and the washings assayed by high performance liquid chromatography. From this the amount of drug collected at each stage was calculated, and a log probability plot constructed, from which the aerosol characteristics were determined.

Results

Sodium cromoglycate

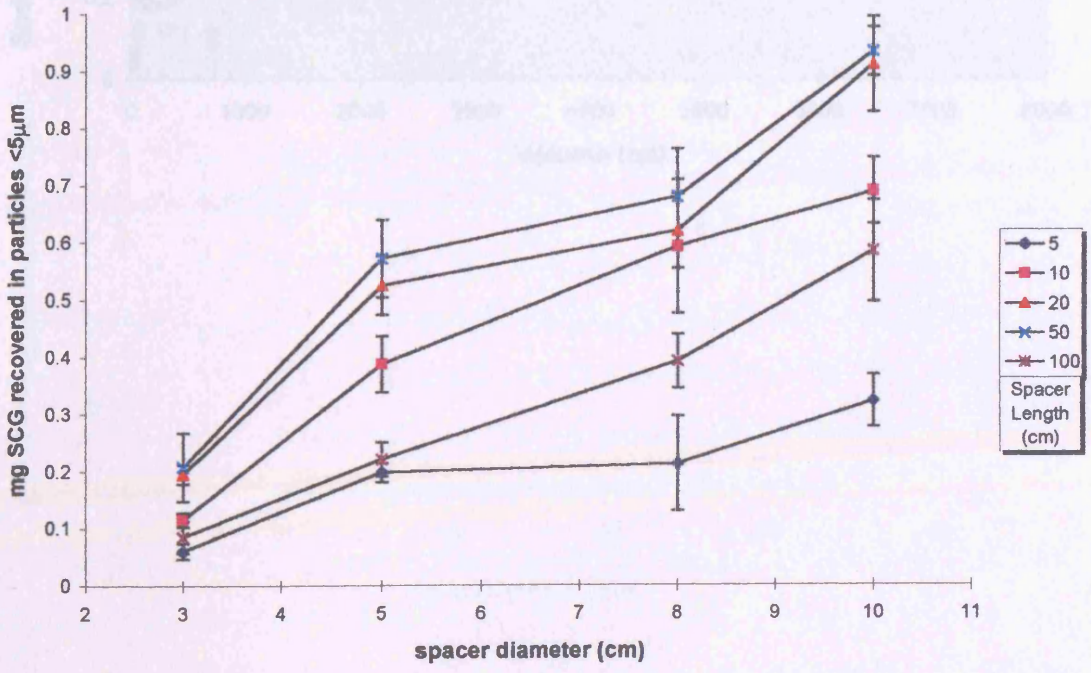
The amount (mg) of sodium cromoglycate recovered in particles smaller than five micrometers is given in the table and shown in the following graph for the different spacers.

The effect of spacer size on the delivery of sodium cromoglycate.

Mean amount of sodium cromoglycate recovered (mg) per 5mg actuation in particles smaller than 5μm (95% CI).

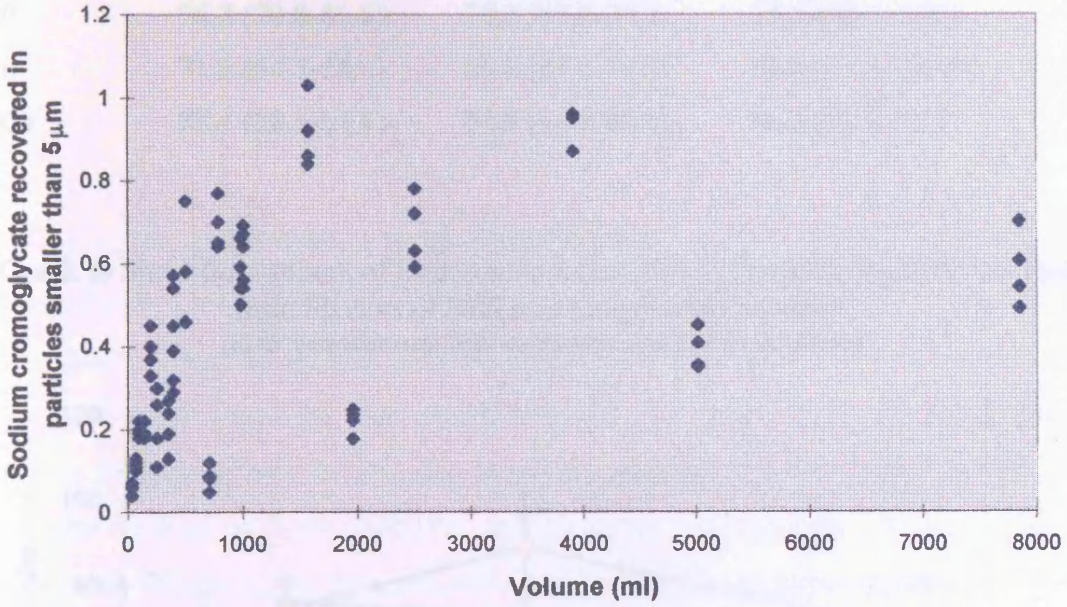
Diameter (cm) / Length (cm)	3	5	8	10
5	**0.06** (0.05-0.07)	**0.20** (0.18-0.21)	**0.21** (0.13-0.30)	**0.32** (0.28-0.37)
10	**0.12** (0.1-0.13)	**0.39** (0.34-0.44)	**0.59** (0.48-0.71)	**0.69** (0.63-0.75)
20	**0.20** (0.18-0.21)	**0.53** (0.47-0.58)	**0.62** (0.55-0.69)	**0.91** (0.83-1.00)
50	**0.21** (0.15-0.27)	**0.57** (0.50-0.64)	**0.68** (0.60-0.76)	**0.94** (0.89-0.98)
100	**0.08** (0.06-0.11)	**0.22** (0.19-0.25)	**0.39** (0.35-0.44)	**0.59** (0.50-0.67)

Graph to Show the Amount of Sodium Cromoglycate Recovered in Particles Smaller than 5μm from Spacers of Different Length and Diameter.
(Error bars represent 95% confidence intervals for the mean)



Increasing the spacer diameter had a large effect, but increasing the spacer length above 20cm only slightly increased the drug recovery. Analysis of the pooled data showed a significant effect of both length and diameter on drug recovery (ANOVA, $p<0.001$). Increasing spacer volume had little effect on drug recovery at volumes above 1,000mls.

Graph to Show the Amount of Sodium Cromoglycate Recovered in Particles Smaller than 5μm Against Spacer Volume for Spacers of Different Length and Diameter.



Budesonide

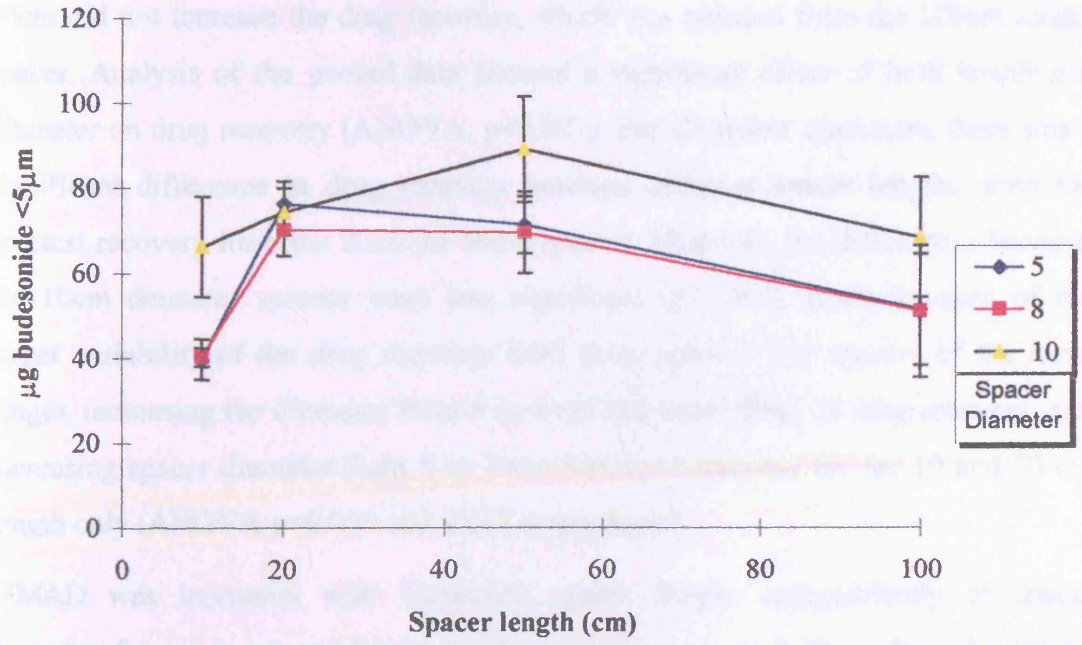
Spacers of 5, 8 and 10 cm diameter were evaluated with budesonide. The amount (μg) of budesonide recovered in particles smaller than five micrometers is given in the table and shown in the following graph for the different spacers.

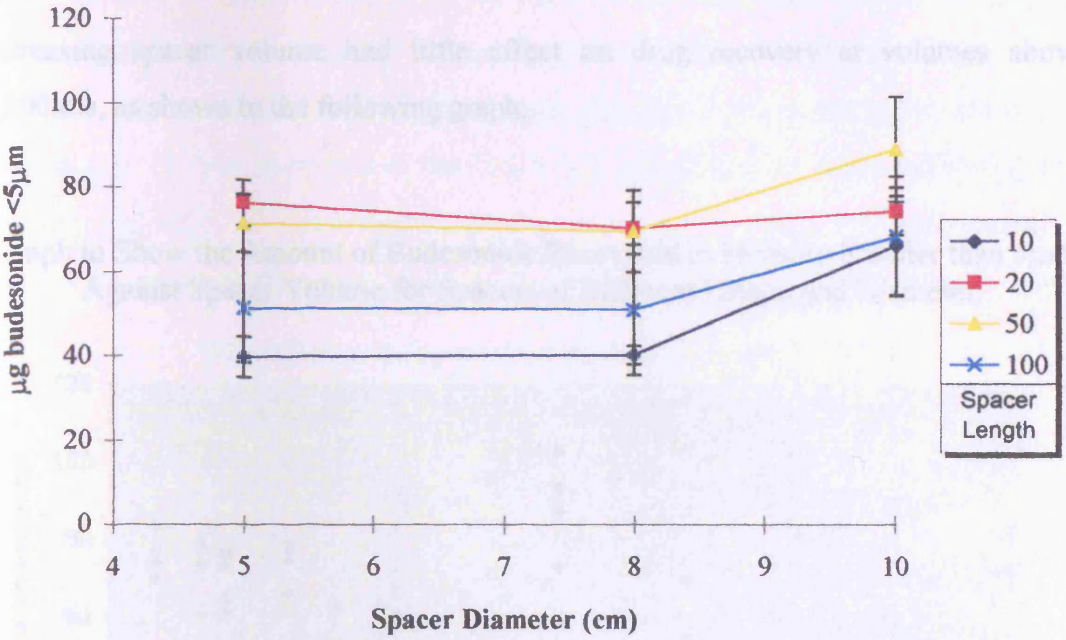
The effect of spacer size on the delivery of budesonide.

Mean amount of budesonide recovered (μg) per 200μg actuation in particles smaller than 5μm (95% confidence intervals).

Diameter (cm) / Length (cm)	5	8	10
10	**39.6** (34.9-44.4)	**40.2** (38.0-42.4)	**66.1** (54.4-77.9)
20	**76.2** (70.8-81.6)	**70.2** (64.0-76.4)	**74.3** (68.7-79.9)
50	**71.3** (64.5-78.0)	**69.5** (59.8-79.2)	**89.0** (76.5-101.4)
100	**51.4** (38.3-64.4)	**50.9** (35.4-66.4)	**68.3** (54.1-82.5)

Graph to Show the Amount of Budesonide Recovered in Particles Smaller than 5μm from Spacers of Different Length and Diameter.
(Error bars represent 95% confidence intervals for the mean)





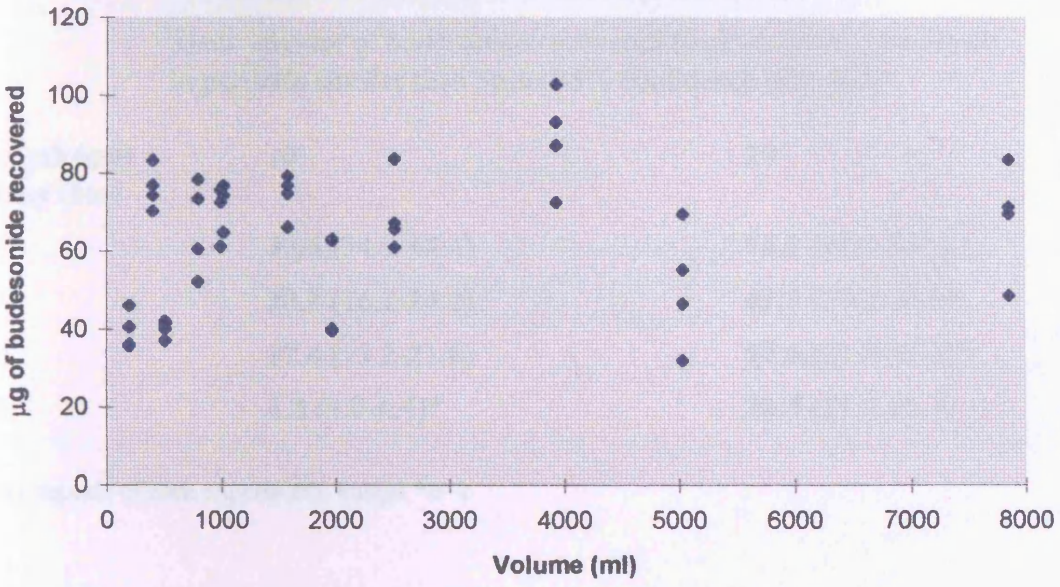
Increasing the spacer diameter had a negligible effect on drug recovery between 5 and 8 cm. However the 10cm diameter spacer delivered significantly more budesonide than smaller ones for lengths apart from 20cm. Increasing the spacer length above 20cm did not increase the drug recovery, which was reduced from the 100cm length spacer. Analysis of the pooled data showed a significant effect of both length and diameter on drug recovery (ANOVA, $p<0.001$). For all spacer diameters, there was a significant difference in drug recovery between different spacer lengths, with the greatest recovery from the 20cm or 50cm spacers. However, the differences between the 10cm diameter spacers were less significant ($p=0.067$), partly because of the larger variability of the drug recovery from these spacers. For spacers of the same length, increasing the diameter from 5 to 8 cm had little effect on drug recovery, and increasing spacer diameter from 8 to 10cm increased recovery for the 10 and 50 cm length only (ANOVA $p=0.001$ and 0.043 respectively).

MMAD was increased with increasing spacer length, independently of spacer diameter, from a mean of 3.94µm from the 10cm spacers to 4.38µm from the 100cm spacers ($p=0.07$), although most of this effect was seen between the 10 and 20cm

spacer, and the variability of the experimental measurements, especially for the 20cm spacer, makes this relationship very weak.

Increasing spacer volume had little effect on drug recovery at volumes above 1,000mls, as shown in the following graph.

Graph to Show the Amount of Budesonide Recovered in Particles Smaller than 5μm Against Spacer Volume for Spacers of Different Length and Diameter.



Delay

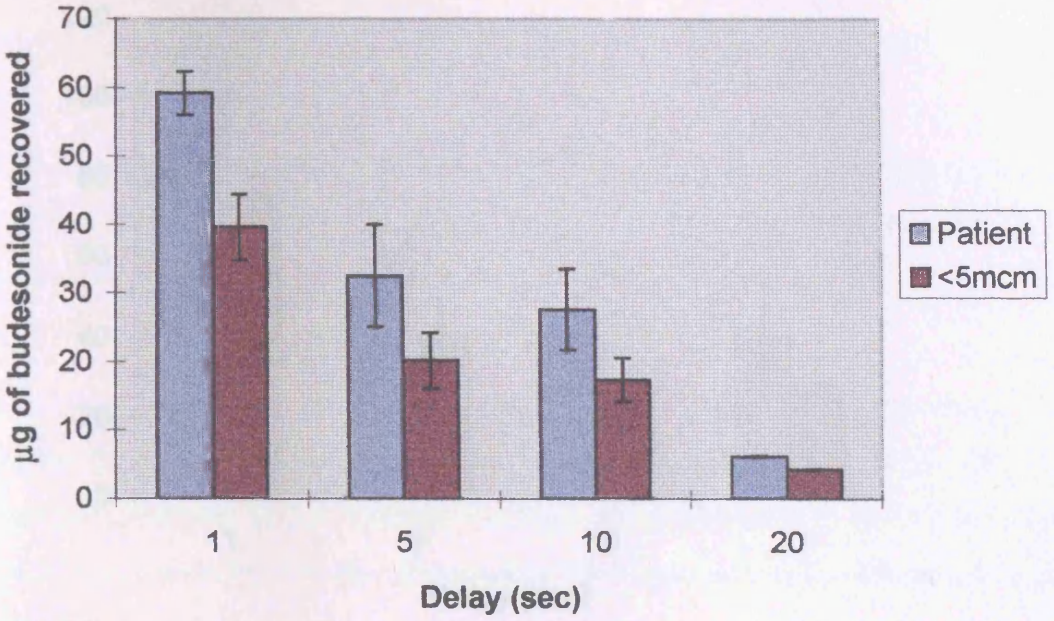
To determine the effect of delay on the recovery of drug from spacers of different lengths, budesonide was actuated into spacers 5cm in diameter and either 10 or 20 cm in length. After a delay of 5, 10 or 20 seconds, the spacer was connected to the MSLI. The amount of drug deposited in the MSLI was determined as described above. The amount (μg) of budesonide recovered in particles smaller than five micrometers is given in the table and shown in the following graph for the different spacers and delays.

The effect of delay on the delivery of budesonide.

Mean amount of budesonide recovered (μg) per 200μg actuation in particles smaller than 5μm (95% confidence intervals).

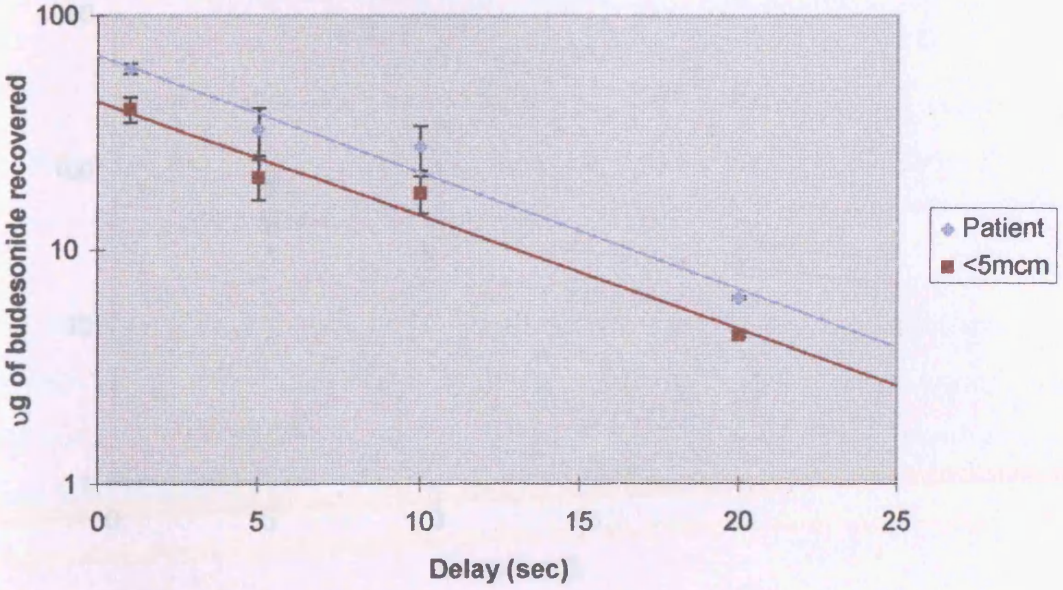
Length (cm) Delay (sec)	10	20
1	**39.6** (34.9-44.4)	**76.2** (70.8-81.6)
5	**20.2** (16.2-24.2)	**41.4** (27.2-55.6)
10	**17.4** (13.2-21.6)	**27.6** (21.8-33.4)*
20	**4.3** (4.0-4.4)*	**28.5** (21.2-35.7)

Four repeats of each experiment, except *n=2

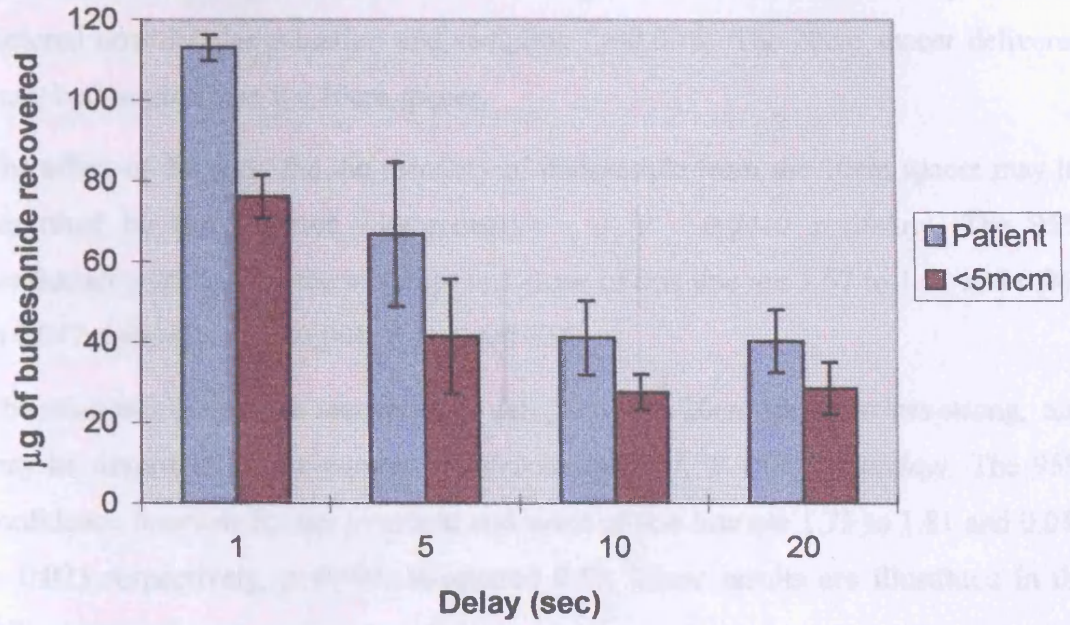


Graph to Show the Amount of Budesonide Recovered in Particles Smaller than 5µm from Spacers of Different Length (10cm diameter spacer).
(Error bars represent 95% confidence intervals for the mean)

Plotted on a semi-log scale, the decrease in drug recovery is shown in the next graph, with a line of best fit also plotted.

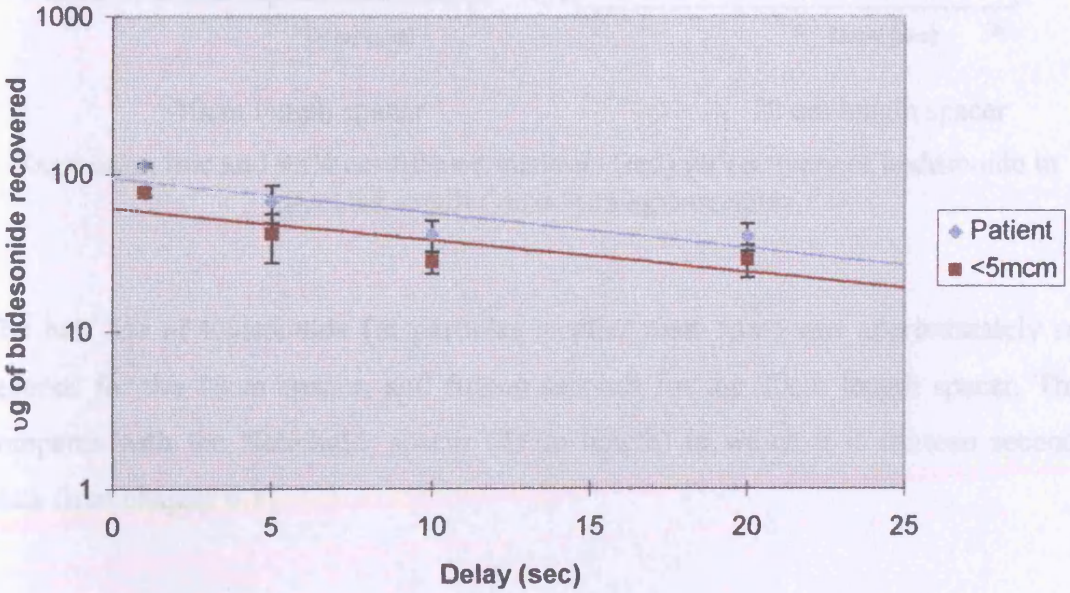


The results for the 20cm spacer are presented in the same way:



Graph to Show the Amount of Budesonide Recovered in Particles Smaller than 5µm from Spacers of Different Length (20cm diameter spacer).
(Error bars represent 95% confidence intervals for the mean)

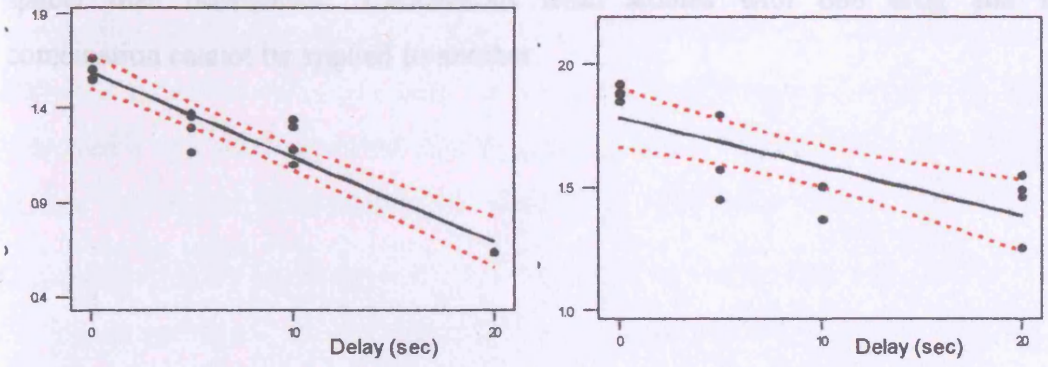
Plotted on a semi-log scale, the decrease in drug recovery is shown in the next graph, with a line of best fit also plotted.



The amount of drug recovered from both spacers decreased with delay between metered dose inhaler actuation and sampling ($p<0.001$). The 20cm spacer delivered more budesonide than the 10cm spacer.

The effect of delay on the the recovery of budesonide from the 10cm spacer may be described by the equation *log(recovery) = 1.59 - 0.0449 x (delay)*. The 95% confidence intervals for the intercept and slope of this line are 1.57 to 1.61 and 0.043 to 0.047 respectively, $p<0.001$, R-squared 0.90.

The relationship between recovery and delay with the 20cm spacer is less strong, and may be described by the equation *log(recovery) = 1.78 - 0.02 x (delay)*. The 95% confidence intervals for the intercept and slope of this line are 1.75 to 1.81 and 0.017 to 0.023 respectively, $p=0.001$, R-squared 0.59. These results are illustrated in the following graphs:



10cm length spacer 20 cm length spacer

Regression line and 95% confidence intervals (red) for recovery of budesonide in particles smaller than 5μm against delay.

The half life of budesonide (in particles smaller than 5μm) was approximately six seconds for the 10cm spacer, and fifteen seconds for the 20cm length spacer. This compares with the Nebuhaler spacer (23cm length) in which it is thirteen seconds (data from chapter 6.1).

Summary

The amount of sodium cromoglycate and budesonide delivered from different length and diameter spacer devices was determined using the multistage liquid impinger. The effect of delay on the amount of budesonide delivered from different length spacers was also determined.

For both drugs, increasing spacer length up to 20cm increased drug recovery, with no increase or a reduction with longer spacers. Increasing spacer diameter also increased drug recovery, although the increase was greater for sodium cromoglycate than budesonide. Budesonide delivery was greatest for spacers of 300ml or greater volume, whereas maximum sodium cromoglycate delivery was from spacers of 1,000ml or greater volume. Drug half life was also longer in larger spacers, and optimum spacer size is different for the two drugs. Sodium cromoglycate is best delivered from a larger spacer than budesonide. Conclusions from studies with one drug and spacer combination cannot be applied to another.

Video Analysis of the Aerosol Cloud Produced by Metered Dose Inhalers.

Aim

To determine the speed, shape and size of aerosol cloud produced from two different metered dose inhalers and relate this to the size of the oropharynx and commonly used spacer devices.

Methods

High speed video recording was undertaken using the Kodak EKTAPRO HS 4540 motion analysis system (Kodak Ltd, Hemel Hempstead, UK), as outlined in chapter 5.5.

Five new MDIs of sodium cromoglycate (Intal, Fisons PLC, Loughborough, UK) 5mg per actuation, and budesonide (Pulmicort, Astra Pharmaceuticals, Kings Langley, UK) 200μg per actuation were assessed. The first ten shots from each MDI was fired to waste, and a new adaptor clamped on to a retort stand. The MDI was shaken for ten seconds, then placed in the adaptor and fired. Each MDI was assessed once.

Measurements were made from the MDI adaptor to the leading edge of the cloud, allowing aerosol speed to be computed. Volume of the aerosol cloud was estimated by measuring the maximum vertical dimension of the cloud, the horizontal distance from the MDI adaptor to the maximum vertical dimension, and the distance from there to the leading edge of the cloud. The cloud was assumed to be conical in shape from the MDI adaptor to its maximum vertical dimension, and cylindrical from there on to the leading edge. Measurements of volume and speed were made until the leading edge of the aerosol cloud left the video frame or could not be discerned. Summary measures were used to analyse the volume and speed of the aerosol (Matthews et al 1990). These comprised volume at 60ms and the slope of speed plotted against time, and were compared for the two drugs using a two tailed t test.

Results

Representative images of both aerosols are given in the figures and in the accompanying video (appendix 1).

Cloud morphology.

Both aerosol clouds appeared to consist of two distinct phases; an initial narrow jet which appeared to be spiral in shape, and a subsequent slower moving cloud of dispersing aerosol. The budesonide aerosol (figure) appeared to have a narrower jet phase, and smaller cloud than the sodium cromoglycate MDI (figure), whose jet phase was more wedge shaped. The budesonide actuation, recorded to completion on three occasions, was completed by 80ms, and the cloud difficult to visualise after 100ms, whereas the sodium cromoglycate actuation (recorded to completion on one occasion) continued for 120ms.

Speed.

The initial cloud speed varied from 14 to 33m/s in the first 2ms, the variation being due to the difficulty in accurately measuring the cloud at such an early stage. By 5ms, when aerosol had travelled approximately five centimetres, the speed of the leading edge of the aerosol cloud was less than 10m/s. After 30ms the speed was 2-3m/s (distance travelled 17-18cm), which was maintained until the leading edge passed out of the video frame, approximately 25cm from the actuator. There was no difference in the change in forward speed of the sodium cromoglycate or the budesonide aerosols ($t=0.55$, $P=0.6$).

Time (ms)	Speed (m/sec), (Mean (95% confidence intervals)). Budesonide	Sodium Cromoglycate
10	5.1 (3.6-6.7)	5.1 (3.7-6.5)
20	4.1 (3.6-4.7)	4.2 (3.9-4.6)
30	3.4 (3.1-3.8)	3.1 (2.7-3.4)
40	2.5 (2.2-2.7)	2.5 (1.9-3.1)
50	1.9 (1.8-2.1)	2.5 (2.1-2.9)
60	2.3 (1.9-2.6)	2.3 (1.8-2.7)

Time (ms)	Distance from actuator (mm), (Mean (95% confidence intervals)).	
	Budesonide	Sodium Cromoglycate
10	100 (97-102)	106 (98.3-113.1)
20	141 (136-146)	148 (143.2-152.6)
30	175 (169-182)	179 (171.9-185.4)
40	199 (192-206)	204 (196.4-210.6)
50	220 (211-228)	229 (221.7-236.0)
60	242 (233-252)	252 (242.4-260.8)

Volume.

Aerosol volume was significantly greater for the sodium cromoglycate aerosol (686ml vs 411ml at 60ms, t=-2.39, P=0.04).

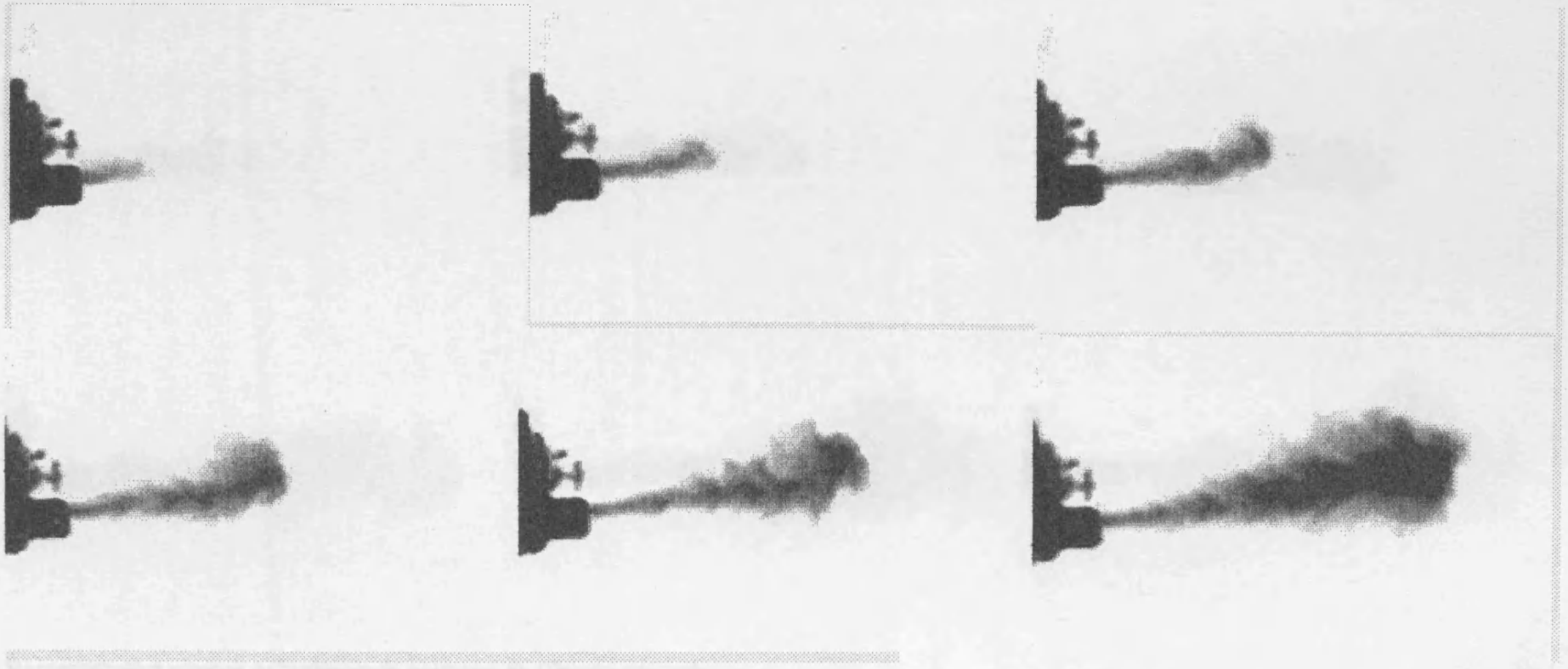
Time (ms)	Estimated Volume (cm^3), (Mean (95% confidence intervals)).	
	Budesonide	Sodium Cromoglycate
10	18 (12-23)	34 (28-40)
20	57 (43-72)	115 (99-131)
30	150 (116-183)	200 (126-274)
40	202 (152-252)	350 (230-469)
50	331 (252-409)	533 (388-678)
60	411 (325-498)	686 (478-894)

The Aerochamber spacer is 11cm in length, and both aerosols would reach the end of the spacer a little over ten seconds after actuation. In contrast, the end of the Nebuhaler or Fisonair spacers (both approx 20cm length) would not be reached until 50msec after actuation.

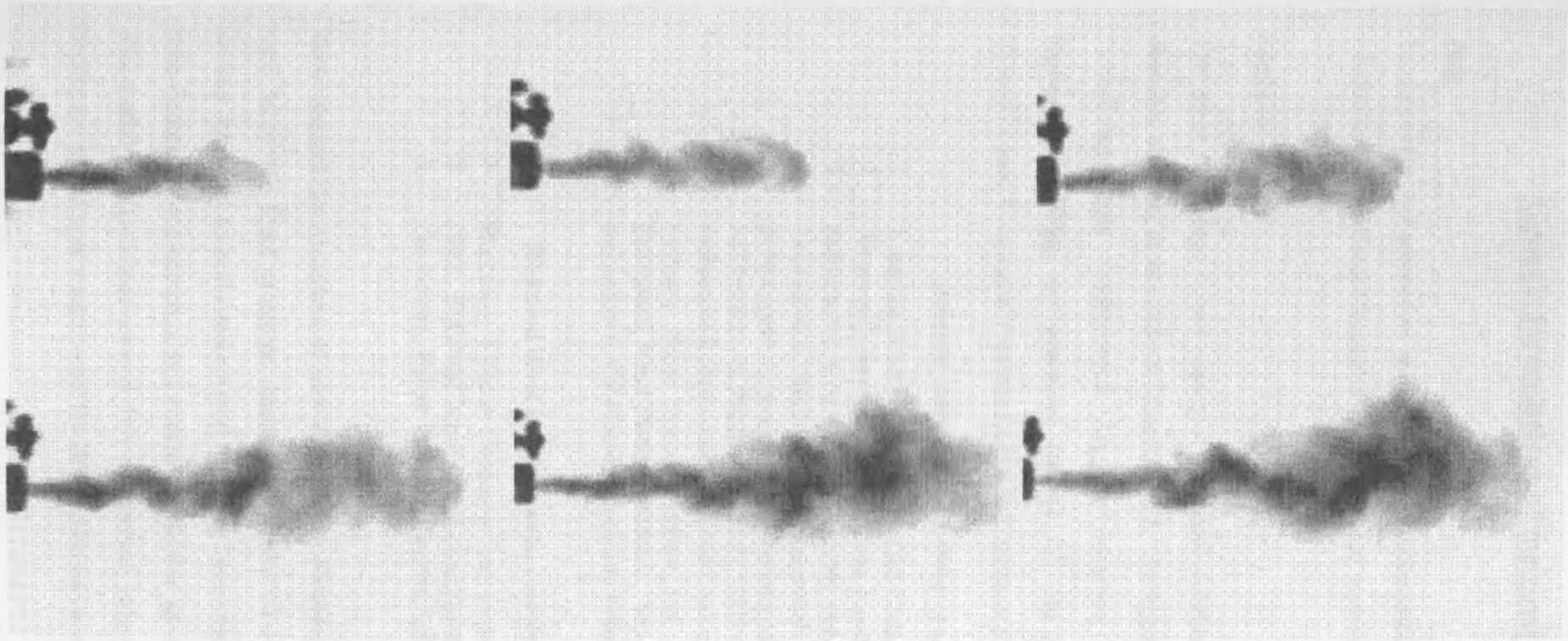
Summary

The speed, shape and size of aerosol cloud produced from two different metered dose inhalers actuated into still air was measured using a high speed video camera. The two inhalers studied differed in the aerosol cloud shape, though not in their forward speed.

As aerosol deposition in the upper airway and spacer devices is largely due to inertial impaction, differences in aerosol cloud formation and geometry may affect drug delivery. The high speed video system provides a means to investigate these factors.



Representative plumes of sodium cromoglycate captured at 10msec intervals.



Representative plumes of budesonide captured at 10msec intervals.

Generic formulations of metered dose inhalers

Aim

To determine the amount of salbutamol and beclomethasone dipropionate available for inhalation from different formulations when used with the Volumatic spacer.

Methods

The glass multistage liquid impinger was used to determine the MDI output of the different metered dose inhalers (chapter 5.1). The experiments were undertaken using 10 different brands of salbutamol, 100μg per actuation and 3 brands of beclomethasone dipropionate, 100μg per actuation (table). All the medications were administered through a new Volumatic™ spacer (Allen & Hanburys Ltd, Uxbridge, UK).

Metered dose inhalers used - salbutamol

1. Ventolin, Allen & Hanburys Ltd, Uxbridge, UK
2. Asmaven, Berk Pharmaceuticals, Leeds, UK
3. Salbutamol, Cox Pharmaceuticals, Barnstaple, UK
4. Salbutamol, Hillcross Pharmaceuticals, Burnley, UK
5. Salbutamol, Generics (UK), Potters Bar, UK
6. Salbutamol, K Pharmaceuticals, Harlow, UK
7. Salbutamol, Approved Prescription Services, Leeds, UK
8. Salamol, Baker Norton, Harlow, UK
9. Kentamol, Kent Pharmaceuticals, Ashford, UK
10. Salbutamol, CP Pharmaceuticals. Wrexham, UK

Metered dose inhalers used - beclomethasone

1. Becotide, Allen & Hanburys Ltd, Uxbridge, UK
2. Filair, 3M Healthcare Ltd, Loughborough, UK.
3. Beclazone, Baker Norton, Harlow, UK.

New metered dose inhalers of each brand were obtained. The first ten actuations from each MDI was fired to waste. Immediately prior to each experiment, the MDI was shaken for thirty seconds and primed by firing two actuations to waste. The MDI was then shaken for ten seconds and actuated into the spacer, which was attached to the MSLI after a delay of one second. For salbutamol, only one actuation was administered before the MSLI was washed and the drug collected assayed. For beclomethasone, this

procedure was repeated for ten actuations during each experiment to facilitate the drug assay.

Each stage of the MSLI was washed with methanol and the amount of drug collected at each stage determined by high pressure liquid chromatography (chapter 5.7).

Experiments were repeated four times with each brand of metered dose inhaler. Fifty two new Volumatic spacers were used once each for the experiments. The laboratory temperature and relative humidity were recorded for each experiment.

Single shots of salbutamol

To ensure that each brand delivered the claimed amount of medication, the total amount of salbutamol released from both a single and two consecutive actuations of each metered dose inhaler was determined by firing the MDI into a glass trap and filter unit. Immediately prior to each experiment, the MDI was shaken for thirty seconds and primed by firing three actuations to waste. The MDI was weighed then shaken for ten seconds and connected to the filter unit. A vacuum pump was switched on to draw the drug onto the filter and the MDI was actuated once. If two actuations were being measured, the MDI was shaken for a further ten seconds, attached to the filter unit and fired again Twenty seconds after the final actuation, the pump was turned off and the MDI re-weighed. The filter and filter unit were washed out with methanol and the amount of drug collected at each stage determined by high pressure liquid chromatography (chapter 5.7). Note that drug remaining on the MDI actuator or the MDI valve stem was not measured.

Results - salbutamol

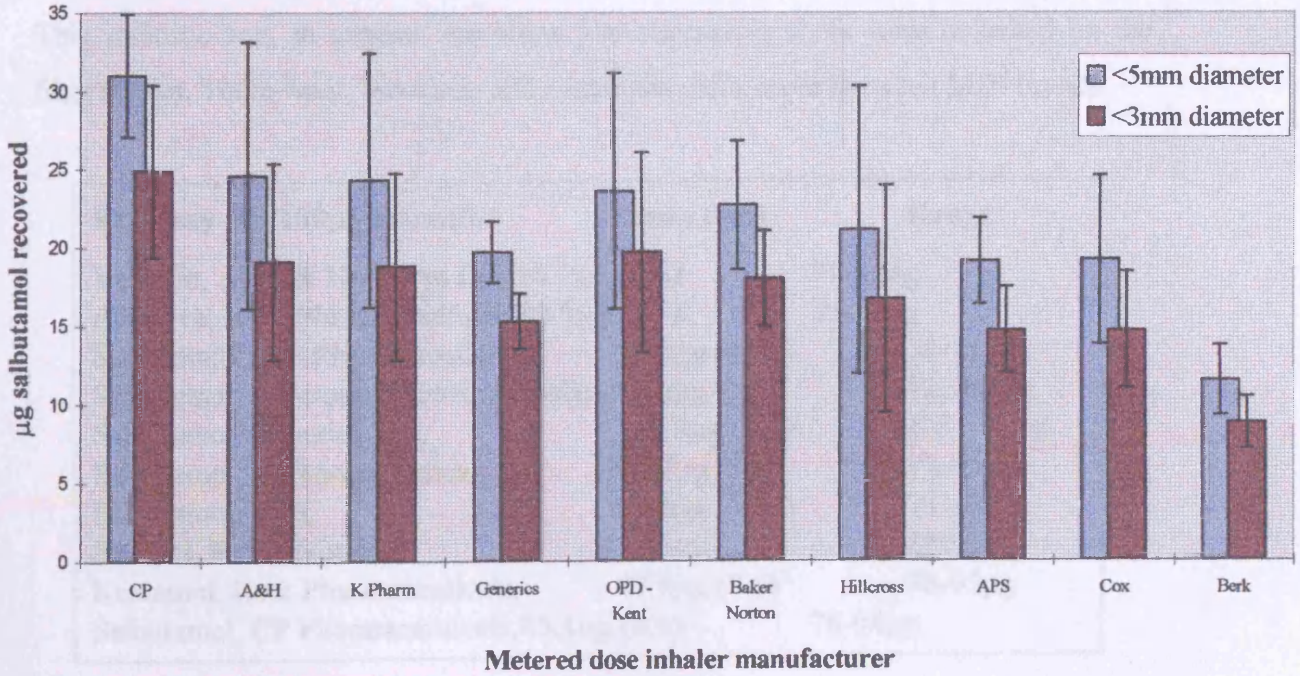
The table gives the amount of salbutamol recovered from the MSLI, the calculated amount in particles smaller than five and three micrometers, the mass median aerodynamic diameter and the geometric standard deviation. Analysis of variance shows significant differences between brands of inhaler, with nearly a three fold difference between the delivery of salbutamol from the CP Pharmaceuticals inhaler and that from Berk Pharmaceuticals. The large variation in drug delivery within each brand

and that from Berk Pharmaceuticals. The large variation in drug delivery within each brand should also be noted.

The delivery of salbutamol from different brands of metered dose inhaler via the Volumatic spacer.

MDI manufacturer.	Mean amount of salbutamol recovered (μg) per 100μg actuation (95% Confidence Intervals).				
	Dose To MSLI	In Particles < 5 μm	In Particles < 3 μm	MMAD	GSD
CP	**36.4** (34.0-38.8)	**31.0** (27.1-34.9)	**24.9** (19.4-30.4)	**2.2** (1.3-3.1)	**2.1** (1.9-2.3)
A&H	**30.0** (19.2-40.8)	**24.6** (16.1-33.1)	**19.1** (12.8-25.4)	**2.2** (2.0-2.4)	**2.4** (2.3-2.5)
K Pharm.	**29.1** (18.5-39.7)	**24.3** (16.2-32.4)	**18.8** (12.8-24.8)	**2.2** (2.0-2.4)	**2.2** (2.1-2.3)
Generics	**27.6** (17.4-37.8)	**19.7** (17.7-21.7)	**15.3** (13.5-17.1)	**2.9** (1.2-4.6)	**2.7** (1.9-3.5)
OPD - Kent	**27.2** (18.8-35.6)	**23.6** (16.1-31.1)	**19.7** (13.3-26.1)	**1.8** (1.6-2.0)	**2.6** (2.2-3.0)
Baker Norton	**25.8** (20.8-30.8)	**22.7** (18.6-26.8)	**18.0** (15.0-21.0)	**2.0** (1.9-2.1)	**2.1** (2.0-2.2)
Hillcross	**25.0** (14.2-35.8)	**21.1** (11.9-30.3)	**16.7** (9.4-24.0)	**2.2** (2.0-2.4)	**2.3** (2.2-2.4)
APS	**23.1** (20.3-25.9)	**19.1** (16.4-21.8)	**14.7** (12.0-17.4)	**2.3** (2.1-2.5)	**2.3** (2.1-2.5)
Cox	**23.0** (16.0-30.0)	**19.2** (13.8-24.6)	**14.7** (11.0-18.4)	**2.3** (2.1-2.5)	**2.2** (2.0-2.4)
Berk	**14.2** (10.7-17.7)	**11.5** (9.2-13.8)	**8.8** (7.1-10.5)	**2.4** (2.0-2.8)	**2.2** (1.9-2.5)

These results are illustrated in the graph:



Single shot

The amount of salbutamol collected on the filter is given in the table. There are significant differences between the brands and large variations within each brand, more marked for some (i.e. APS, Generics) than others (i.e. K Pharmaceuticals, Cox Pharmaceuticals). Shot weights were not significantly different either between brands or within each brand, suggesting that the differences seen are not due to misfiring of the MDI.

Recovery per 100µg actuation	Mean (SD)	Range
Ventolin, Allen & Hanburys Ltd,	82.6µg (6.4)	72-90µg
Asmaven, Berk Pharmaceuticals,	82.3µg (7.2)	71-89µg
Salbutamol, Cox Pharmaceuticals,	88.9µg (4.6)	82-95µg
Salbutamol, Hillcross Pharmaceuticals,	96.3µg (7.2)	88-108µg
Salbutamol, Generics UK,	118.2µg (25.3)	82-158µg
Salbutamol, K Pharmaceuticals,	81.4µg (4.2)	75-87µg
Salbutamol, APS,	66.2µg (19.0)	36-86µg
Salamol, Baker Norton	77.6µg (9.3)	62-86µg
Kentamol, Kent Pharmaceuticals,	72.4µg (8.4)	66-87µg
Salbutamol, CP Pharmaceuticals,	86.2µg (7.2)	76-96µg

Two shots

This variation was, in general less when two successive shots were collected on the filter (table). There were, however, still significant differences between MDI brands

Recovery per 100µg actuation	Mean (SD)	Range
Ventolin, Allen & Hanburys Ltd,	80.3µg (6.6)	71-89µg
Asmaven, Berk Pharmaceuticals,	82.5µg (2.3)	80-87µg
Salbutamol, Cox Pharmaceuticals,	93.3µg (13.9)	78-112µg
Salbutamol, Hillcross Pharmaceuticals,	96.6µg (5.3)	92-107µg
Salbutamol, Generics UK,	116.3µg (9.0)	108-131µg
Salbutamol, K Pharmaceuticals,	86.7µg (5.3)	79-93µg
Salbutamol, APS,	85.5µg (10.8)	73-102µg
Salamol, Baker Norton	91.5µg (2.5)	88-96µg
Kentamol, Kent Pharmaceuticals,	87.8µg (7.5)	76-95µg
Salbutamol, CP Pharmaceuticals,	85.1µg (6.6)	78-94µg

The data suggests differences in drug delivery between formulations of salbutamol when used alone and when used with the Volumatic spacer device. Only one batch of each brand was tested, and it is possible that the low recovery from, i.e. the APS salbutamol was due to a faulty batch of inhalers.

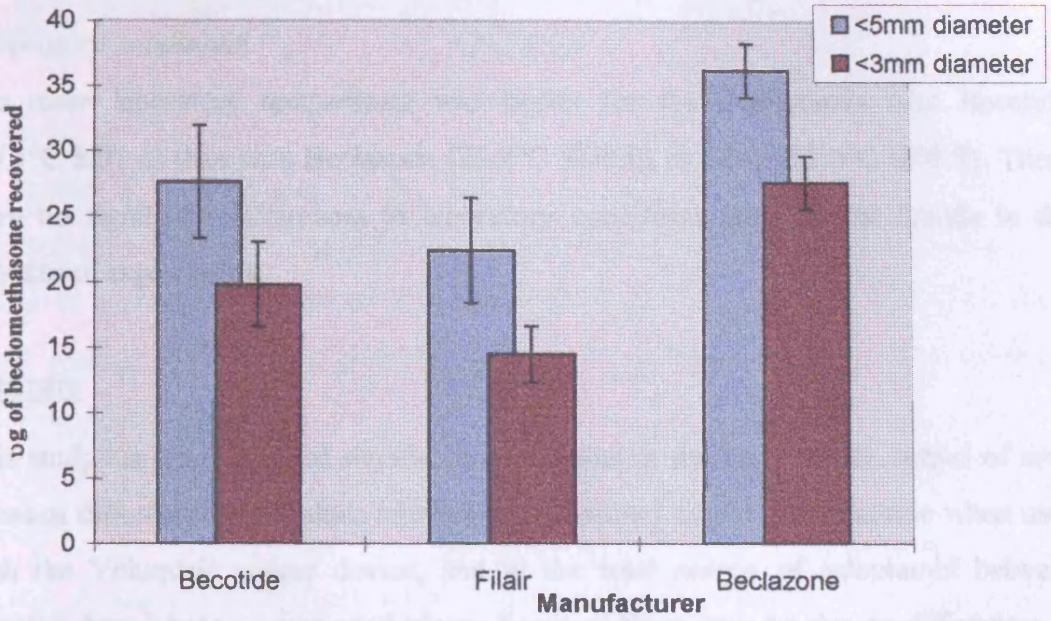
Results - beclomethasone

The table gives the amount of beclomethasone, per 100μg actuation, recovered from the MSLI, the calculated amount in particles smaller than five and three micrometers, the mass median aerodynamic diameter and the geometric standard deviation. Analysis of variance shows significant differences between brands of inhaler.

The delivery of beclomethasone from different brands of metered dose inhaler via the Volumatic spacer.

MDI brand	Mean amount of beclomethasone recovered (μg) per 100μg actuation (95% Confidence Intervals).				
	Dose To 'Patient'	In Particles < 5 μm	In Particles < 3 μm	MMAD	GSD
Becotide	**33.3** (28.1-38.5)	**27.6** (23.3-31.9)	**19.8** (16.6-23.0)	**2.5** (2.4-2.6)	**2.1** (2.0-2.2)
Filair	**28.8** (23.7-33.9)	**22.4** (18.4-26.4)	**14.5** (12.3-16.7)	**3.0** (2.8-3.2)	**2.0** (1.9-2.1)
Beclazone	**43.3** (40.5-46.1)	**36.2** (34.1-38.3)	**27.6** (25.5-29.7)	**2.3** (2.0-2.6)	**2.2** (2.1-2.3)

These results are illustrated in the graph:



Inhaled corticosteroids may contribute to both local and systemic side effects. Use of the Volumatic spacer has been shown to reduce local side effects by reducing oropharyngeal deposition of drug from metered dose inhalers. The amount of beclomethasone released from the inhalers in this study may be expressed as the percentage of the total drug delivered that is in particles below five microns aerodynamic diameter, where a greater percentage implies less risk of local side effects, and as the percentage contained in particles smaller than three microns, which have more chance of entering the lung periphery. These are given in the table. The Filair MDI delivers a coarser aerosol than the other two, with a lower percentage of particles below three microns aerodynamic diameter. The differences between the Becotide and Beclazone inhalers are not statistically significant.

Ratio of	<5μm/total delivery Mean (95% CI)	<3μm/<5μm Mean (95% CI)
Becotide	**82.8%** (80.6-84.9)	**72.0%** (70.8-73.1)
Filair	**77.6%** (75.6-79.7)	**65.3%** (62.4-68.1)
Beclazone	**83.8%** (80.4-87.1)	**76.2%** (72.7-79.7)

Laboratory conditions

The mean laboratory temperature was higher for the experiments with Becotide (29.1°C SD1.4) than with Beclazone (25.2°C SD0.8) or Filair (22.0°C SD1.8). There were no significant differences in laboratory conditions between the brands in the salbutamol experiments.

Summary

This study has demonstrated significant differences in the fine particle output of drug between different metered dose inhalers of salbutamol and beclomethasone when used with the Volumatic spacer device, and in the total output of salbutamol between metered dose inhalers when used alone. Some of these may be due to differences in laboratory conditions, and the possibility that a substandard batch of some types of metered dose inhalers was used cannot be excluded. Nevertheless, if the results of this study are generalisable, they suggest that different metered dose inhalers cannot be assumed to be equivalent when used with spacer devices, even if equivalence is suggested when they are used alone.

The effect of breathing pattern on the clearance of aerosol from spacers.

Aim

In chapter 6.3, differences in the output of drug from different spacers were demonstrated. To try and explain these differences, it was hypothesised that aerosol would be cleared with different efficiencies from different spacers. The aim of this study was to visualise the emptying pattern of different spacers under simulated breathing conditions

Methods

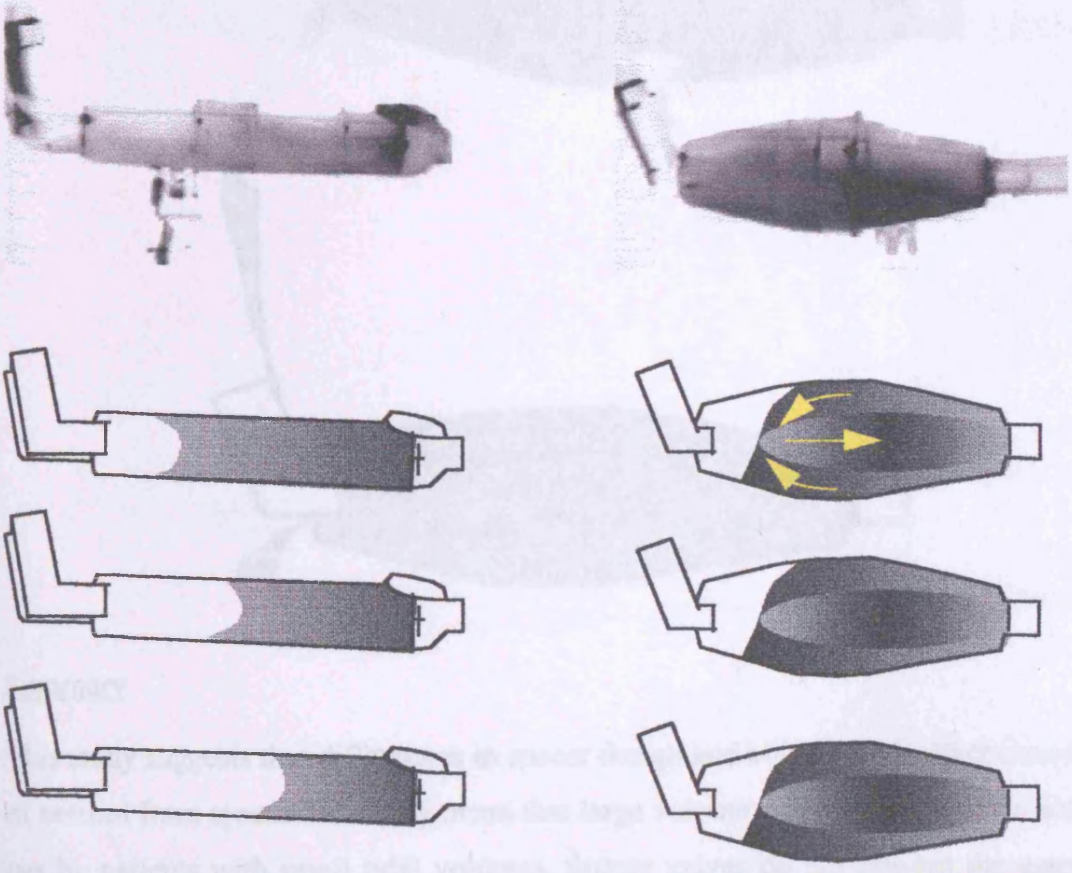
A video camera was used to record the clearance of aerosol from five different spacers, two small volume (<200ml); the Babyhaler (Glaxo Wellcome, Uxbridge, UK) and Aerochamber (Trudell Medical, London, Canada), and three large volume (>700ml); Volumatic (Allen & Hanburys Ltd, Uxbridge, UK), Nebuhaler (Astra Pharmaceuticals, Kings Langley, UK) and Fisonair (Fisons plc, Loughborough, UK). The spacers were filled with dry ice, then connected to a breathing simulator (Pari Sinus Breath Simulator). The clearance of aerosol from the spacer was then recorded with tidal breathing at a rate of 20 or 30 breaths, inspiratory fraction 40%, and tidal volume 50, 100, 200, 400 and 800ml. Recording was stopped after 20seconds.

Results

Analysis of the video recordings showed more efficient clearance of the CO_2 from the cylindrical spacers (Babyhaler and Aerochamber) at lower tidal volumes, with CO_2 still visible in the three larger spacers after 20 seconds (seven breaths at 20 breaths per minute) at tidal volumes less than 300ml. At a tidal volume of 800ml, the Babyhaler and Aerochamber were cleared of CO_2 after one breath, the Nebuhaler after two breaths, and the Volumatic and Fisonair after three breaths. The accompanying figures show the spacers at the end of each simulated breath for different breathing patterns.

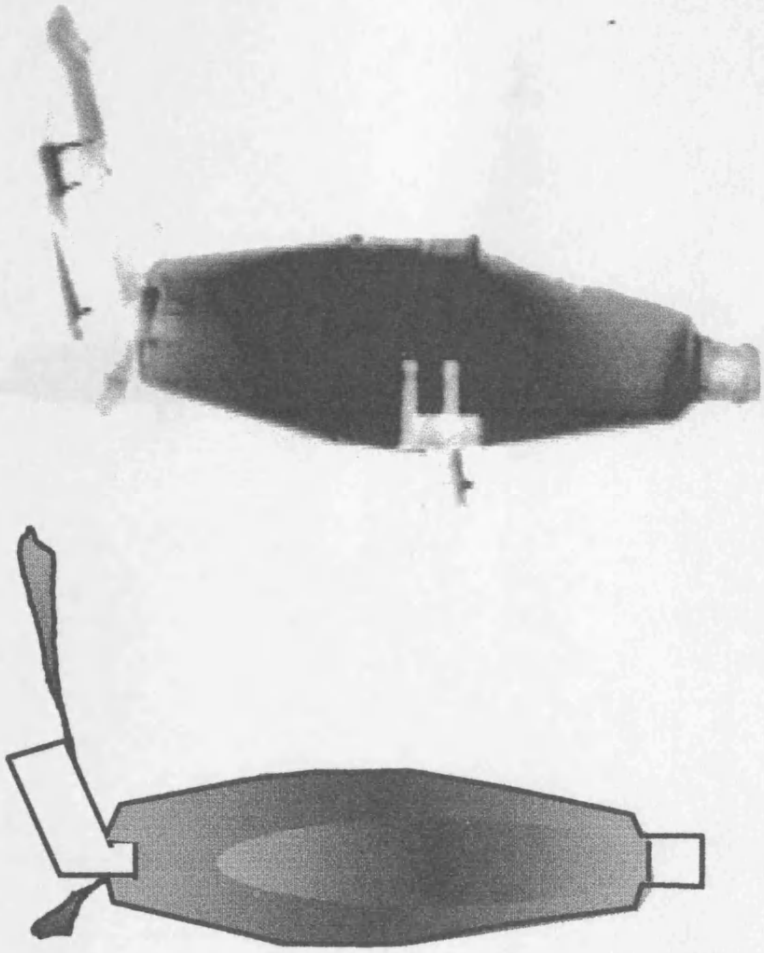
Tidal volume (ml)	Number of breaths to empty spacer (20bpm)				
	Babyhaler	Aerochamber	Nebuhaler	Volumatic	Fisonair
50	>7	>7	>7	>7	>7
100	6	4	>7	>7	>7
200	4	4	7	>7	6-7
400	3	2	4	6-7	3-4
800	1	1	2	4	2-3

Their was considerable mixing of aerosol and entrained air during inspiration and expiration with the large volume spacers. The cylindrical spacers cleared aerosol progressively along their length, with little mixing with entrained air.



This is demonstrated in the figure above, in which the position of the aerosol at the end of three breaths is outlined from the Babyhaler and Volumatic spacers. No atttempt was made to quantify aerosol delivery during these experiments.

Although all the spacers have expiratory valves, there were obvious losses of aerosol from the spacer, displaced by expired air passing through the incompletely closed valve, as shown in the following diagram:

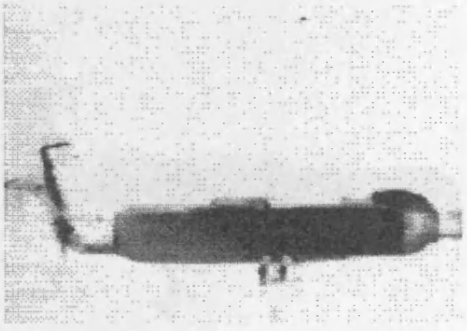


Summary

This study suggests that differences in spacer design and volume may affect clearance of aerosol from spacers, and may mean that large volume spacers are less efficient for use by patients with small tidal volumes. Spacer valves do not prevent the entry of expired air, and the loss of aerosol from the spacer. The effects of these findings on drug delivery remain to be determined.

Babyhaler
Tidal Volume 800 ml
Rate 20 bpm

1.



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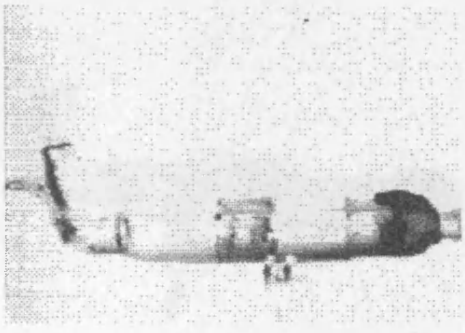
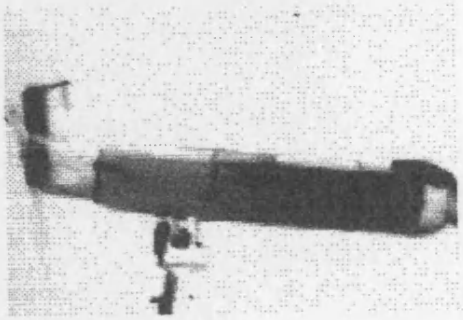


Babyhaler
Tidal Volume 100 ml
Rate 20 bpm

1.



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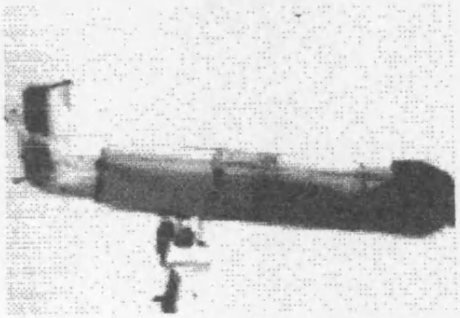
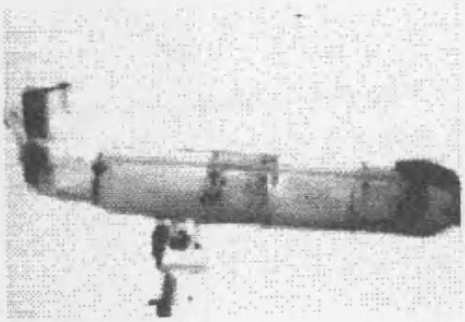


3.

4.

5.



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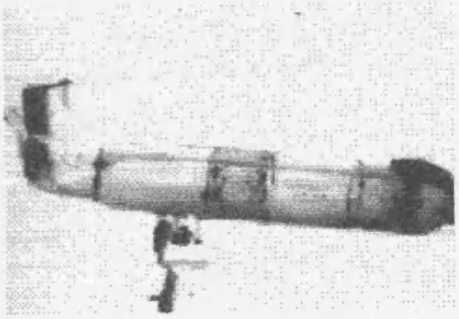

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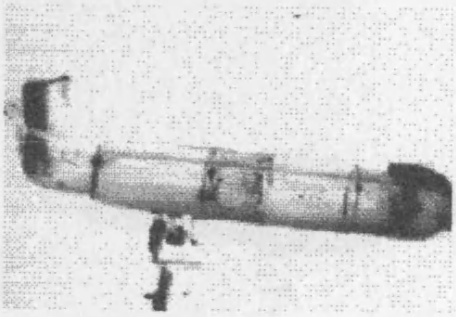
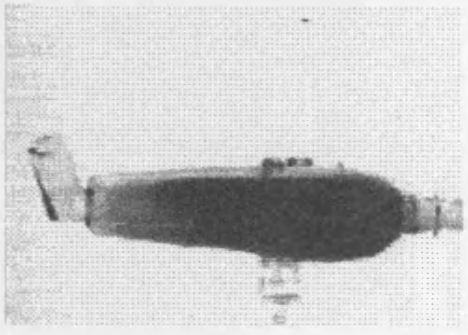


Nebuhaler
Tidal Volume 800 ml
Rate 20 bpm

1.



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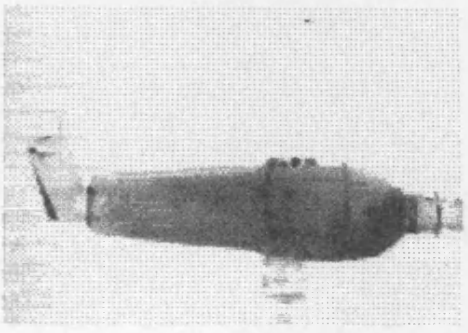

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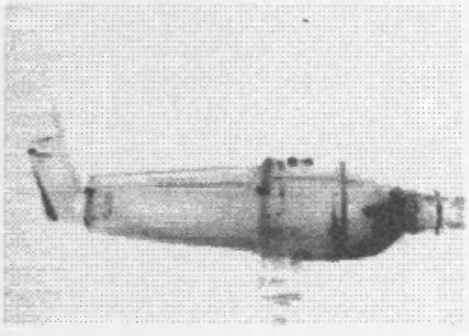

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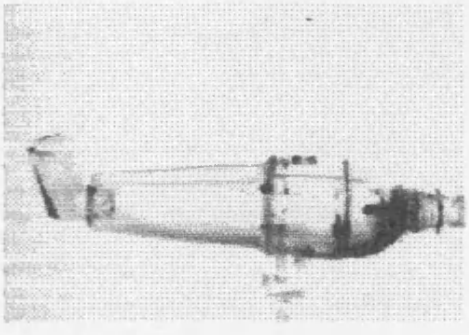

Nebuhaler
Tidal Volume 400 ml
Rate 20 bpm

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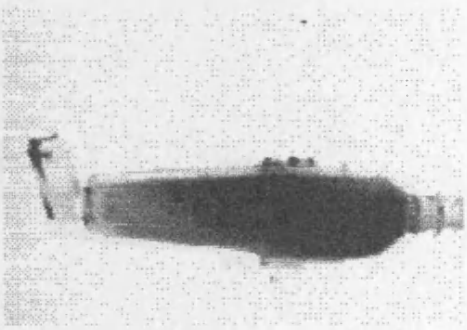

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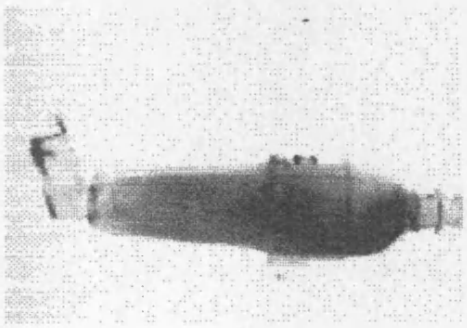

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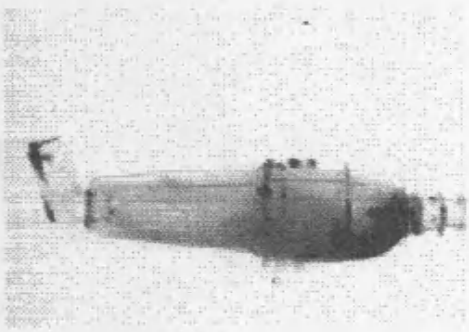

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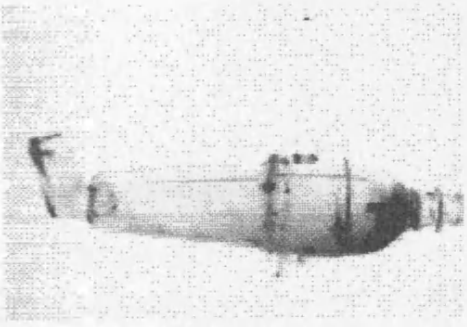

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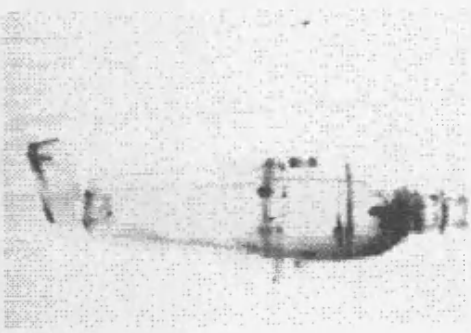
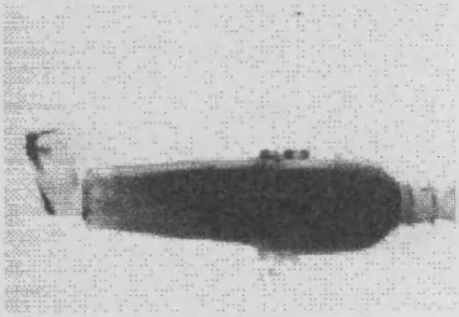


Nebuhaler
Tidal Volume 200 ml
Rate 20 bpm

1.



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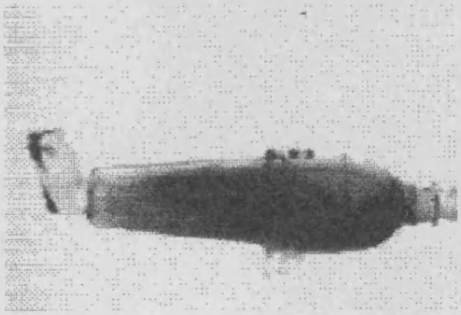

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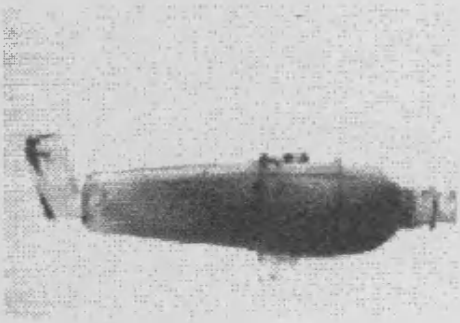

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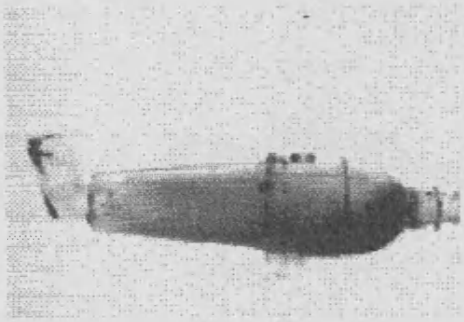

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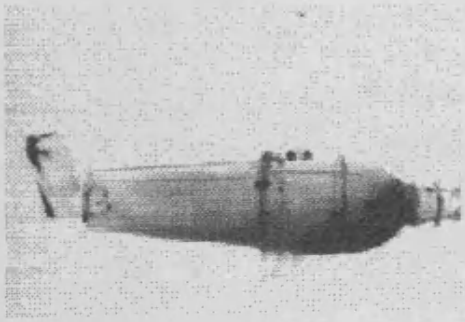

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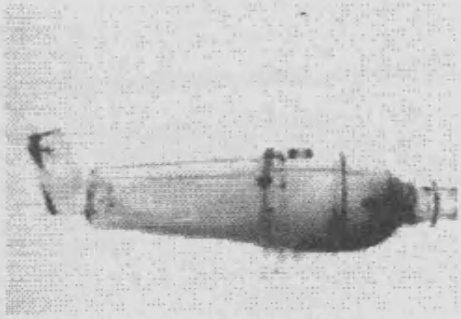

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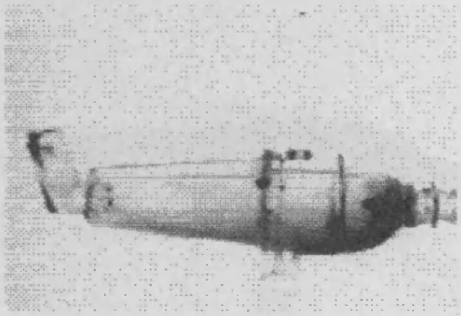
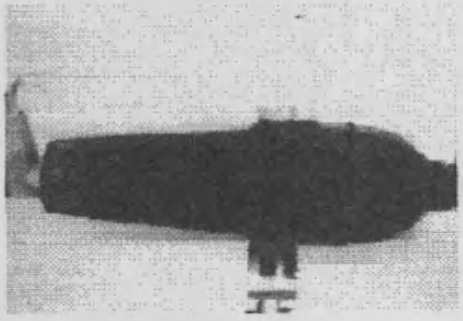


Nebuhaler
Tidal Volume 100 ml
Rate 20 bpm

1.

2.

3.

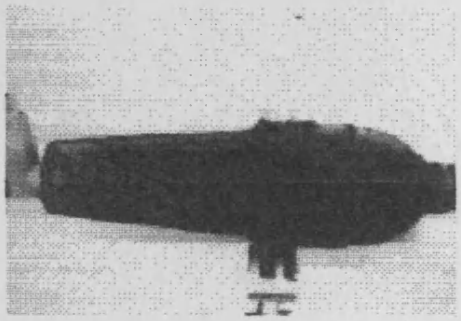


4.

5.

6.

7.



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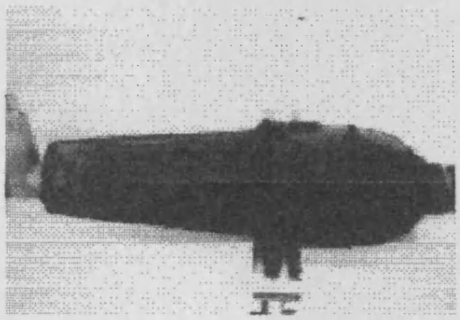

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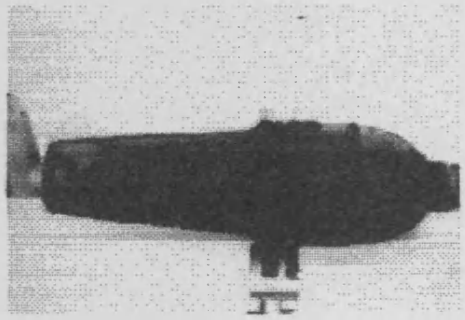

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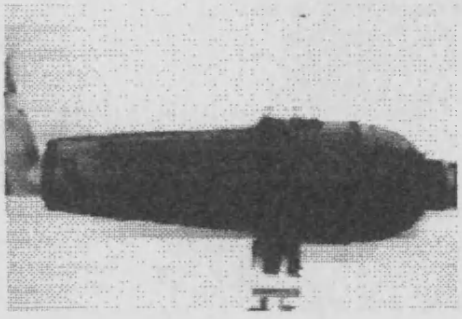
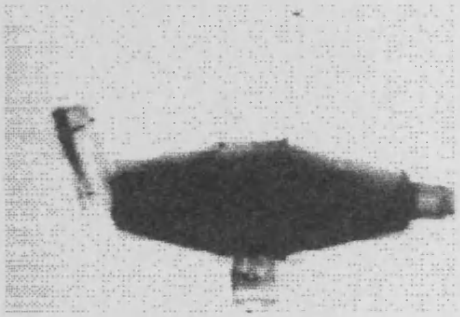


Volumatic
Tidal Volume 800 ml
Rate 20 bpm

1.



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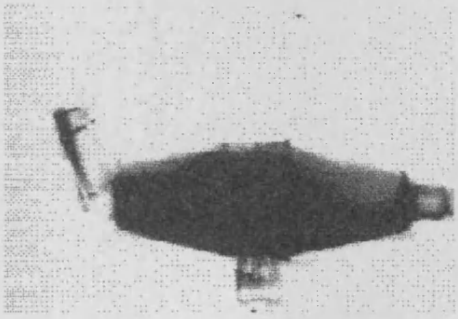

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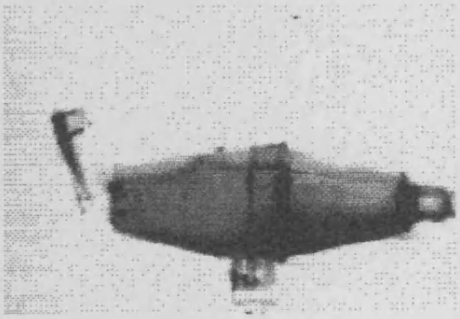

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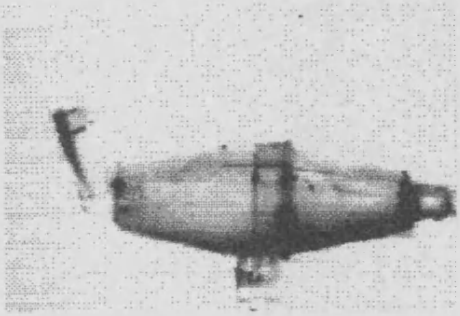
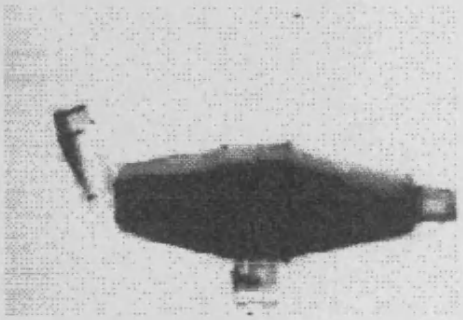


Volumatic
Tidal Volume 400 ml
Rate 20 bpm

1.



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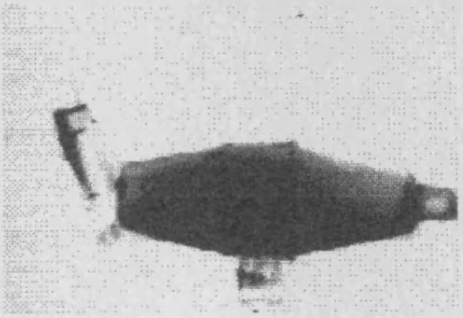

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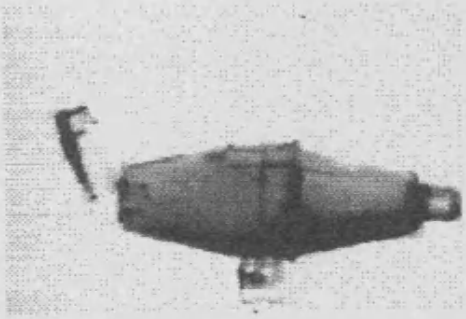

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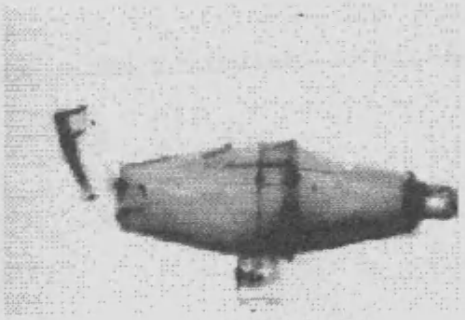

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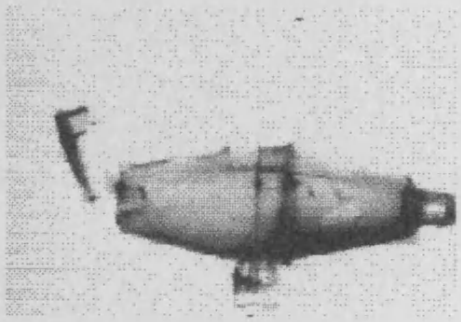
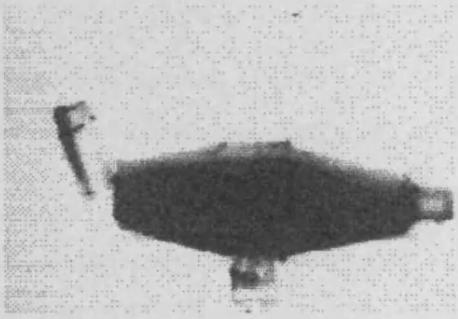


Volumatic
Tidal Volume 200 ml
Rate 20 bpm

1.



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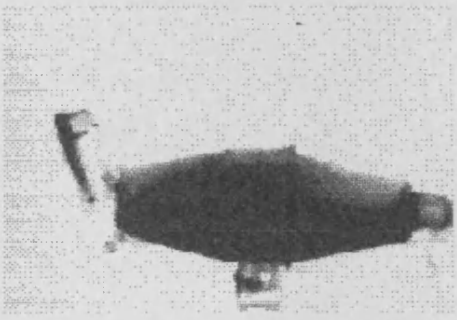

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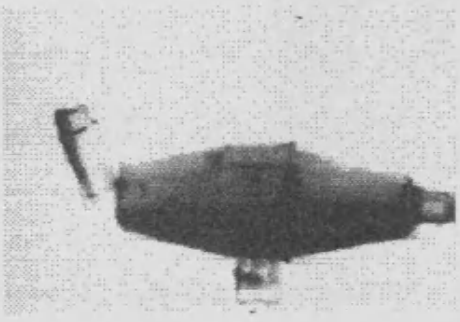

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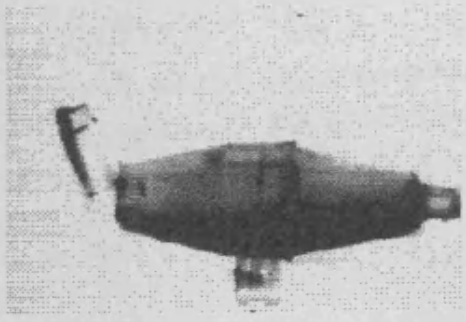

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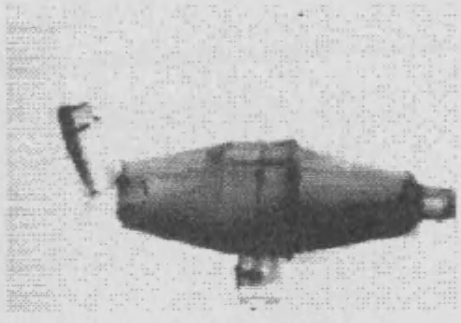


7.

Static charge

Aim

To determine the level of static charge on different spacers, the factors affecting spacer static charge and the effect of static charge on the amount of drug available for inhalation from different spacers.

Methods

Field strength within the spacers was measured using either the JC211 electrostatic miniprobe or the Simco SS-2 electrostatic locator, as described in chapter 5.6 and detailed below.

Data was recorded from the static probes in one of three ways. From the SS-2, the maximum field strength measured 2cm from the end, and 2cm from the surface of the open spacer was recorded. For the JC211, the spacer was secured on a wooden stand, and the field meter probe was placed at the (metered dose inhaler) entrance to the Fisonair.

The stand was then moved under computer control, so that the probe traversed the whole length of the spacer. During this time the field strength (volts) at 4,000 points along the spacer length was recorded on the computer, stored as an array of 1x4000 data points. This array was transferred to Excel for data manipulation. The maximum and total field strength were computed, and a graph drawn of field strength against position of the probe relative to the spacer.

For some of the experiments, outlined below, the data download from the JC211 was not functional, and measurements were therfore recorded by hand from ten preset positions within the spacer.

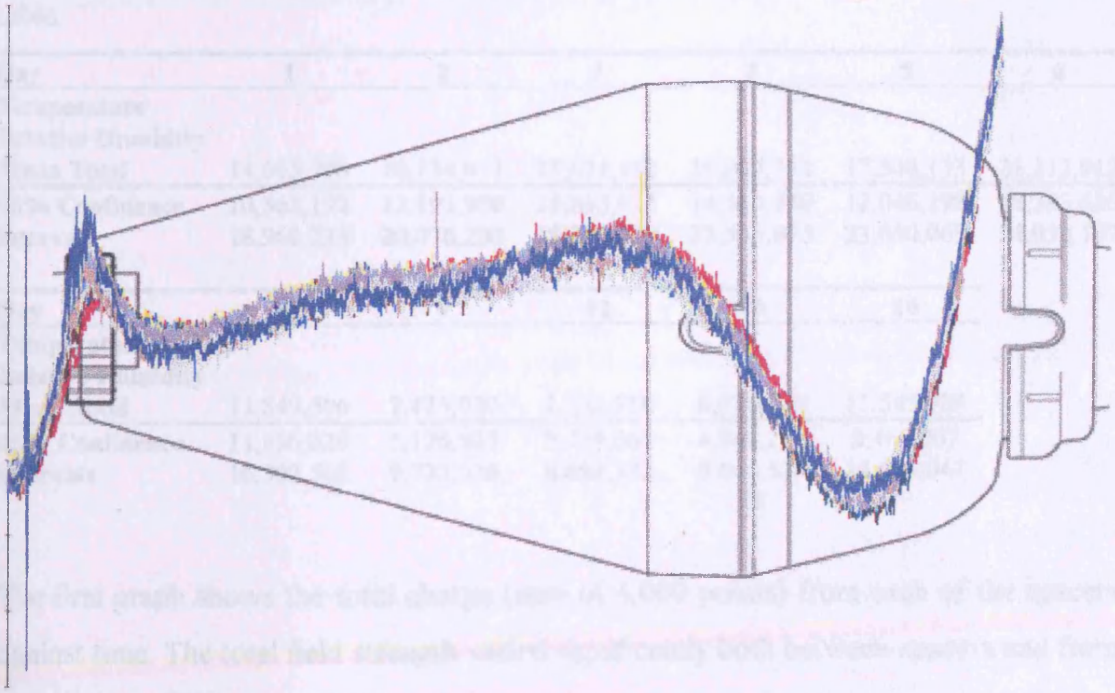
For each experiment, the laboratory temperature and relative humidity were recorded. The experimental protocols are given below:

Experiment *- repeated measurements of a single Fisonair spacer*

A used Fisonair spacer was left in place on the stand, and field strength measurements were made on three consecutive days, and intermittently over a number of hours on one day. The measurements were reproducible over this period. The mean total field strength was 2180380 (95% confidence intervals 2109376 to 2251383). (Table and Graph).

Day	0	1	2	2	2	2	2	2	2
Time		16:30	08:30	09:00	09:40	10:10	10:40	11:05	11:40
Temp			24	24.1	24.3	24.4	24.4	24.4	24.4
RH			20	21	21	21	21	21	21
Total	1664705	2262035	2254174	2223437	2167655	2134132	2185329	2229264	2133971

Day	2	2	2	2	2	2	2	2
Time	12:15	12:45	13:15	13:45	14:15	14:45	15:15	15:45
Temp	24.6	24.4	24.1	24	23.8	23.5	23.4	23.4
RH	21	21	20	20	20	19	19	20
Total	2247173	2059263	2328948	2290761	2168518	2302850	2195826	2218411



The graph shows the measured field strength at different points along the Fisonair spacer. Each of the seventeen different coloured lines represent a measurement made at a different time. A schematic of the Fisonair spacer has been superimposed for reference.

However, removing the spacer from the stand and replacing it prior to each measurement led to variation in the recorded field strength.

Measurement of the stand without a spacer demonstrated that the apparatus does not generate an electrical field. Application of the securing band to the spacer, without otherwise disturbing the spacer on the stand, does not affect the field within the spacer.

Experiment - Repeated measurement of field strength over ten days

Ten new Fisonairs were removed from their packaging and labelled. In turn, each was placed on the measurement stand, and the field strength recorded five times.

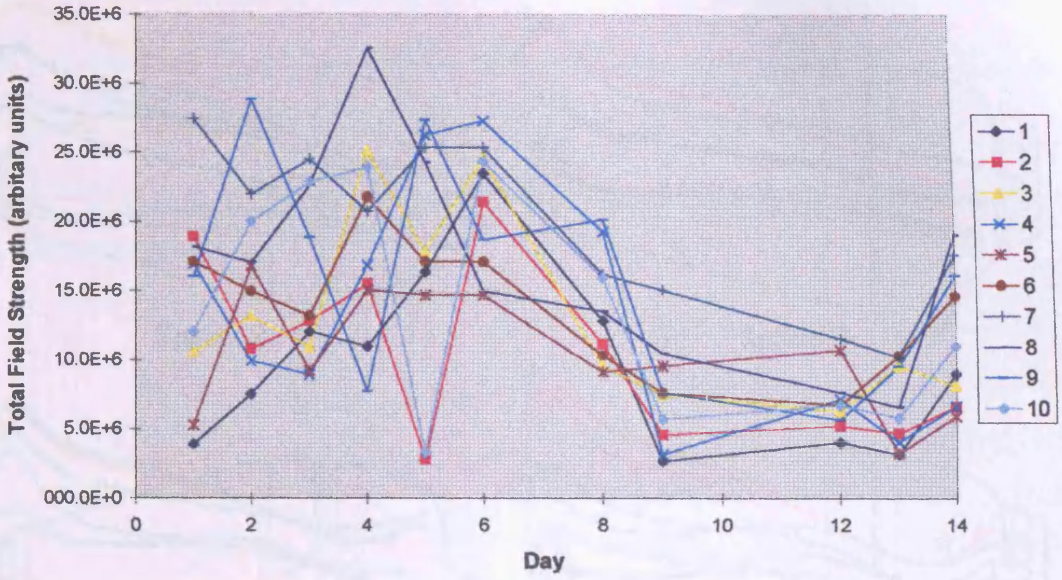
The spacer was then removed, disassembled, and replaced in its box.

At approximately the same time on ten days over the next fortnight, the process was repeated. Laboratory temperature and relative humidity for each day are given in the table.

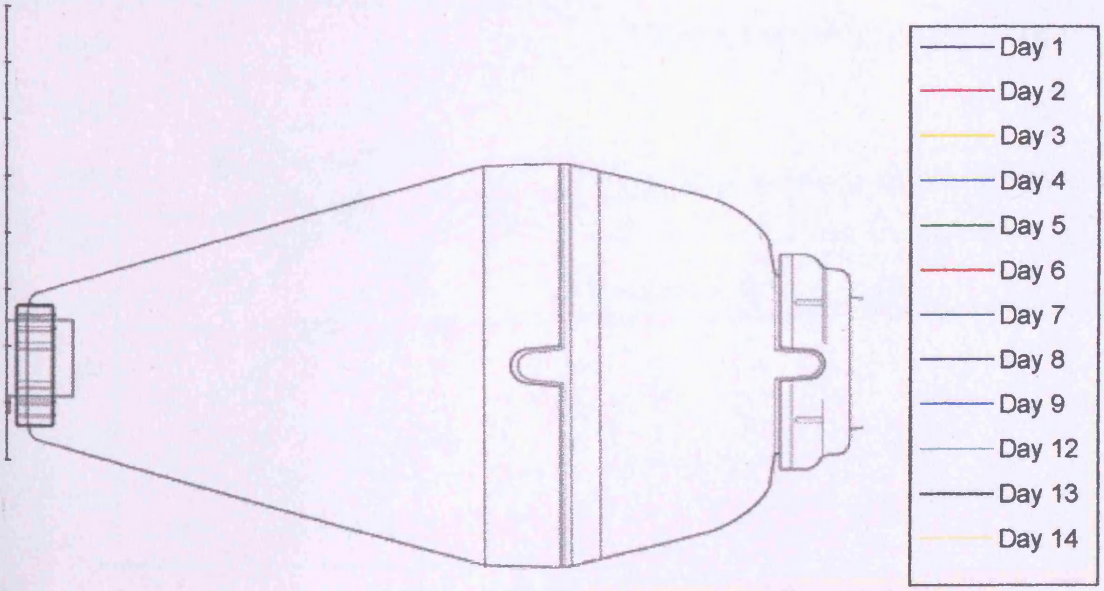
Day	1	2	3	4	5	6
Temperature						
Relative Humidity						
Mean Total	14,665,708	16,134,071	15,624,152	19,037,312	17,548,133	21,212,912
95% Confidence	10,363,192	12,191,856	11,895,615	14,510,950	12,046,198	18,393,626
intervals	18,968,223	20,076,286	19,352,689	23,563,675	23,050,069	24,032,197

Day	8	9	12	13	14
Temperature					
Relative Humidity					
Mean Total	13,849,596	7,425,030	7,230,718	6,776,383	11,545,904
95% Confidence	11,396,629	5,126,525	5,779,063	4,948,228	8,466,767
intervals	16,302,562	9,723,536	8,682,373	8,604,538	14,625,041

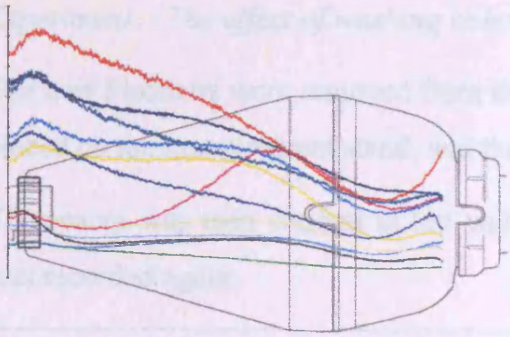
The first graph shows the total charge (sum of 4,000 points) from each of the spacers against time. The total field strength varied significantly both between spacers and from day to day (ANOVA $p<0.001$).



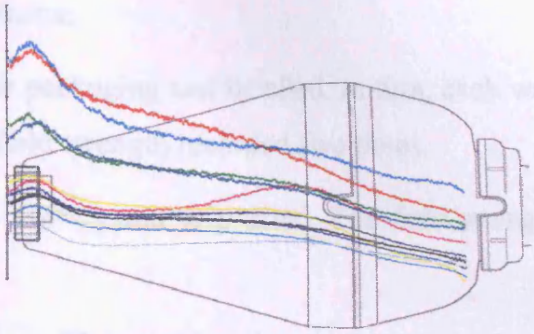
On the following graphs, the results for five representative spacers are presented as field strength (vertical axis, in arbitary units) against spacer position (horizontal axis). A schematic of the Fisonair spacer has been superimposed for reference. The different lines represent the field strength measured on different days.



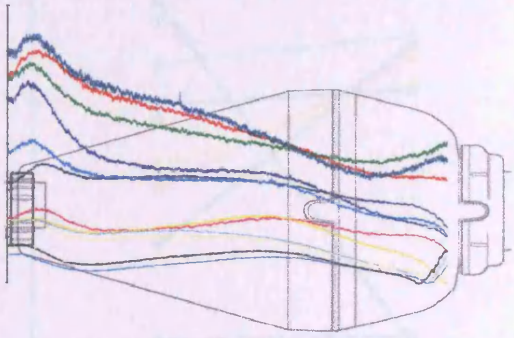
Spacer 1.



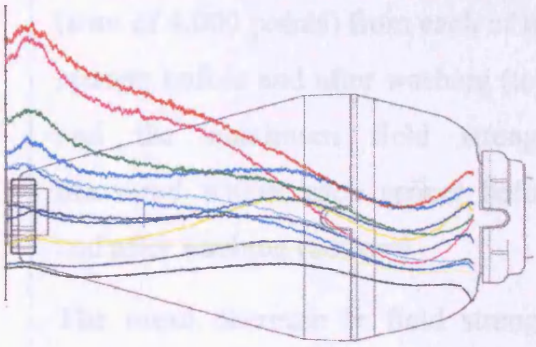
Spacer 2



Spacer 3



Spacer 4

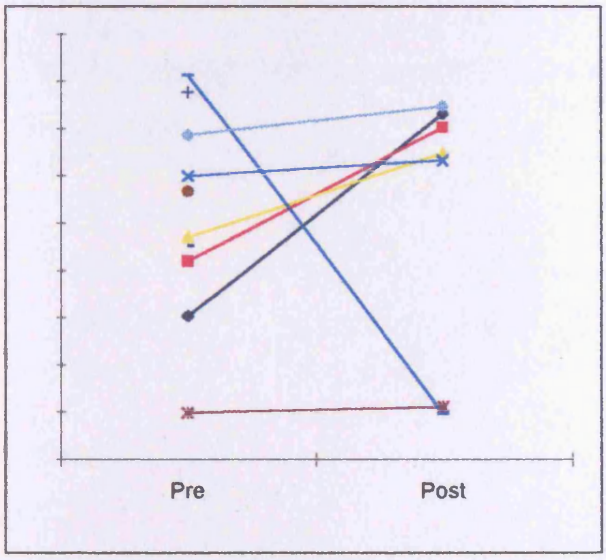


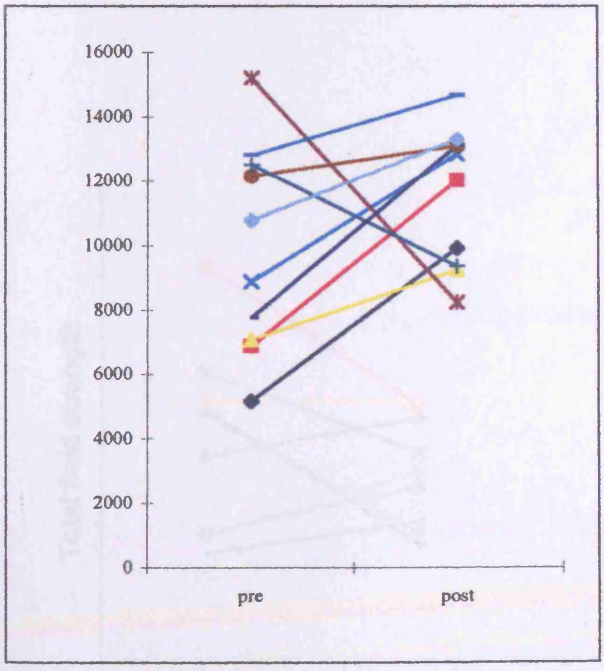
Spacer 5

Experiment - The effect of washing in hot water.

Ten new Fisonairs were removed from their packaging and labelled. In turn, each was placed on the measurement stand, and the field strength recorded five times.

The spacer was then washed in hot water (44°C) and drip dried. The field strength was recorded again.





The graphs shows the total charge (sum of 4,000 points) from each of the spacers before and after washing (top) and the maximum field strength measured within each spacer before and after washing (bottom).

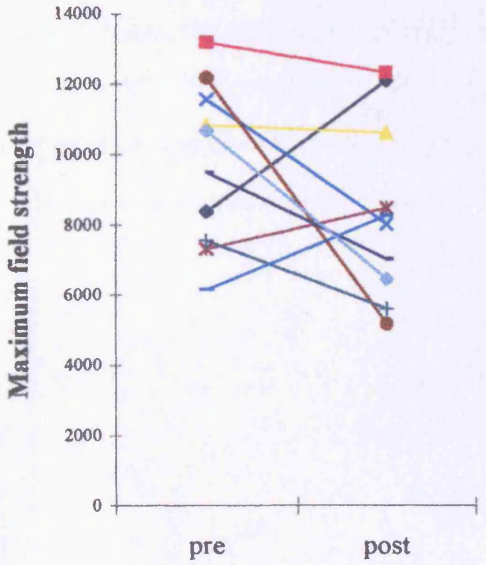
The mean decrease in field strength after the wash was -501 units (95% confidence intervals -3332 to 2329 units) This was not significantly different from no change (p=0.21).

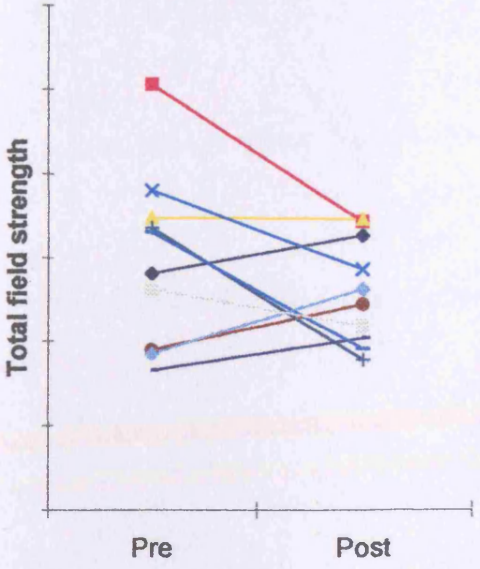
Thus no consistent effect of washing with hot water was seen in the total or maximum field strength.

Experiment - The effect of washing in cold water (9 ℃).

Ten new Fisonairs were removed from their packaging and labelled. In turn, each was placed on the measurement stand, and the field strength recorded five times.

The spacer was then washed in cold water and drip dried. The field strength was recorded again.





The graphs show the total charge (sum of 4,000 points) from each of the spacers before and after washing (left) and the maximum field strength measured within each spacer before and after washing (right).

The mean decrease in total field strength after the wash was 963 volts (95% confidence intervals -489 to 2414 volts). This was not significantly different from no change ($p=0.22$).

Thus no consistent effect of washing with cold water was seen in the total or maximum field strength.

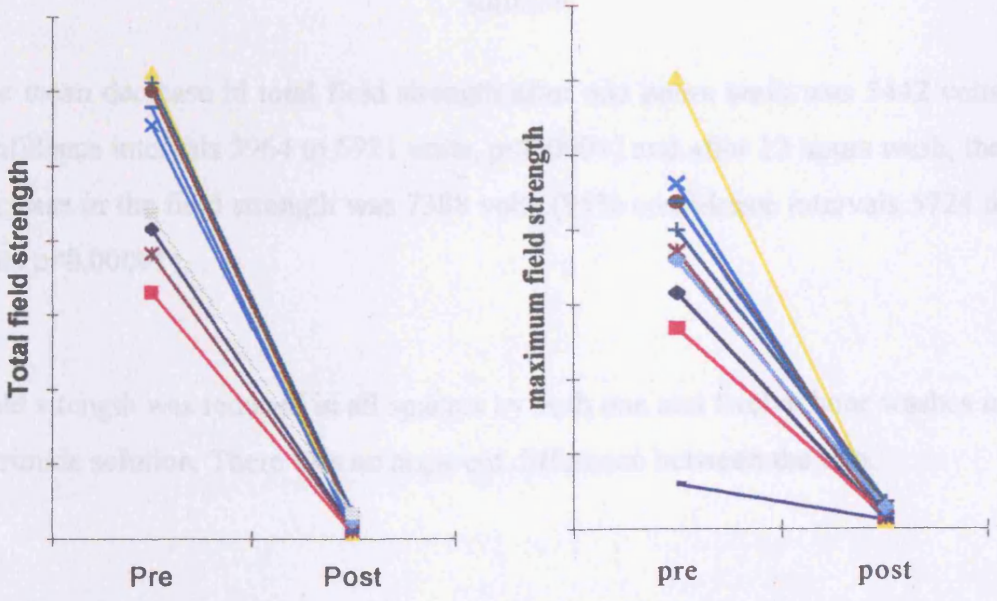
Experiment - The effect of washing in cetrimide solution (0.1%).

Ten new Fisonairs were removed from their packaging and labelled. In turn, each was placed on the measurement stand, and the field strength recorded five times.

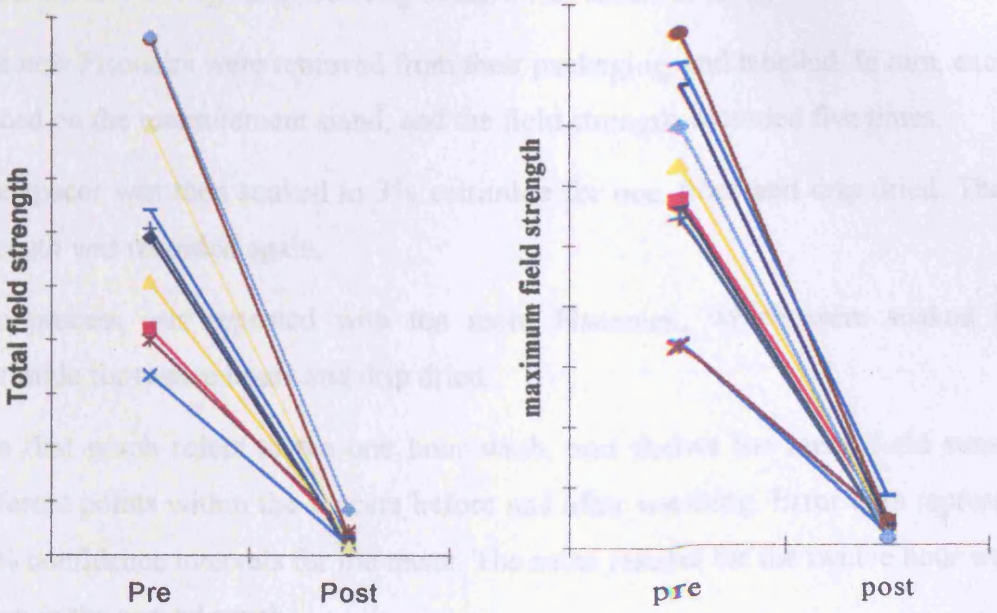
The spacer was then soaked in 0.1% cetrimide for one hour and drip dried. The field strength was recorded again.

The process was repeated with ten more Fisonairs, which were soaked in 0.1% cetrimide for twelve hours and drip dried.

The first two graphs refer to the one hour wash, and show the total field strength (sum of 4,000 points) from each of the spacers before and after washing (left) and the maximum field strength measured within each spacer before and after washing (right). The same results for the twelve hour wash are given in the second pair of graphs.



Total and maximum field strength before and after a 1 hour wash in 0.1% cetrimide solution



Total and maximum field strength before and after a 12 hour wash in 0.1% cetrimide solution

The mean decrease in total field strength after one hours wash was 5442 volts (95% confidence intervals 3964 to 6921 units, $p<0.0001$) and after 12 hours wash, the mean decrease in the field strength was 7388 volts (95% confidence intervals 5724 to 9052 units $p<0.00001$).

Field strength was reduced in all spacers by both one and twelve hour washes in 0.1% cetrimide solution. There was no apparent difference between the two.

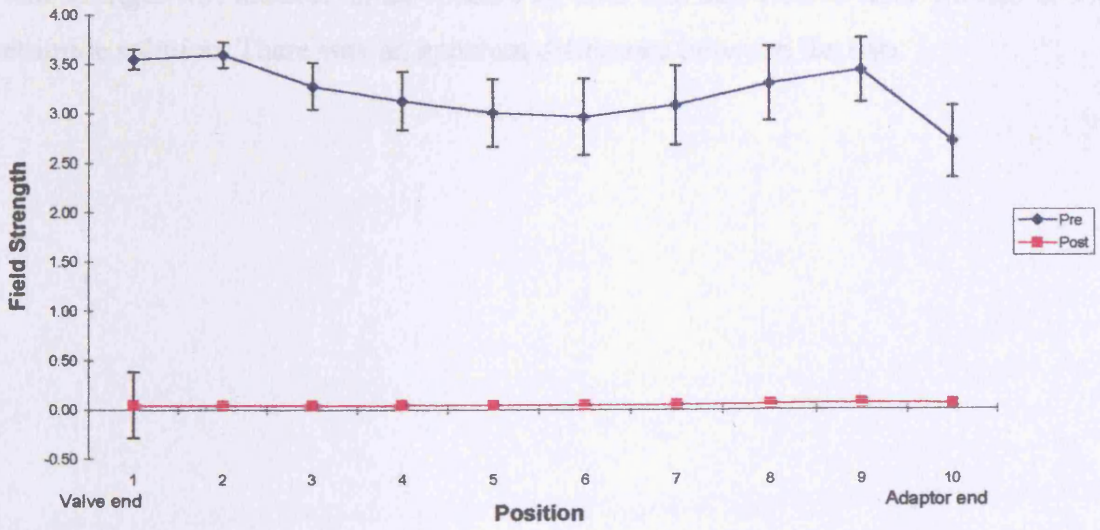
Experiment - The effect of washing in cetrimide solution (3%).

Ten new Fisonairs were removed from their packaging and labelled. In turn, each was placed on the measurement stand, and the field strength recorded five times.

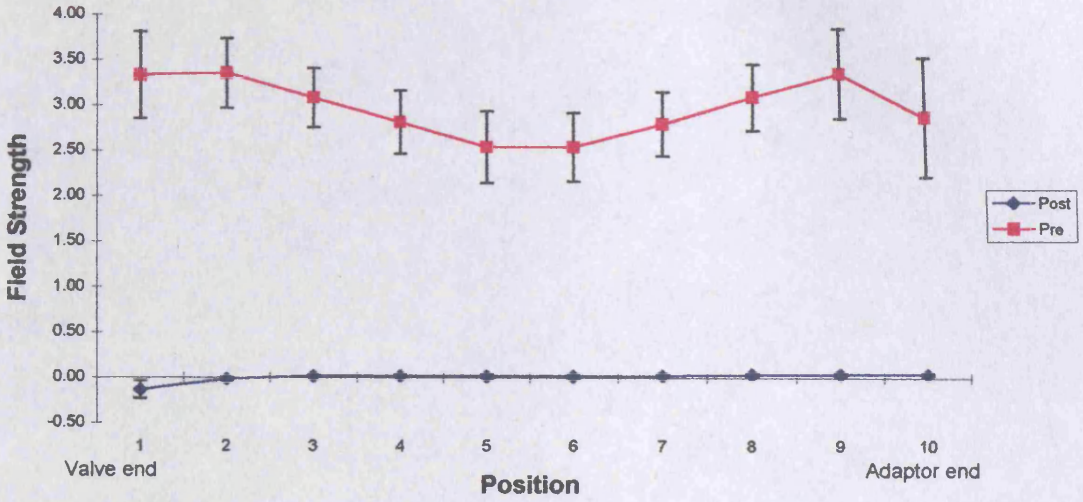
The spacer was then soaked in 3% cetrimide for one hour and drip dried. The field strength was recorded again.

The process was repeated with ten more Fisonairs, which were soaked in 3% cetrimide for twelve hours and drip dried.

The first graph refers to the one hour wash, and shows the mean field strength at different points within the spacers before and after washing. Error bars represent the 95% confidence intervals for the mean. The same results for the twelve hour wash are given in the second graph.



The effect of washing for one hour in 3% cetrimide solution



The effect of washing for twelve hours in 3% cetrimide solution

Field strength was reduced in all spacers by both one and twelve hour washes in 3% cetrimide solution. There was no apparent difference between the two.

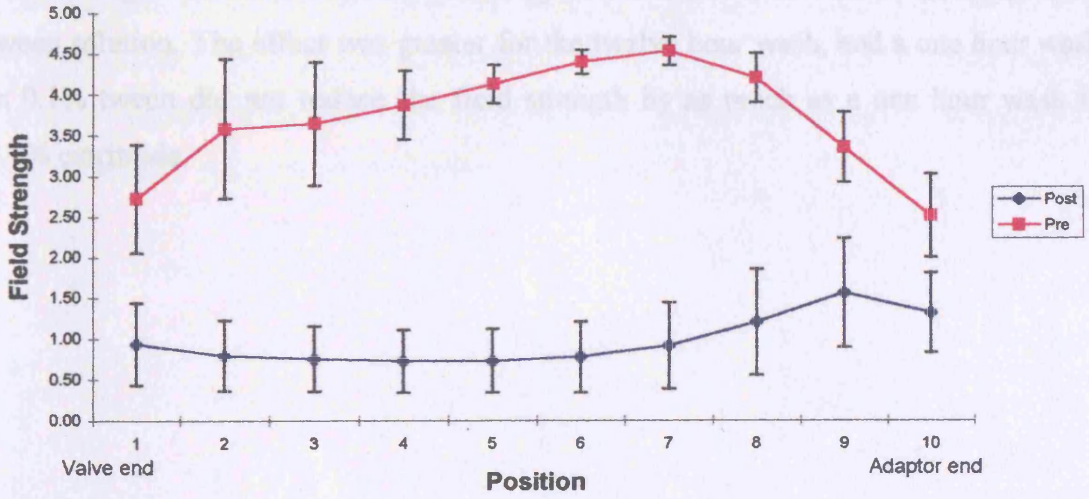
Experiment - The effect of washing in tween solution (0.1%).

Ten new Fisonairs were removed from their packaging and labelled. In turn, each was placed on the measurement stand, and the field strength recorded five times.

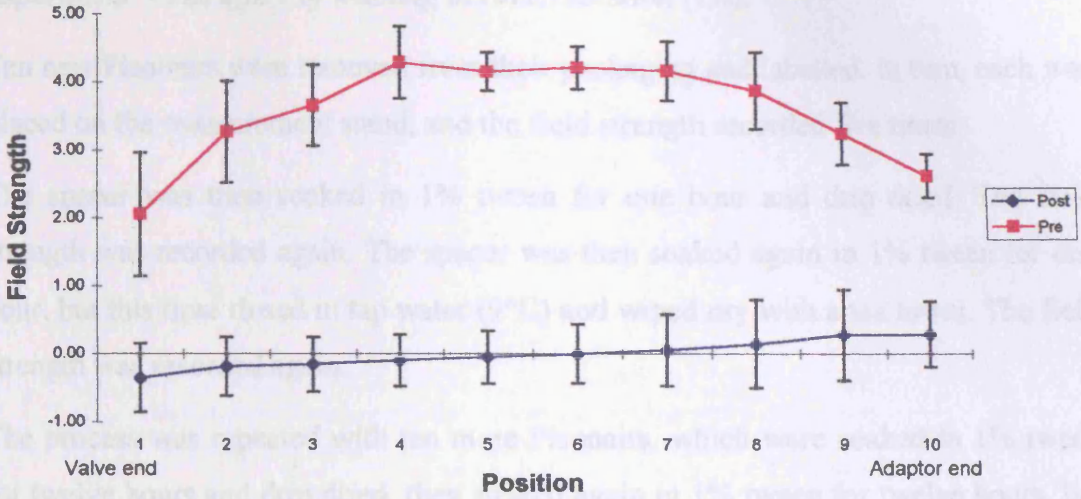
The spacer was then soaked in 0.1% tween for one hour and drip dried. The field strength was recorded again.

The process was repeated with ten more Fisonairs, which were soaked in 0.1% tween for twelve hours and drip dried.

The first graph refers to the one hour wash, and shows the mean field strength at different points within the spacers before and after washing. Error bars represent the 95% confidence intervals for the mean. The same results for the twelve hour wash are given in the second graph.



One hour wash in 0.1% tween, no rinse, drip dry.



Twelve hour wash in 0.1% tween, no rinse, drip dry.

Field strength was reduced in all spacers by both one and twelve hour washes in 0.1% tween solution. The effect was greater for the twelve hour wash, and a one hour wash in 0.1% tween did not reduce the field strength by as much as a one hour wash in 0.1% cetrimide.

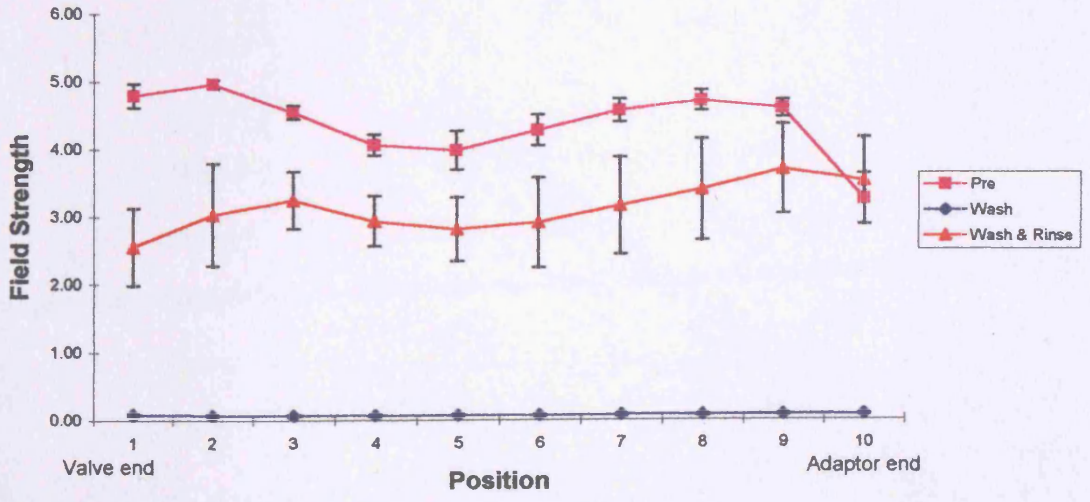
Experiment - The effect of washing in tween solution (1%).

Ten new Fisonairs were removed from their packaging and labelled. In turn, each was placed on the measurement stand, and the field strength recorded five times.

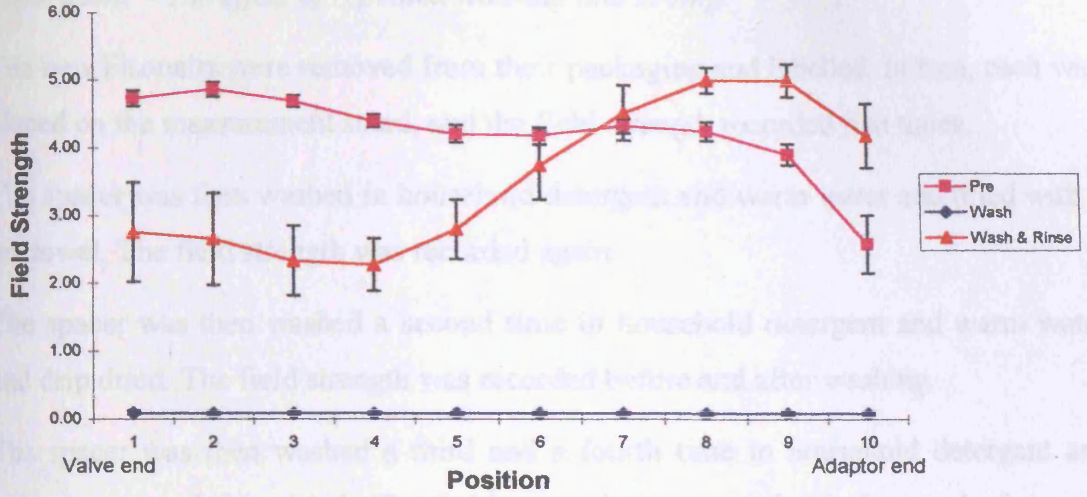
The spacer was then soaked in 1% tween for one hour and drip dried. The field strength was recorded again. The spacer was then soaked again in 1% tween for one hour, but this time rinsed in tap water (9°C) and wiped dry with a tea towel. The field strength was recorded again.

The process was repeated with ten more Fisonairs, which were soaked in 1% tween for twelve hours and drip dried, then soaked again in 1% tween for twelve hours, but this time rinsed in tap water (9°C) and wiped dry with a tea towel. The field strength was recorded after each wash.

The first graph refers to the one hour wash, and shows the mean field strength at different points within the spacers before and after washing, and washing with rinsing. Error bars represent the 95% confidence intervals for the mean. The same results for the twelve hour wash are given in the second graph.



Twelve hour wash in 1% tween, no rinse/drip dry, and rinse/towel dry.



Twelve hour wash in 1% tween, no rinse/drip dry, and rinse/towel dry.

Field strength was reduced in all spacers by both one and twelve hour washes in 1% tween solution. There was no apparent difference between the two. Field strength was increased to near baseline values by rinsing the spacers in tap water and wiping them dry.

Experiment - The effect of repeated washing and drying.

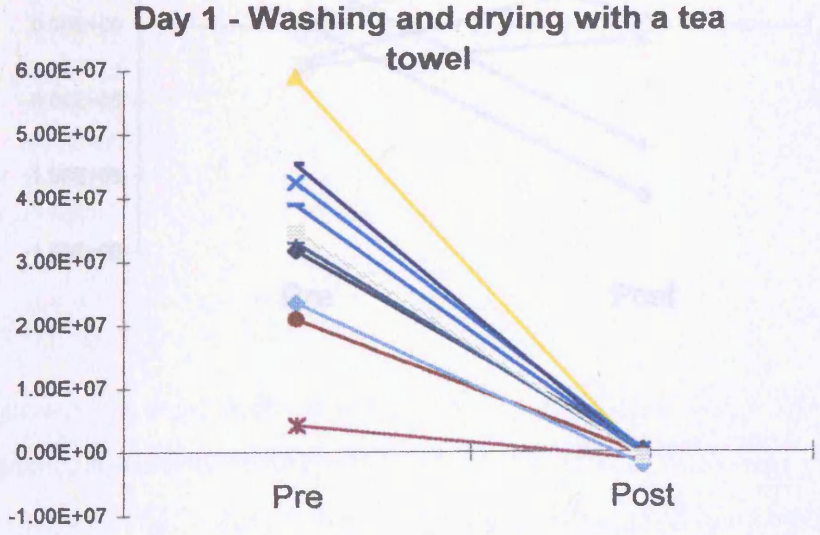
Ten new Fisonairs were removed from their packaging and labelled. In turn, each was placed on the measurement stand, and the field strength recorded five times.

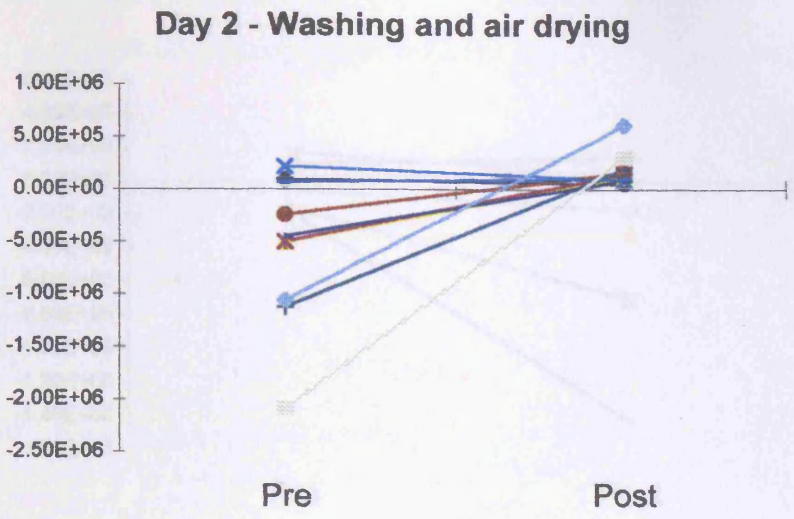
The spacer was then washed in household detergent and warm water and dried with a tea towel. The field strength was recorded again.

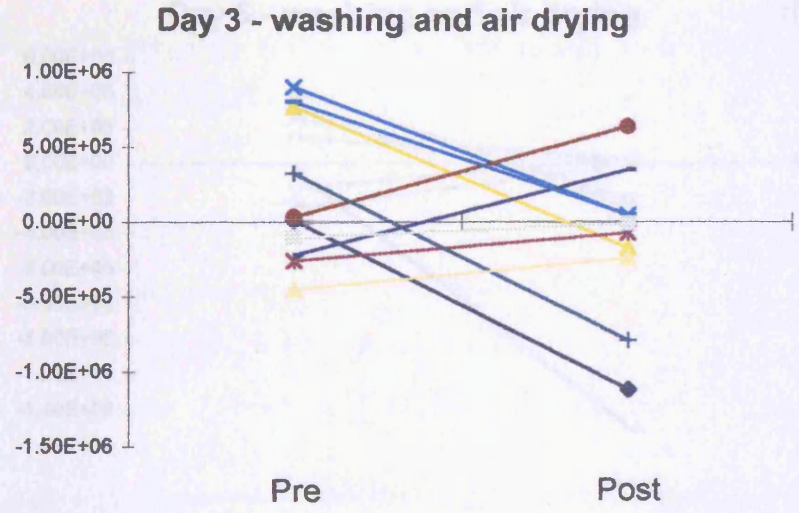
The spacer was then washed a second time in household detergent and warm water and drip dried. The field strength was recorded before and after washing.

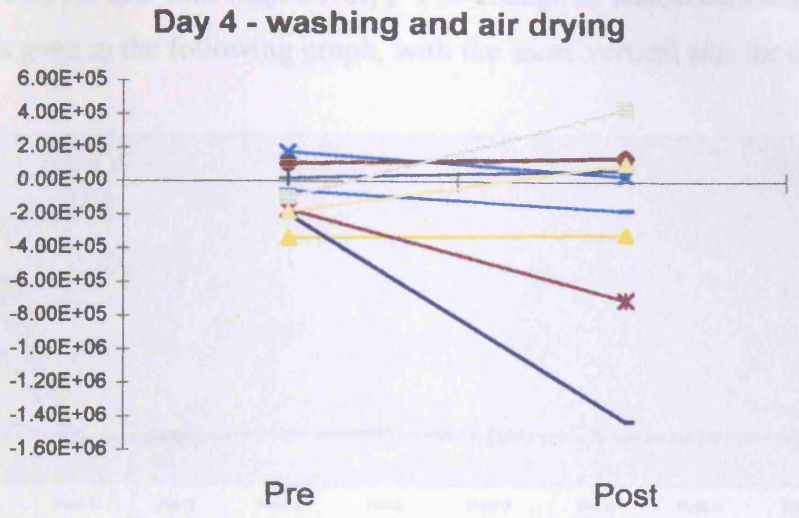
The spacer was then washed a third and a fourth time in household detergent and warm water and drip dried. The field strength was recorded before and after each wash.

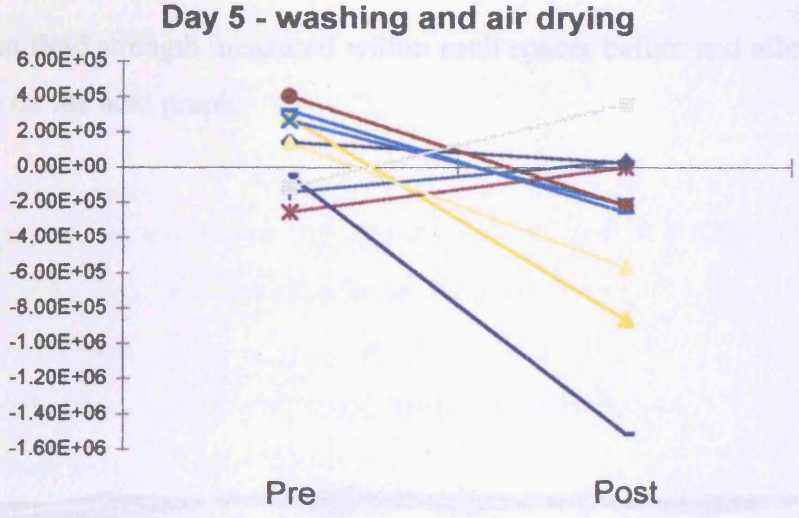
The first five graphs show the total charge (sum of 4,000 points) from each of the spacers before and after each wash. Note the change in the vertical scale, which is in arbitary units, between graphs.




Day 2 - Washing and air drying
1.00E+06
5.00E+05
0.00E+00
-5.00E+05
-1.00E+06
-1.50E+06
-2.00E+06
-2.50E+06
Pre
Post


Day 3 - washing and air drying
1.00E+06
5.00E+05
0.00E+00
-5.00E+05
-1.00E+06
-1.50E+06
Pre
Post

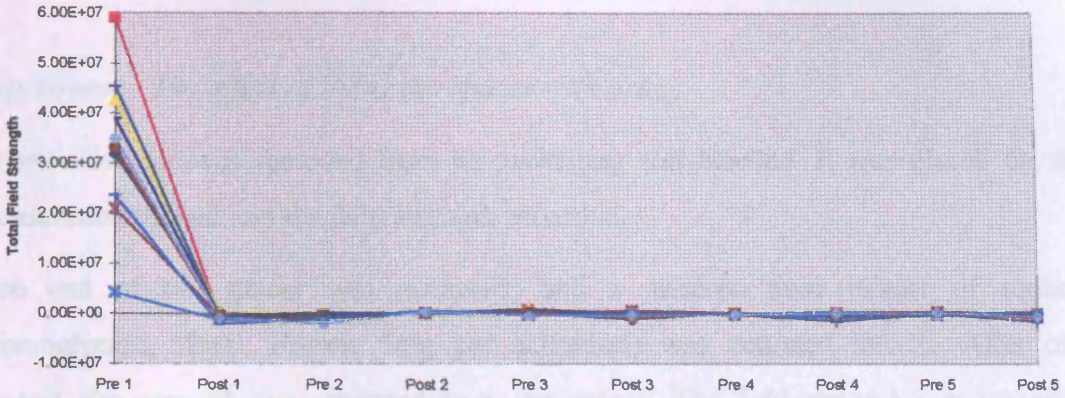




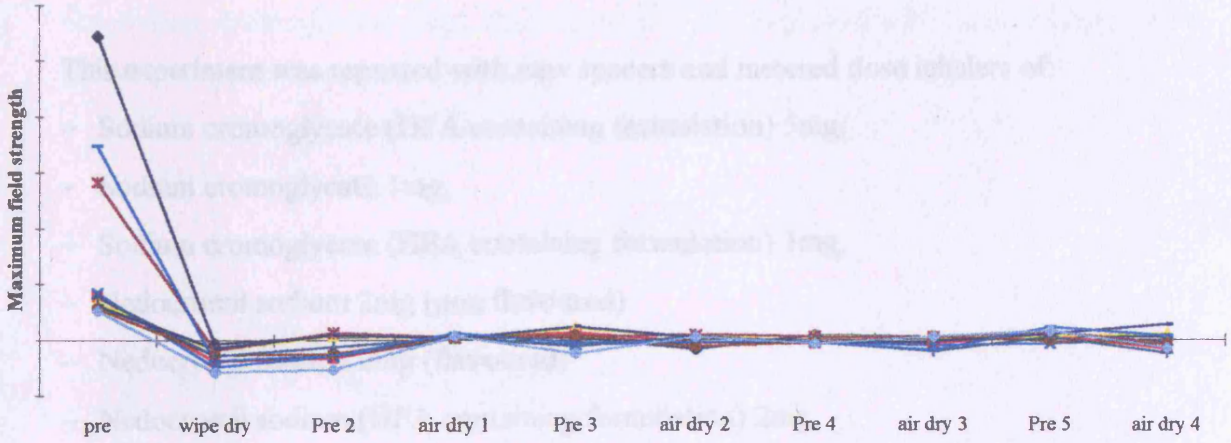
The mean decrease in total field strength after the first wash was 8550 volts (95% confidence intervals 6247 to 10,852 volts). Mean decrease in total field strength after the second wash was -187 volts (95% confidence intervals -313 to-61 volts), after the third it was 79 volts (95% confidence intervals -31 to 188 volts), after the fourth it was 26 volts (95% confidence intervals -49 to 101 volts) and after the fifth it was 106 volts (95% confidence intervals 9 to 202 volts).

The change in total field strength was significant for the first and second washes (paired t test, $p<0.0001$ and 0.02 respectively), but not for the subsequent washes

(days 3-5; p=0.2, 0.5 and 0.06 respectively). The change in total field stength over the five washes is gven in the following graph, with the same vertical axis for each day.



The maximum field strength measured within each spacer before and after each wash is also shown on the next graph.



Field strength was reduced in all spacers after one wash, and was not increased by wiping dry with a towel. Field strength was further reduced after the second wash, and remained low after the subsequent washes.

Experiment - The effect of lining the spacer with drug

A new Fisonair was removed from its packaging and labelled. It was placed on the measurement stand and the field strength recorded.

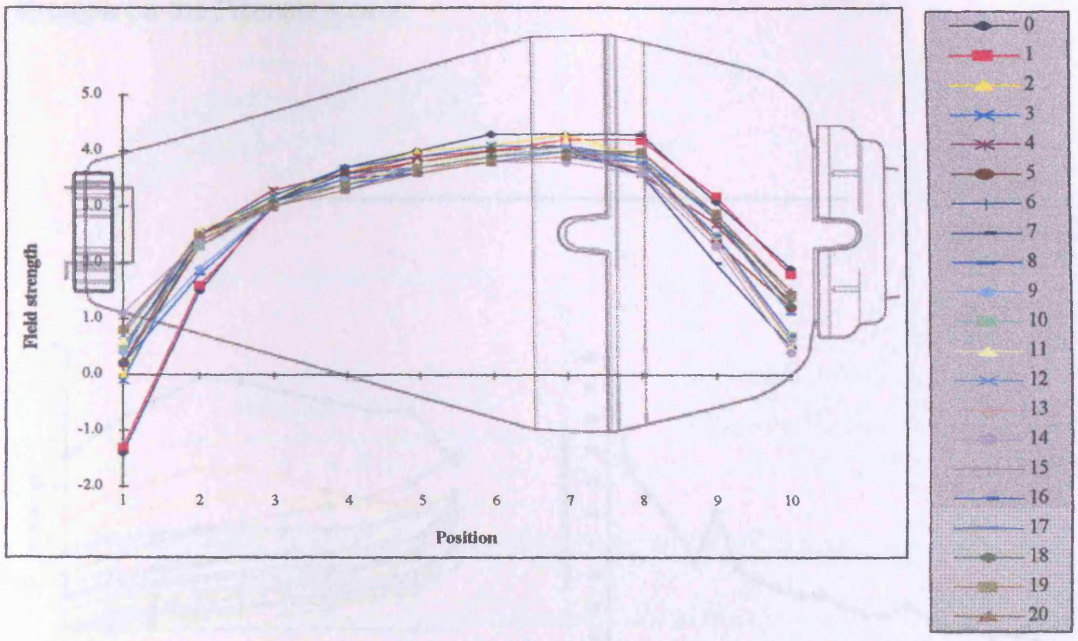
One end of the spacer was occluded, and a metered dose inhaler of sodium cromoglycate, (Intal, Fisons, 5mg per actuation) was actuated into it. After one second, the aerosol was aspirated from the spacer. The field strength was recorded again.

The procedure of actuating the metered dose inhaler into the spacer and measuring the static charge was repeated a total of twenty times.

This experiment was repeated with new spacers and metered dose inhalers of:

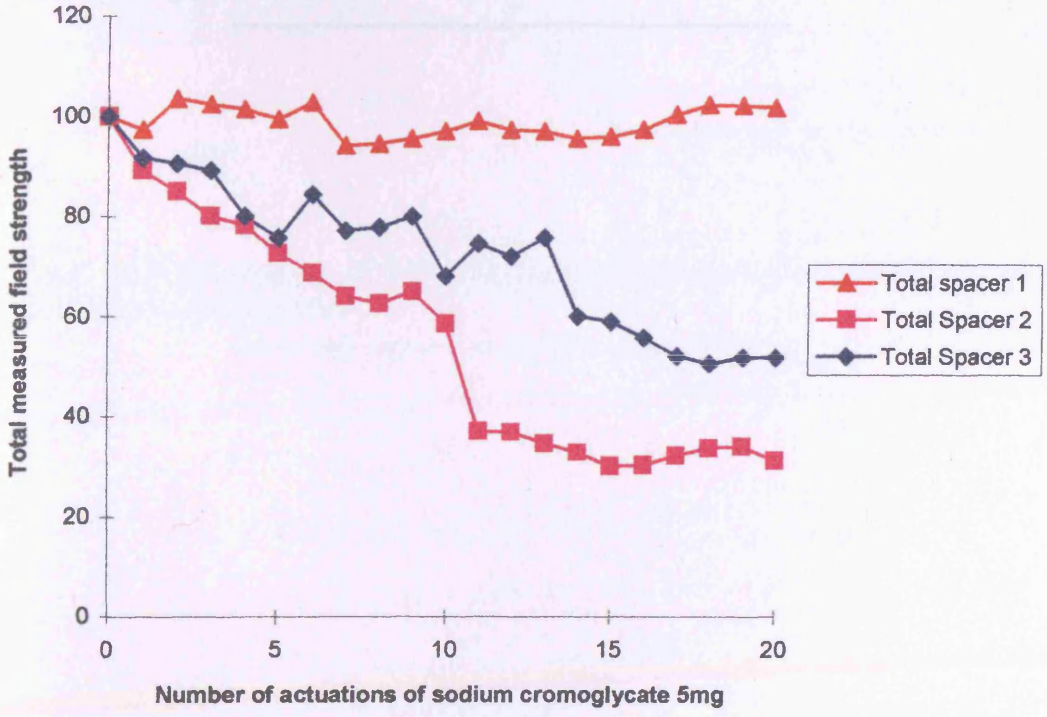
- Sodium cromoglycate (HFA containing formulation) 5mg,
- Sodium cromoglycate 1mg,
- Sodium cromoglycate (HFA containing formulation) 1mg,
- Nedocromil sodium 2mg (non flavoured)
- Nedocromil sodium 2mg (flavoured)
- Nedocromil sodium (HFA containing formulation) 2mg
- Beclomethasone (Becotide) 100mcg
- Salbutamol (Ventolin) 100mcg

On the following graphs, the results for the spacers are presented as field strength (vertical axis, in arbitary units) against spacer position (horizontal axis). A schematic of the Fisonair spacer has been superimposed for reference on the first graph. The different lines represent the field strength measured after each actuation of the metered dose inhaler into the spacer.

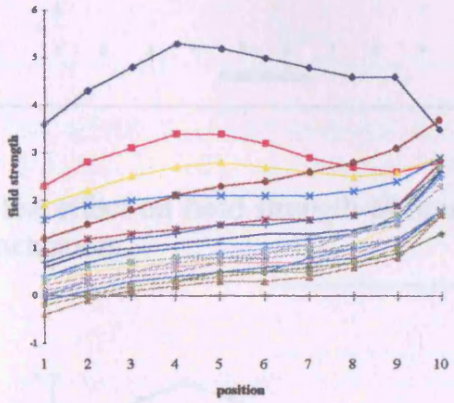


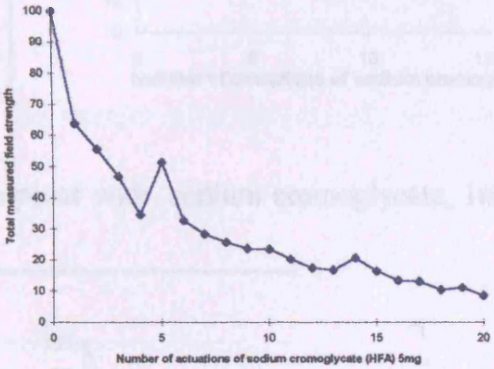
The effect on field strength of lining the spacer with sodium cromoglycate, 5mg per actuation.

For sodium cromoglycate 5mg, the experiment was repeated with three spacers, and the results are shown on the following graph:

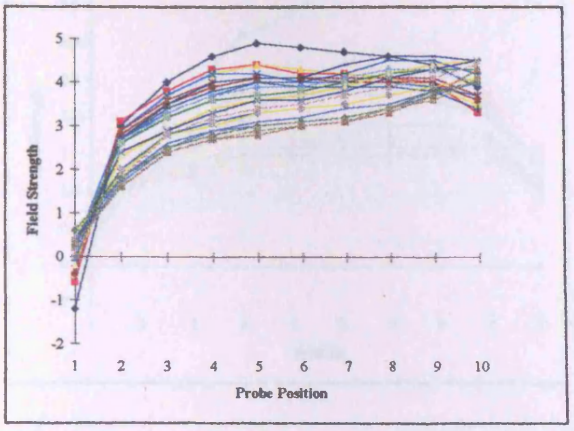


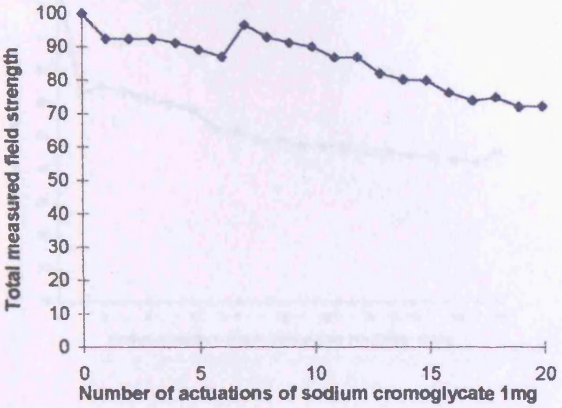
Lining the spacer with sodium cromogcate had a variable effect on the measured field strength on the Fisonair spacer.



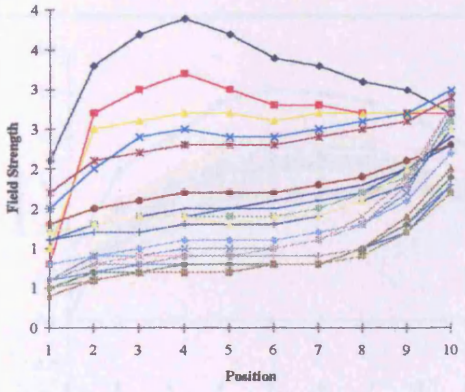


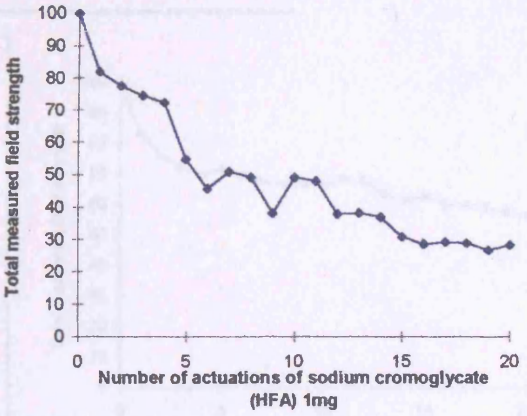
The effect on field strength of lining the spacer with sodium cromoglycate, HFA formulation, 5mg per actuation.



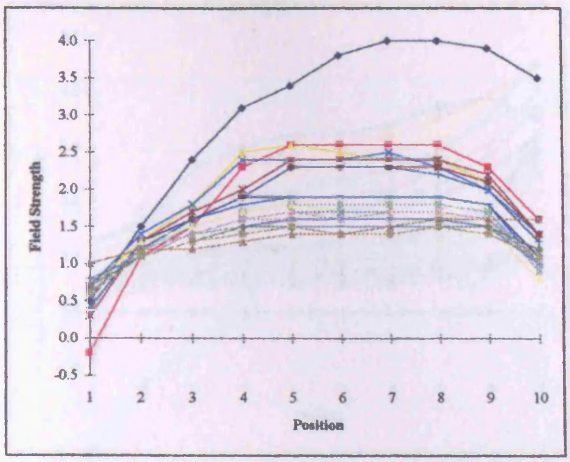


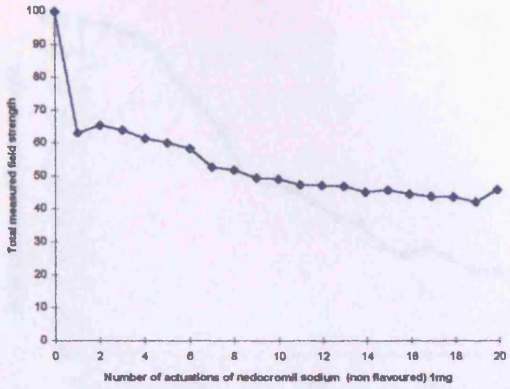
The effect on field strength of lining the spacer with sodium cromoglycate, 1mg per actuation.



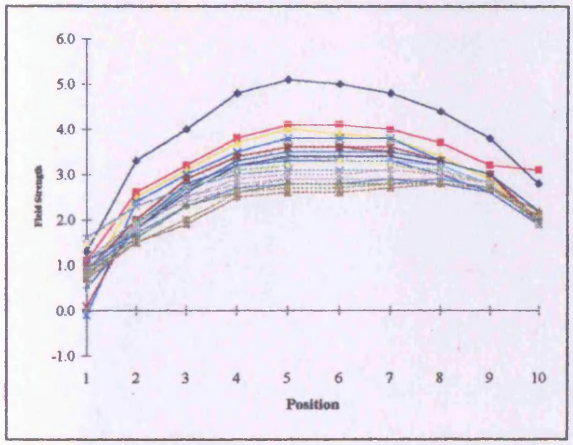


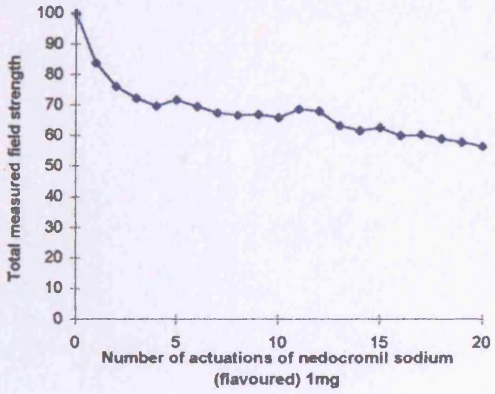
The effect on field strength of lining the spacer with sodium cromoglycate, HFA formulation, 1mg per actuation.



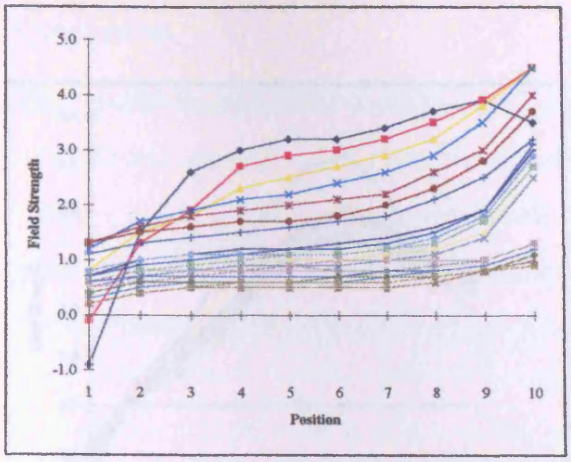


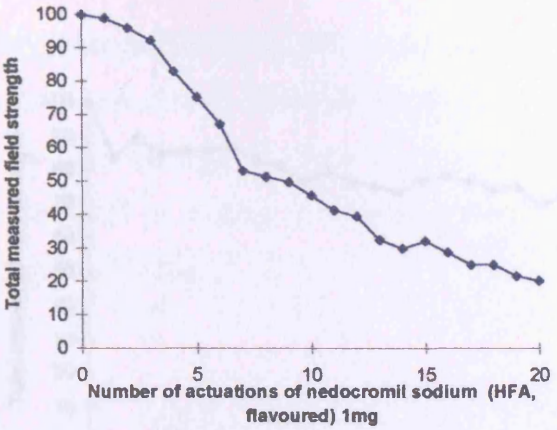
The effect on field strength of lining the spacer with nedocromil sodium (non flavoured formulation) 2mg per actuation.



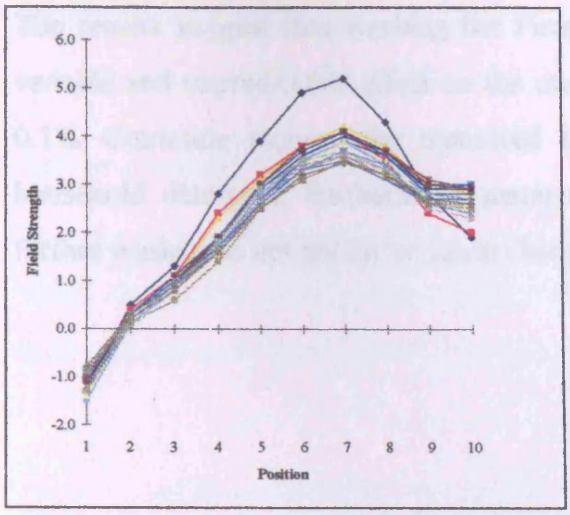


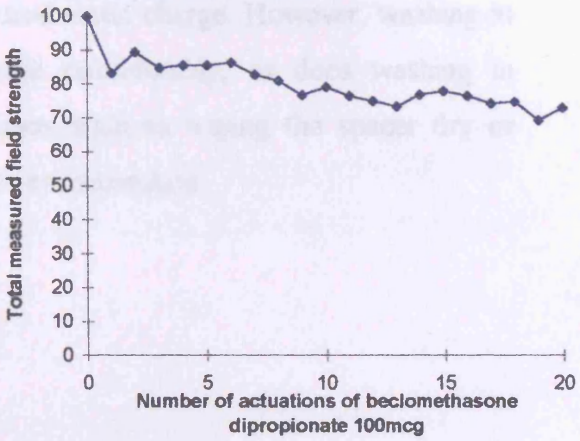
The effect on field strength of lining the spacer with nedocromil sodium, (flavoured formulation) 2mg per actuation.



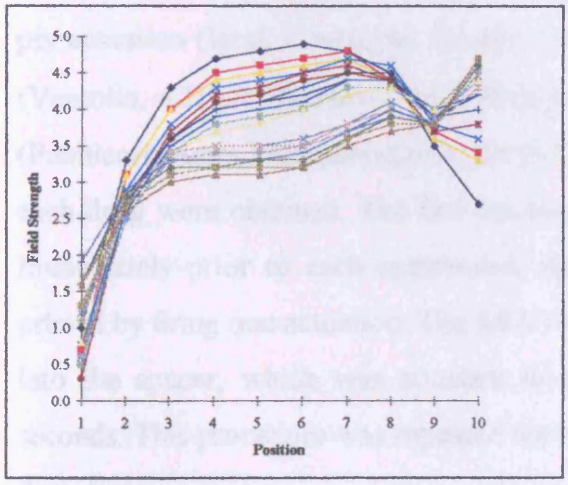


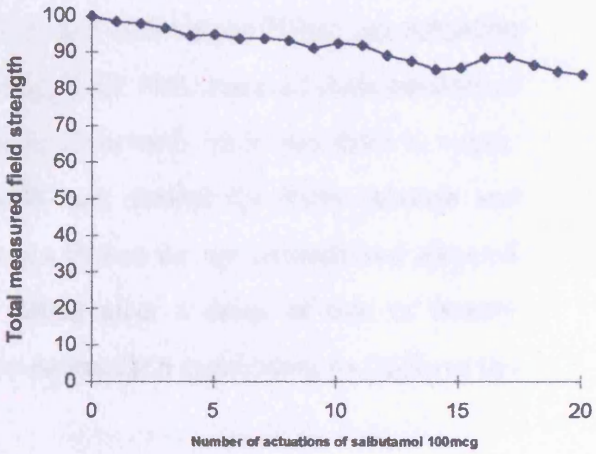
The effect on field strength of lining the spacer with nedocromil sodium, (flavoured HFA formulation) 2mg per actuation.





The effect on field strength of lining the spacer with beclomethasone diproprionate, 100µg per actuation.





The effect on field strength of lining the spacer with salbutamol, 100µg per actuation.

Conclusions.

The results suggest that washing the Fisonair spacer with hot or cold water has a variable and unpredictable effect on the measured static charge. However, washing in 0.1% Cetrimide reduces the measured charge considerably, as does washing in household detergent. Furthermore, manipulations such as wiping the spacer dry or further washes do not appear to cause charge to reaccumulate.

The aim of the next series of experiments was to determine the effect of spacer static charge on the amount of drug available from the spacer for inhalation.

The glass multistage liquid impinger was used to determine the MDI output from different spacers. The experiments were undertaken using sodium cromoglycate, 5mg per actuation (Intal, Fisons plc, Loughborough, UK), salbutamol, 100μg per actuation (Ventolin, Allen & Hanburys Ltd, Uxbridge, UK), and budesonide 200μg per actuation (Pulmicort, Astra Pharmaceuticals, Kings Langley, UK). New metered dose inhalers of each drug were obtained. The first ten actuations from each MDI was fired to waste. Immediately prior to each experiment, the MDI was shaken for thirty seconds and primed by firing one actuation. The MDI was then shaken for ten seconds and actuated into the spacer, which was attached to the MSLI after a delay of one or twenty seconds. This procedure was repeated ten times during each experiment to facilitate the drug assay.

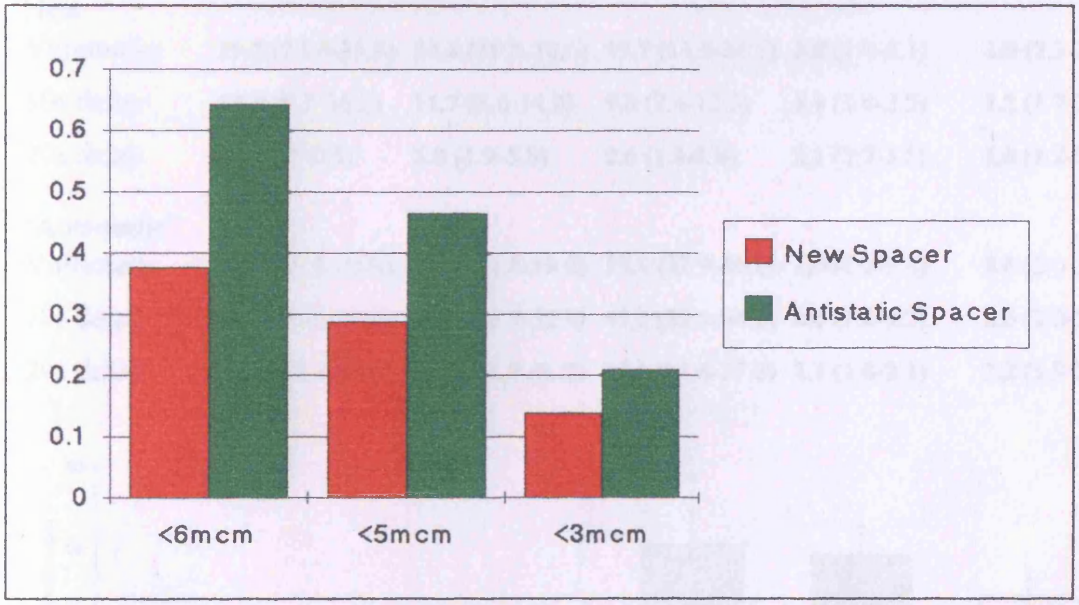
For sodium cromoglycate each stage of the MSLI was washed quantitatively with water. The amount of drug collected in each stage was assayed by UV spectrophotometry. For salbutamol and budesonide, each stage was washed with methanol or ethanol and the amount of drug collected at each stage determined by high pressure liquid chromatography.

The laboratory temperature and relative humidity were recorded for each experiment.

Sodium cromoglycate

Drug recovery was measured from a new Fisonair spacer, and from spacers lined with static dissapative plastic. Each was assessed four times. The amount of sodium cromoglycate recovered is shown in the graph:

SCG available for inhalation from new and antistatic fisonair.



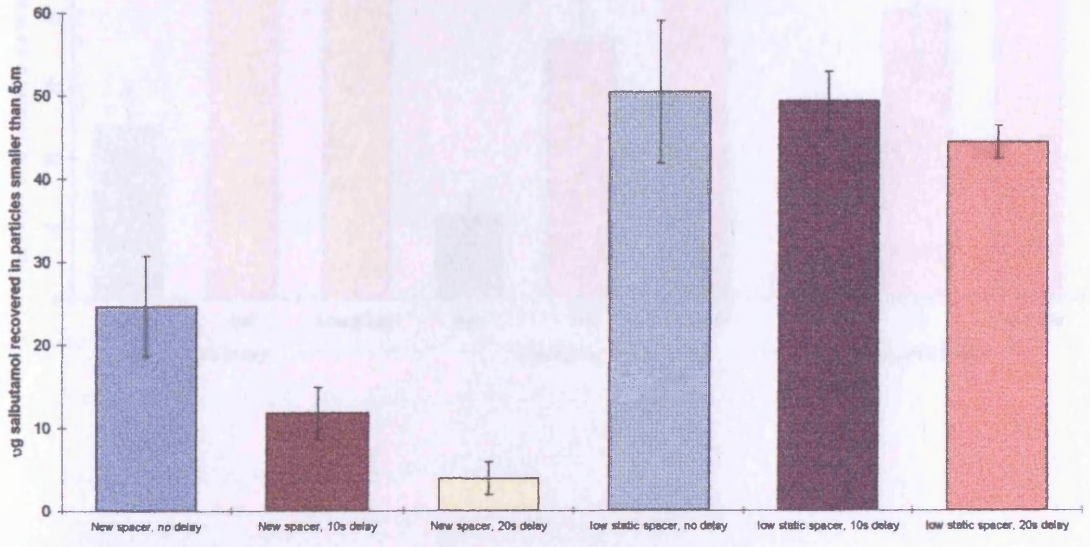
The graph shows the amount of sodium cromoglycate available from a spacer of 'high' and 'low' static charge in particles of less than six, five and three micrometers aerodynamic diameter. For SCG 5mg MDI, the dose recovered in particles <5mcm was increased by 61% using a spacer with low static charge (0.288mg (0.022) to 0.465mg (0.107)).

Salbutamol

Drug recovery was measured from new Volumatic spacers, and from spacers wiped with a static dissapative cloth (Safe Clens). Each was assessed four times. The amount of salbutamol recovered is shown in the table:

The effect of spacer static charge on the delivery of salbutamol from the Volumatic spacer.

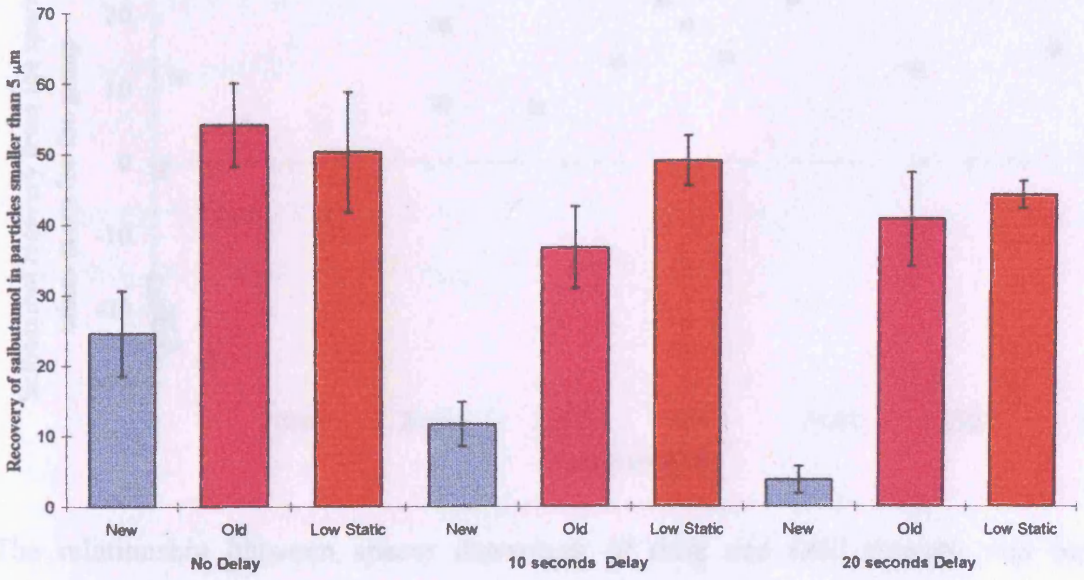
	Mean amount of salbutamol recovered (mg) per 100µg actuation (95% confidence intervals).				
Spacer Type	Dose To Patient	In Particles < 5 mcm	In Particles < 3 mcm	MMAD	GSD
New Volumatic	**29.2** (22.4-35.9)	**24.6** (18.5-30.6)	**19.7** (14.9-24.6)	**2.0** (1.9-2.1)	**2.5** (2.3-2.7)
10s delay	**12.9** (9.7-16.2)	**11.7** (8.6-14.8)	**9.8** (7.4-12.3)	**1.9** (1.6-2.2)	**2.2** (1.7-2.6)
20s delay	**4.5** (2.5-6.6)	**3.8** (1.9-5.8)	**2.6** (1.3-3.9)	**3.1** (2.7-3.5)	**1.8** (1.2-2.3)
'Anti-static' Volumatic	**62.7** (53.5-72.0)	**50.4** (41.8-59.0)	**39.6** (32.9-46.2)	**2.3** (2.2-2.4)	**2.6** (2.0-3.1)
10s delay	**56.3** (53.6-59.0)	**49.2** (45.7-52.8)	**40.0** (35.8-44.2)	**2.0** (1.6-2.3)	**2.3** (2.0-2.5)
20s delay	**50.6** (47.3-53.9)	**44.3** (42.4-46.3)	**35.7** (33.6-37.8)	**2.1** (1.8-2.3)	**2.2** (1.9-2.5)



Salbutamol available for inhalation from new and antistatic Volumatic.
Mean values. Error bars are 95% confidence intervals.

As shown in chapter 6.1, a delay between metered dose actuation and sampling reduced the amount of salbutamol available in particles smaller than 5μm (ANOVA, $p<0.001$). Wiping the Volumatic spacer with an anti static cloth increased the recovery of salbutamol from the spacer compared to the new spacer, both immediately and after a delay of up to 20 seconds ($p<0.001$), and reduced the effect of delay on salbutamol recovery (ANOVA, $p=0.34$).

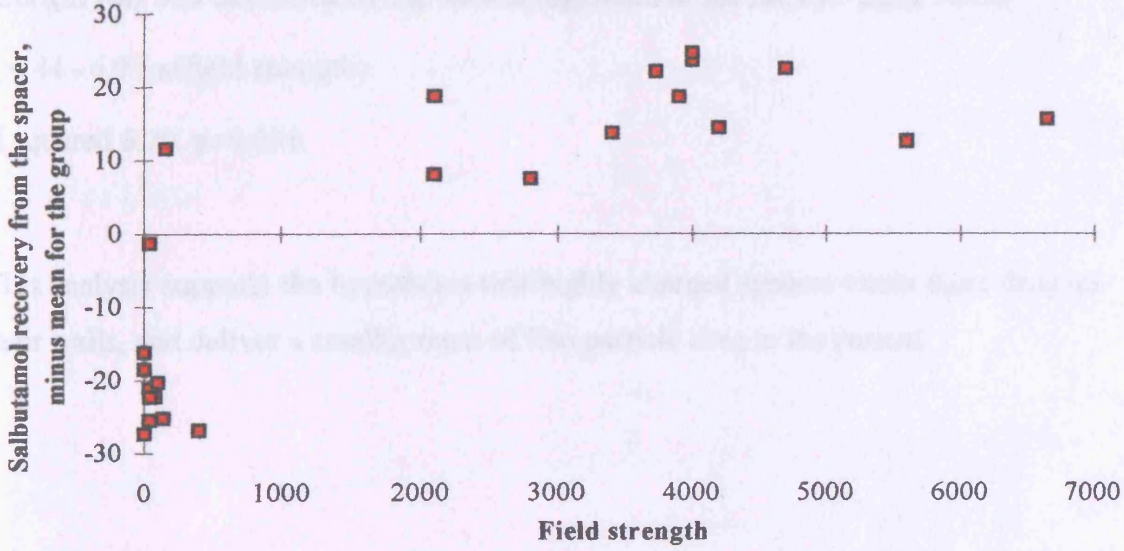
Comparing the effect of the antistatic wipe against the recovery of salbutamol from an 'old' spacer, described in chapter 6.1, in the following graph, there was no difference in the recovery of salbutamol from the old or antistatic wipes either immediately or after a 20 second delay ($p>0.05$). Although less salbutamol was recovered from the old spacer than the low static spacer after a ten second delay (t test, $p=0.019$), this was not significant when corrected for multiple comparisons.



Field strength was measured at two points within the spacer for twenty-five of the experiments with salbutamol and the Volumatic described in chapters 6.1 and 6.2. The effect of static charge on spacer deposition and the recovery of drug in particles

smaller than 5μm was studied. The different experiments involved different numbers of actuations into the spacer, and different time delays, prior to sampling, as outlined in chapters 6.1 and 6.2. To remove the effect of the different experimental conditions, the mean recovery (deposited in the spacer or the emitted drug in particles smaller than 5μm) for all experiments sharing the same conditions was subtracted from the particular experiment's results, and this value was compared against the measured spacer charge.

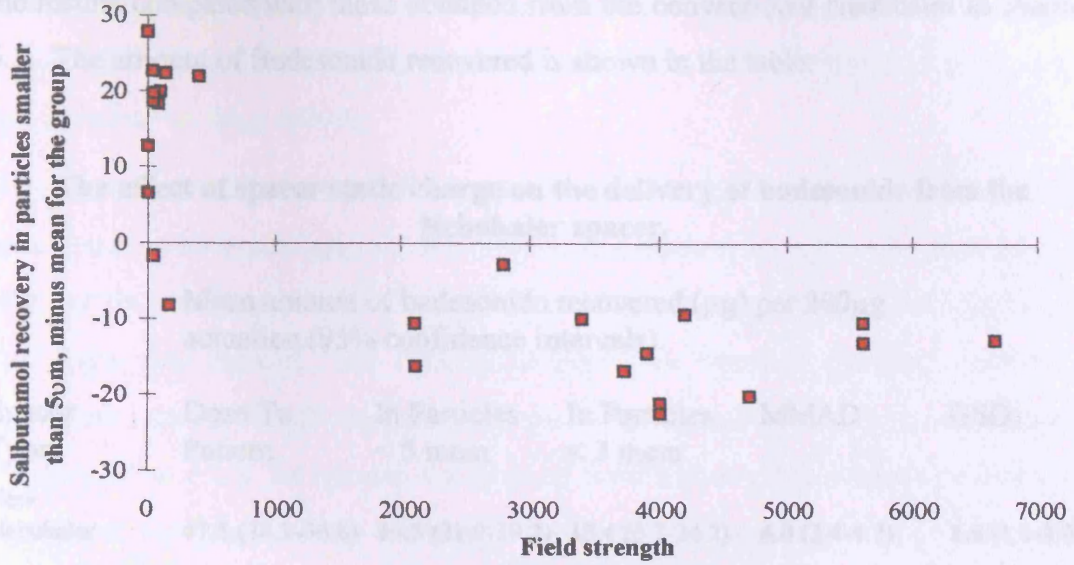
Thus, by example, for experiment code V1D1020, 79.6% of the total drug recovered was recovered from the spacer. The mean % recovery of drug from the spacer in all four experiments sharing the same experimental conditions (ten second delay between metered dose inhaler actuation and sampling) was 60.8%. The difference between the two was therefore 18.8%, and this value was plotted against the measured field strength, in this case 4000.



The relationship between spacer deposition of drug and field strength was best described by the natural logarithm of the field strength, where

$y = 8.11 \ln(\text{field strength}) - 51.4$

R squared 0.72, $p<0.001$.



The relationship between drug recovery in particles smaller than 5μm and field strength was best described by the natural logarithm of the field strength, where

y = 44 - 6.9 Ln(field strength)

R squared 0.70, $p<0.001$.

This analysis supports the hypothesis that highly charged spacers retain more drug on their walls, and deliver a smaller mass of fine particle drug to the patient.

Budesonide

Drug recovery was measured from a Nebuhaler spacers coated with a static dissapative paint (U100, Static Safe Environments, Birmingham, England), assessed four times. In addition, the drug recovery from the treated Nebuhaler was measured with a twenty second delay between metered dose inhaler actuation and sampling, and

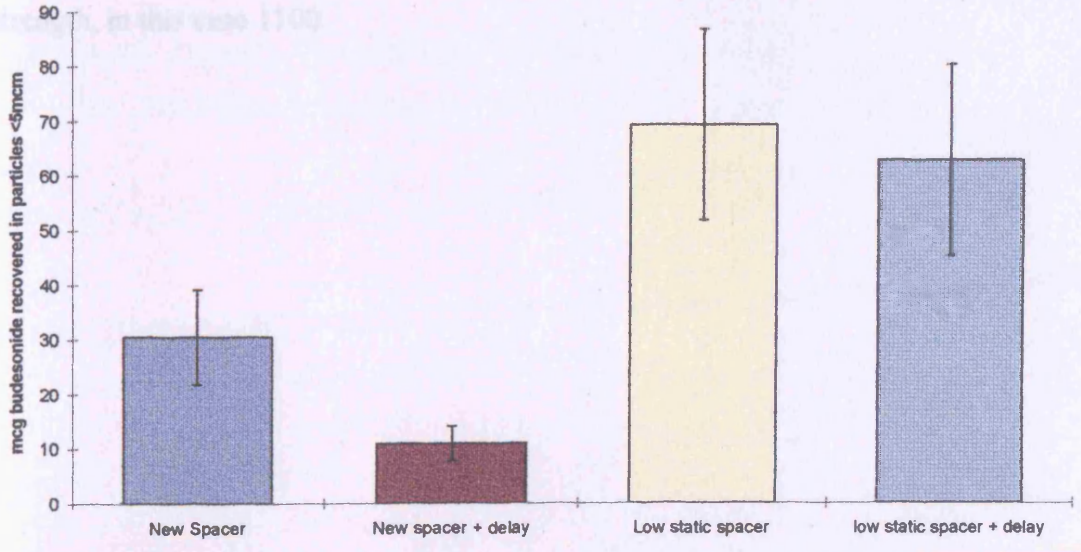
the results compared with those obtained from the conventional Nebuhaler in chapter 6.1. The amount of Budesonide recovered is shown in the table:

The effect of spacer static charge on the delivery of budesonide from the Nebuhaler spacer.

Mean amount of budesonide recovered (μg) per 200μg actuation (95% confidence intervals).

Spacer Type	Dose To Patient	In Particles < 5 mcm	In Particles < 3 mcm	MMAD	GSD
New Nebuhaler	**47.5** (38.2-56.8)	**30.5** (21.9-39.2)	**15.4** (6.7-24.2)	**4.0** (3.4-4.7)	**1.8** (1.6-2.0)
'Anti-static' Nebuhaler	**110.9** (102.2-119.7)	**69.3** (51.8-86.9)	**25.8** (16.0-35.5)	**4.4** (3.7-5.0)	**1.6** (1.6-1.6)
Nebuhaler +20 sec delay	**14.2** (10.1-18.2)	**10.9** (7.8-14.1)	**5.9** (4.4-7.5)	**3.3** (3.3-3.4)	**1.7** (1.7-1.7)
'Anti-static' Nebuhaler +20 sec delay	**89.5** (76.0-103.0)	**62.8** (45.3-80.3)	**22.0** (11.3-32.7)	**4.1** (3.6-4.6)	**1.5** (1.4-1.6)

and in the graph:

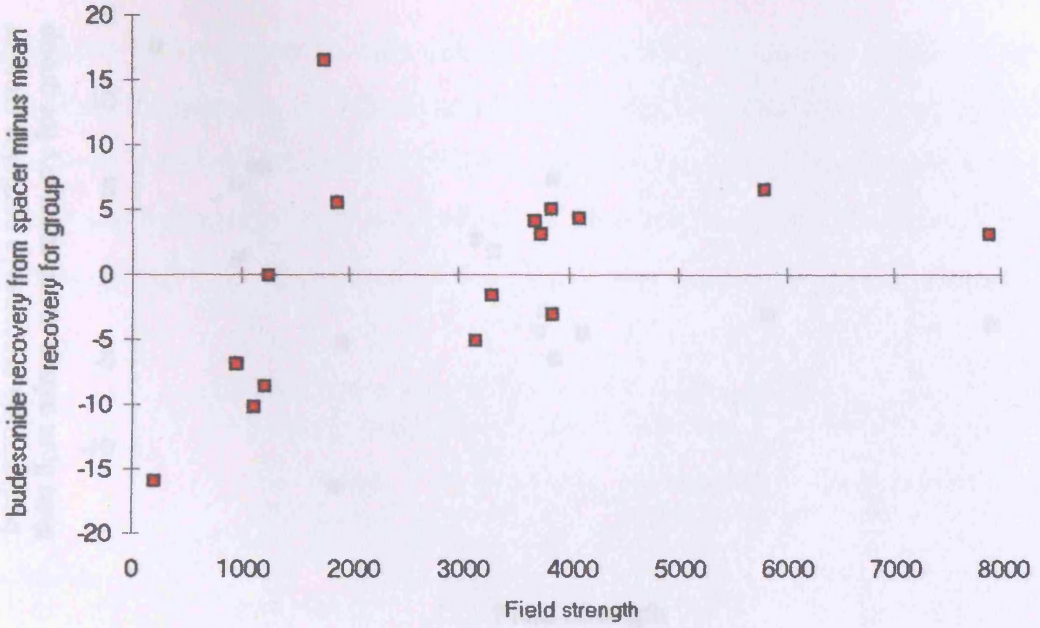


Budesonide available for inhalation from new and antistatic Nebuhaler.
Mean values. Error bars are 95% confidence intervals.

Painting the Nebuhaler spacer with static dissapative paint increased the delivery of budesonide and reduced the effects of delay between metered dose inhaler actuation and sampling on drug delivery.

Field strength was also measured at two points within the spacer for seventeen of the experiments with budesonide and the Nebuhaler described in chapters 6.1 and 6.2. The effect of static charge on spacer deposition and the recovery of drug in particles smaller than 5μm was studied. The different experiments involved different numbers of actuations into the spacer, and different time delays, prior to sampling, as outlined in chapters 6.1 and 6.2. To remove the effect of the different experimental conditions, the mean recovery (deposited in the spacer or the emitted drug in particles smaller than 5μm) for all experiments sharing the same conditions was subtracted from the particular experiment's results, and this value was compared against the measured spacer charge.

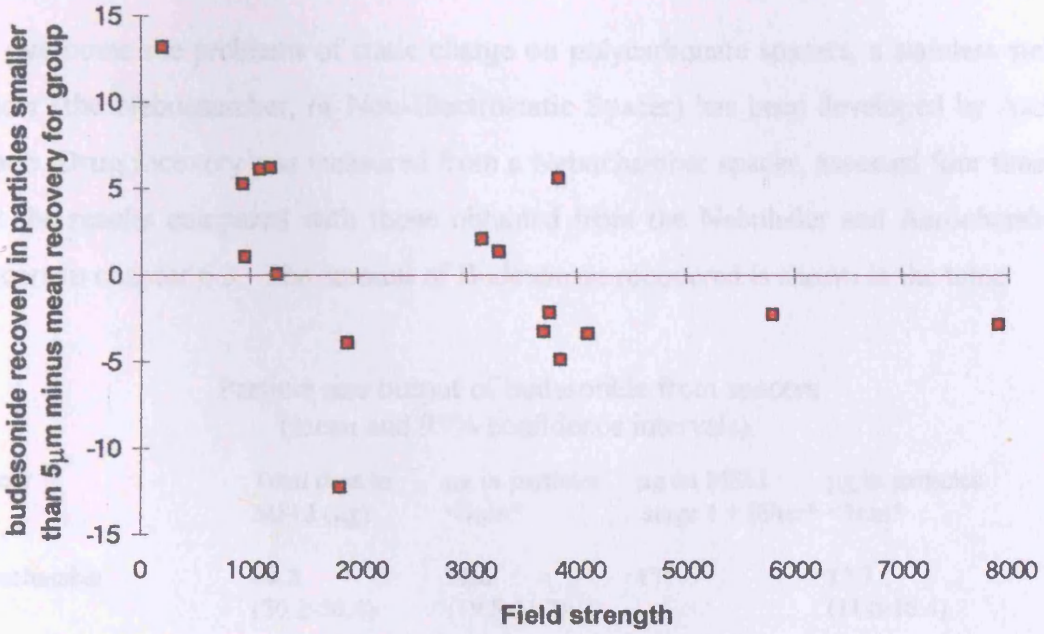
Thus, by example, for experiment code BD102, 70.5% of the total drug recovered was recovered from the spacer. The mean % recovery of drug from the spacer in all four experiments sharing the same experimental conditions (ten second delay between metered dose inhaler actuation and sampling) was 80.7%. The difference between the two was therefore -10.2%, and this value was plotted against the measured field strength, in this case 1100.



The relationship between spacer deposition of drug and field strength was best described by the natural logarithm of the field strength, where

y = 5.7 Ln(field strength) - 44.3

R squared 0.42, p=0.005.



The relationship between drug recovery in particles smaller than 5µm and field strength was best described by the natural logarithm of the field strength, where

y = 30.7 - 3.95 Ln(field strength)

R squared 0.37, p=0.009.

This analysis supports the hypothesis that highly charged spacers retain more drug on their walls, and deliver a smaller mass of fine particle drug to the patient.

Metal spacer

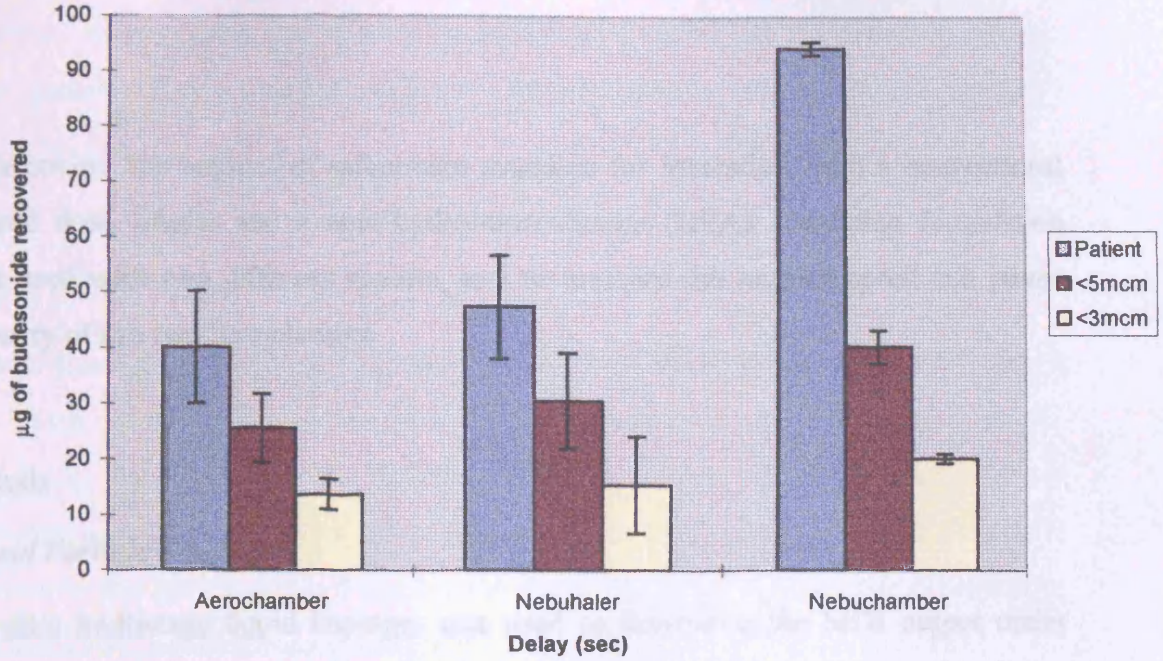
To overcome the problems of static charge on polycarbonate spacers, a stainless steel spacer (the Nebuchamber, or Non-Electrostatic Spacer) has been developed by Astra Draco. Drug recovery was measured from a Nebuchamber spacer, assessed four times, and the results compared with those obtained from the Nebuhaler and Aerochamber spacers in chapter 6.3. The amount of Budesonide recovered is shown in the table:

Particle size output of budesonide from spacers
(mean and 95% confidence intervals).

Spacer	Total dose to MSLI (μg)	μg in particles <5μm*	μg on MSLI stage 4 + Filter*	μg in particles <3μm*
Aerochamber	**40.2** (30.2-50.4)	**25.6** (19.5-31.7)	**17†**	**13.7** (11.0-16.4)
Nebuhaler	**47.5** (38.2-56.8)	**30.5** (21.9-39.2)	**22.7†** (17.4-27.9)	**15.4** (6.7-24.2)
NES	**94.3** (93.2-95.5)	**40.4** (37.4-43.4)	**36.1*** (33.7-38.4)	**20.3** (19.5-21.1)

Spacer	MMAD (μm)*	GSD*
Aerochamber	**3.9** (3.6-4.2)	**2.0** (1.8-2.2)
Nebuhaler	**4.0** (3.4-4.7)	**1.8** (1.6-2.0)
NES	**3.5** (3.5-3.6)	**1.68** (1.66-1.70)

* Due to a large deposition of budesonide in the MSLI inlet, the aerosol released from the Nebuchamber was not normally distributed, and values calculated using the line of best fit (described in chapter 5.1) are incorrect. The calculated values for drug delivery from the Nebuchamber given in the table (μg of drug in particles smaller than 5μm or 3 μm, mass median aerodynamic diameter and the geometric standard deviation) are therefore calculated from the mass of drug progressing beyond the MSLI inlet, ignoring the inlet deposition. Aerosol particles from the Nebuhaler and Aerochamber are normally distributed, and derived values have been calculated using all data points in the way described in chapter 5.1. For further comparison, the amount of drug deposited on stage 4 and the filter of the MSLI (representing particles smaller than * - 4.7μm or † - 4.5μm) is also given.



The Nebuchamber significantly increases the delivery of budesonide compared with the Aerochamber and Nebuhaler spacers. However it also increases the delivery of large particle drug, a proportion of which would impact in the oropharynx, contributing to local side effects but not systemic effects.

Comparison of the amount of salbutamol available for inhalation from different formulations used with different spacer devices.

Aim

To determine the amount of salbutamol available for inhalation from a conventional metered dose inhaler and a new hydrofluoroalkanes (HFA) containing formulation when used with two different spacers, and to measure the aerosol speed and plume geometry of the two formulations.

Methods

Aerosol Particle Size

The glass multistage liquid impinger was used to determine the MDI output under different conditions. Experiments were undertaken using a CFC containing formulation of salbutamol, (Ventolin, Allen & Hanburys Ltd, Uxbridge, UK) and a non-CFC formulation (Airomir, 3M Pharmaceuticals, Loughborough UK). Both formulations are marketed to deliver 100 μg of salbutamol per actuation. They were assessed with the following spacers; Nebuhaler (Astra Pharmaceuticals, Kings Langley, UK), Aerochamber (Trudell Medical, London, Canada), and a Nebuhaler coated with static dissipative paint (U-100, Static Safe Environments Ltd, Birmingham, UK).

New Nebuhalers and Aerochambers were used for each experiment. The child Aerochamber was used, with the mask removed. New metered dose inhalers of each drug were obtained. The first ten actuations from each MDI were fired to waste.

Between experiments MDIs were stored on their sides. Immediately prior to each experiment, the MDI was shaken for thirty seconds and primed by firing one actuation to waste. The MDI was then shaken for ten seconds and actuated into the MSLI or into the spacer which was immediately attached to the MSLI. Both MDI and spacers were connected to the MSLI via a short plastic sock. This procedure was repeated ten times during each experiment to facilitate the drug assay. After each experiment the experimental apparatus was washed with methanol and the amount of drug collected at

each stage determined by high pressure liquid chromatography as described in chapter 5.7. Experiments were repeated four times with each spacer and formulation, and the laboratory temperature and relative humidity were recorded for each experiment. Mean recovery of salbutamol from the two formulations via each spacer was compared using a two tailed t test.

Aerosol Plume Geometry

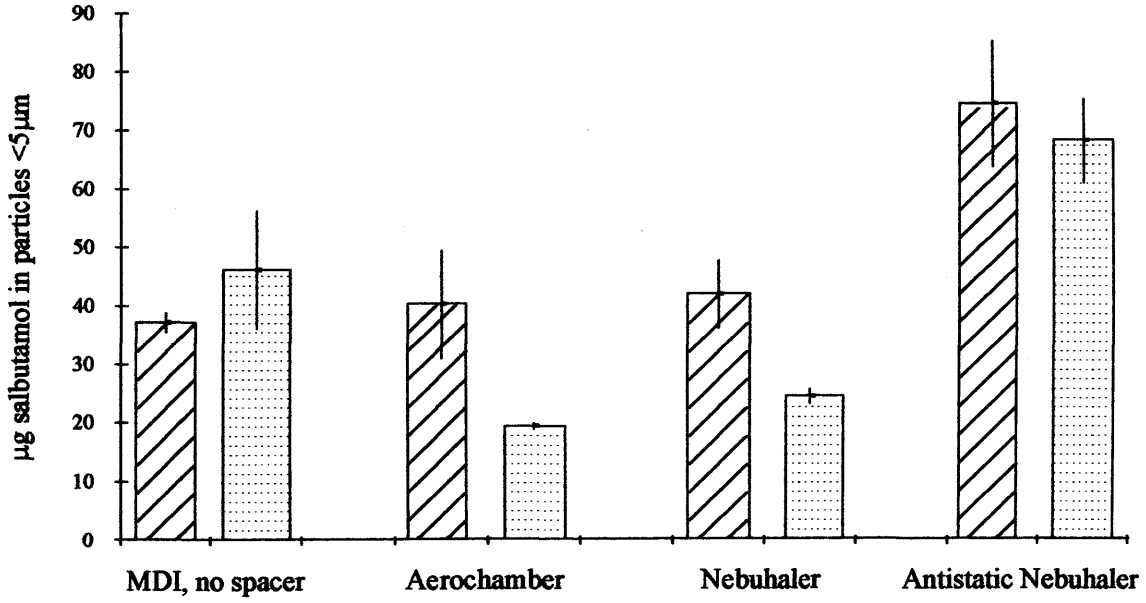
Metered dose inhalers of both formulations were actuated into still air and recorded on high speed video using the Kodak EKTAPRO HS 4540 motion analysis system (Kodak Ltd, Hemel Hempstead, UK)as described in chapter 5.5. Summary measures were used to analyse the speed and volume of the aerosol. These comprised distance travelled by the leading edge of the aerosol cloud and volume of the cloud 60ms after MDI actuation, and were compared for the two drugs using a two tailed t test.

Results

Aerosol particle size (Table)

From each 100μg actuation, 37.2μg (95% confidence intervals 35.5-38.8μg) of salbutamol in particles smaller than five microns was delivered from the Airomir MDI without a spacer, compared with 46.3μg (36.3-56.3) from the Ventolin MDI, p=0.13. With the Airomir MDI and Aerochamber spacer, 40.4μg (31.2-49.6) was delivered, compared with 19.5μg (18.9-20.0) from the Ventolin MDI and Aerochamber, p=0.013. Similarly with the Nebuhaler, the Airomir MDI delivered more drug, 42.1μg (36.3-47.9) as opposed to 24.7 μg (23.0-26.5) from the Ventolin MDI p=0.001. Delivery of salbutamol from both formulations was increased by the use of the low static Nebuhaler, to 74.8μg (64.0-85.6) from the Airomir MDI, and to 68.5μg (61.2 to 75.7), p=0.38.

Method of delivery.	Mean amount of Salbutamol recovered (μg) per 100μg actuation (95% Confidence Intervals).			
	In Particles < 5 μm	In Particles < 3 μm	MMAD μm	GSD
Direct from metered dose inhalers:				
Airomir	**37.2** (35.5-38.8)	**28.5** (27.1-29.9)	**5.7** (5.6-5.9)	**4.9** (4.7-5.2)
Ventolin	**46.3** (36.3-56.3)	**34.4** (27.5-41.3)	**5.2** (3.9-6.5)	**4.8** (3.2-6.4)
Via the Aerochamber:				
Airomir	**40.4** (31.2-49.6)	**33.6** (26.1-41.1)	**1.8** (1.7-1.9)	**2.5** (2.4-2.7)
Ventolin	**19.5** (18.9-20.0)	**15.9** (15.4-16.5)	**1.9** (1.8-2.1)	**2.9** (2.6-3.1)
Via the Nebuhaler:				
Airomir	**42.1** (36.3-47.9)	**35.4** (30.6-40.3)	**1.6** (1.5-1.8)	**2.5** (2.4-2.6)
Ventolin	**24.7** (23.0-26.5)	**20.5** (19.4-21.6)	**1.7** (1.6-1.8)	**2.6** (2.4-2.8)
Via the Antistatic Nebuhaler:				
Airomir	**74.8** (64.0-85.6)	**63.1** (52.8-73.5)	**1.6** (1.5-1.8)	**2.3** (2.3-2.4)
Ventolin	**68.5** (61.2-75.7)	**56.8** (50.2-63.4)	**1.8** (1.7-1.8)	**2.6** (2.4-2.7)

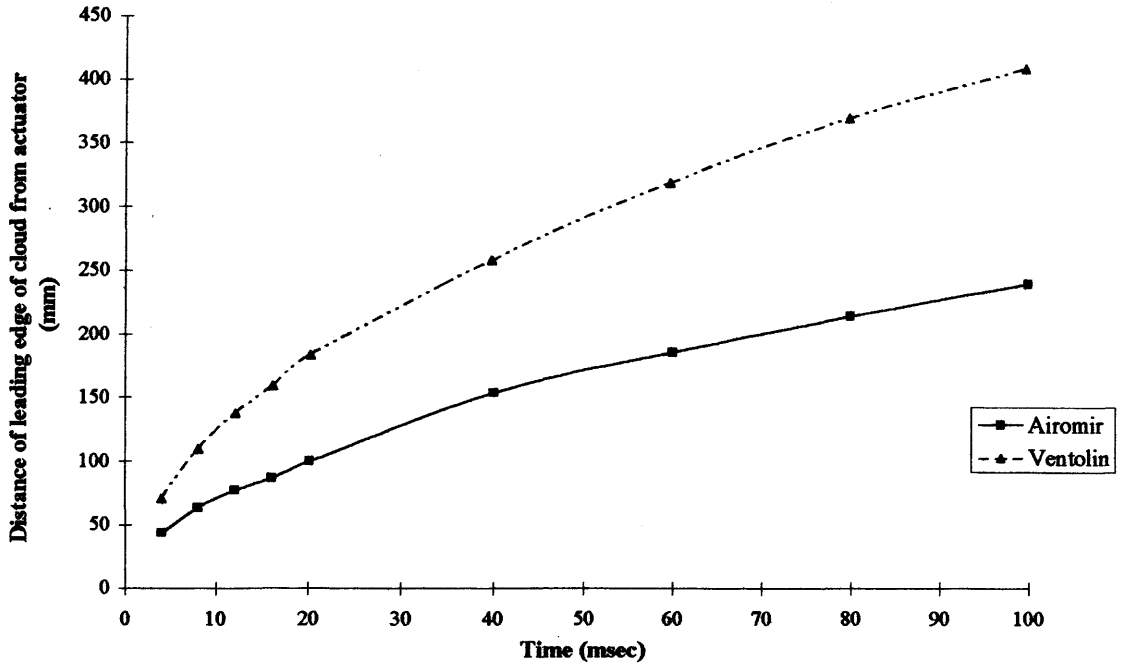


The amount of salbutamol (μg per 100μg dose) recovered in particles smaller than five μm aerodynamic diameter from the different spacer devices used with ■ Airomir or □ Ventolin. Error bars represent 95% confidence intervals.

High speed video analysis

When first visualised, the leading edge of the Airomir aerosol was travelling at 14m/s, decelerating to 3m/s after 10ms. The initial speed of the Ventolin aerosol was 17m/s, decelerating to 7m/s after 10ms. After 60ms, the Airomir aerosol had travelled 186mm,

and was moving at 1.6m/s; the Ventolin aerosol had travelled 320mm, and was moving at 3.1m/s ($p<0.0001$). At the same time the Airomir aerosol volume was 251cm^2 (213-288), and the Ventolin aerosol volume was 695cm^2 (608-782), $p<0.0001$.



The distance travelled by the leading edge of the aerosol cloud (mm) against time after MDI actuation (msec) for Airomir and Ventolin MDIs.

Summary

The delivery of salbutamol from different formulations of salbutamol used with different spacer devices was compared. Aerosol speed and geometry were analysed using a high speed video recording. The HFA formulation delivers more salbutamol than the conventional formulation when used with either the Aerochamber or Nebuhaler spacers. This may be because less drug is deposited in the spacer from the HFA formulation, which is emitted from the MDI at a slower speed and occupies a smaller volume than the conventional formulation.

Breathing simulation - Spacers

Aim

To determine the effect of different breathing patterns on the delivery of drug from different spacers

Methods

The Pari Sinus Breathing Simulator, described in Chapter 5.4, was used to produce tidal airflow through different spacers. Drug released from the spacers was captured on in line filters and assayed. A series of different experiments was undertaken with budesonide and salbutamol.

Four spacer types were assessed with budesonide, two polycarbonate spacers, the Aerochamber and the Nebuhaler, the Nebuhaler treated with static dissapative paint (U100, Static Safe Environments, Birmingham, England) and the metal Nebuchamber or Non-Electrostatic-Spacer (Astra-Draco, Lund, Sweden). The spacers had previously been used in other, unrelated experiments. Spacers were cleaned with water and allowed to dry in air on the laboratory bench before each experiment. Spacers were assessed at different tidal volumes, rates and inspiratory fractions, and also after they had been washed briefly in water; after they had been washed briefly in 0.1% cetrimide solution, and after they had been handled to simulate normal use.

Three spacer types were assessed with salbutamol, the Aerochamber, the Babyhaler and the Volumatic spacer. The spacers had previously been used in other, unrelated experiments. Spacers were cleaned with water and allowed to dry in air on the laboratory bench before each experiment. Spacers were assessed at different tidal volumes, rates and inspiratory fractions, and also when held horizontally, or at an angle, simulating administration of drug to a younger patient. The spacers were all assessed using the face-mask supplied with them.

Metered dose inhalers of budesonide 200μg or salbutamol 100μg were actuated into

the spacer, which was held against a face plate attached to the breathing simulator for five simulated breathing cycles (as described in chapter 5.4). Metered dose inhalers were actuated at the begining of an 'inspiration'. Where spacers were assessed at an angle, the metered dose inhaler and spacer were connected to the breathing simulator and held at the prescribed angle during metered dose inhaler actuation. Drug was collected on a filter placed between the spacer and breathing simulator, and was assayed by high performance liquid chromatography.

Budesonide Results

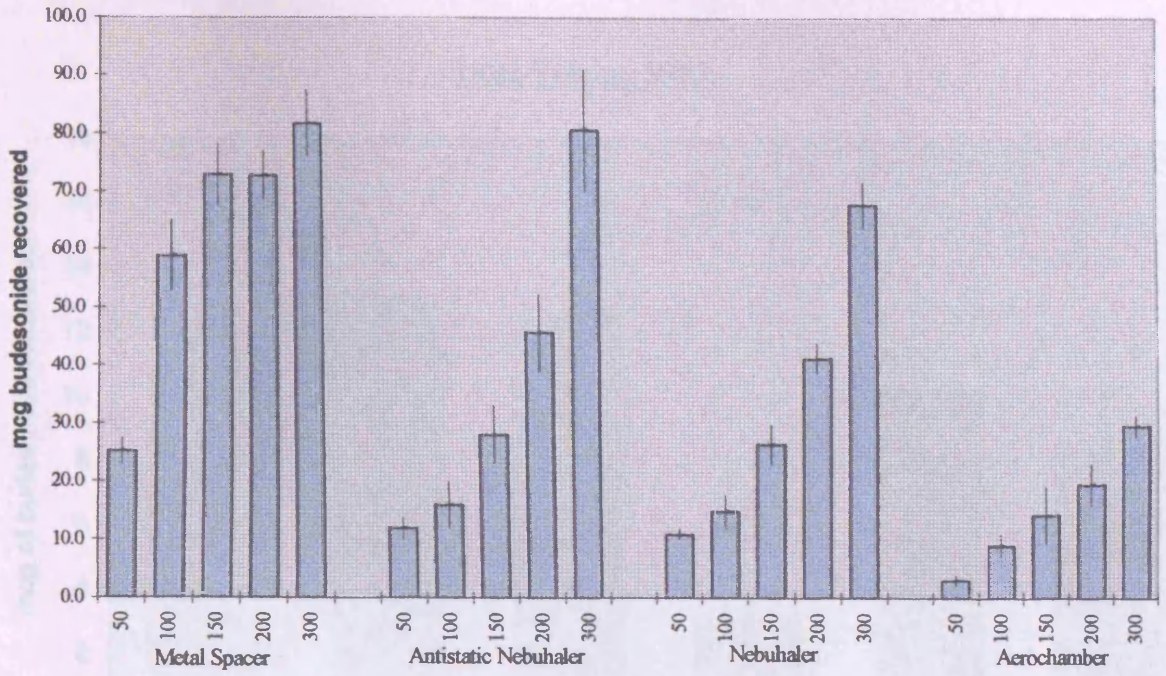
The effect of varying tidal volume

Drug recovery increased with increasing tidal volume for all spacers (ANOVA $p<0.001$), but was not significantly increased with the Nebuchamber at tidal volumes above 150ml. The recovery from the Aerochamber was significantly less than from the other spacers at all tidal volumes. There was no difference between the conventional (used) Nebuhaler and the Nebuhaler treated with antistatic paint. Budesonide recovery was significantly greater for the Nebuchamber than the Nebuhaler at all tidal volumes ($p<0.001$). The difference between the Nebuchambeer and conventional Nebuhaler was least marked at 300mls (t test, $p=0.006$), and was not significant between the Nebuchamber and 'anti-static' Nebuhaler at this tidal volume.

Output of budesonide from spacers (μg budesonide, mean and 95% confidence intervals).
The effect of changing tidal volume.

Rate = 20 cycles/minute, Inspiratory fraction = 40%.

Spacer	Vt 50mls	Vt 100mls	Vt 150mls	Vt 200mls	Vt 300mls
Nebuchamber	**25.2** (22.9-27.5)	**58.9** (52.9-64.9)	**72.9** (67.5-78.3)	**72.7** (68.5-76.9)	**81.6** (75.7-87.5)
A/S Nebuhaler	**12.0** (10.1-13.9)	**16.0** (11.9-20.1)	**28.1** (23.1-33.1)	**45.7** (39.0-52.4)	**80.6** (70.0-91.2)
Nebuhaler	**10.9** (10.0-11.8)	**14.9** (11.9-17.9)	**26.5** (23.1-29.9)	**41.3** (38.7-43.9)	**67.8** (63.9-71.7)
Aerochamber	**3.0** (2.2-3.8)	**9.0** (7.0-11.0)	**14.3** (9.4-19.2)	**19.6** (16.0-23.2)	**29.7** (27.9-31.5)



The effect of washing the spacer

Washing the spacer with 0.1% cetrimide solution did not significantly increase the drug output compared to the (previously used) spacers that had been washed in water. Drying the spacer with a paper towel before testing did not alter the drug output (p=0.51).

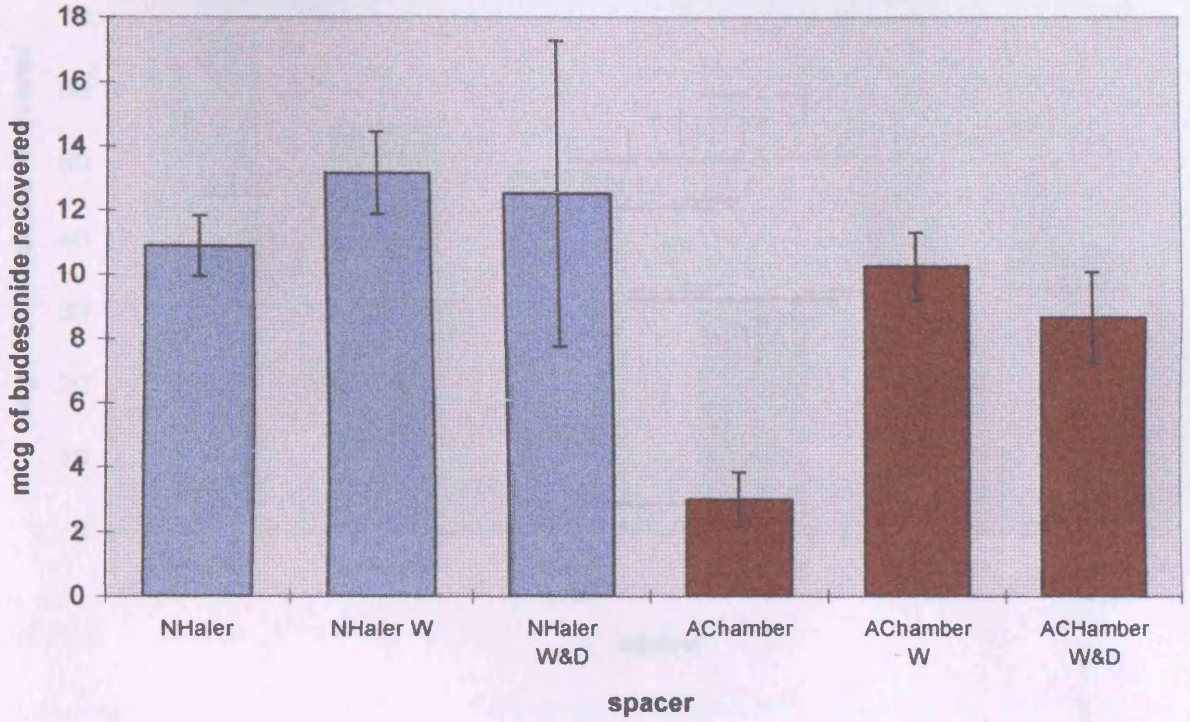
Output of budesonide from spacers (μg budesonide, mean and 95% confidence intervals).
2. The effect of washing and drying the spacer.

Rate = 20 cycles/minute Inspiratory fraction =40%.

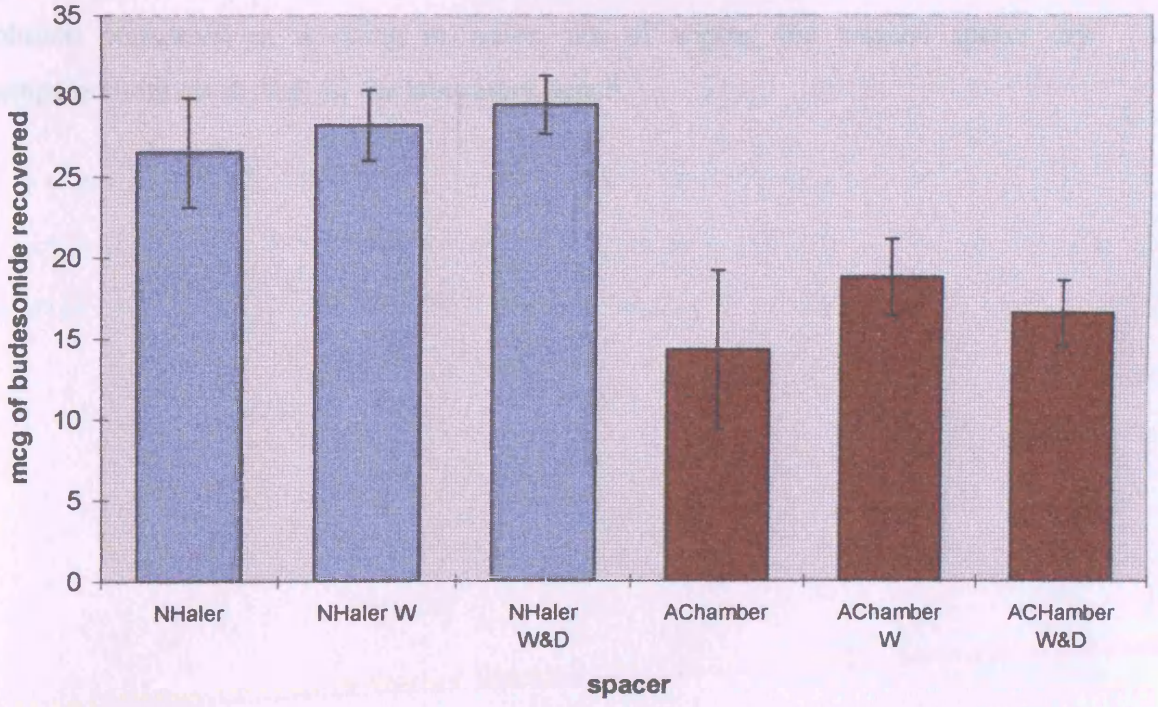
Spacer	Vt 50mls		Vt 150mls	
	Washed	Washed & dried	Washed	Washed & dried
Aerochamber	**10.3** (9.2-11.2)	**8.7** (7.3-10.1)	**18.8** (16.4-21.1)	**16.6** (14.5-18.6)
Nebuhaler	**13.2** (11.9-14.4)	**12.5** (7.8-17.3)	**28.2** (26.0-30.4)	**29.4** (27.6-31.2)

Spacer	Vt 300mls	
	Washed	Washed & dried
Aerochamber	**26.0** (22.2-29.7)	**38.9** (35.6-42.2)
Nebuhaler	**55.3** (48.3-62.3)	**49.1** (42.7-55.5)

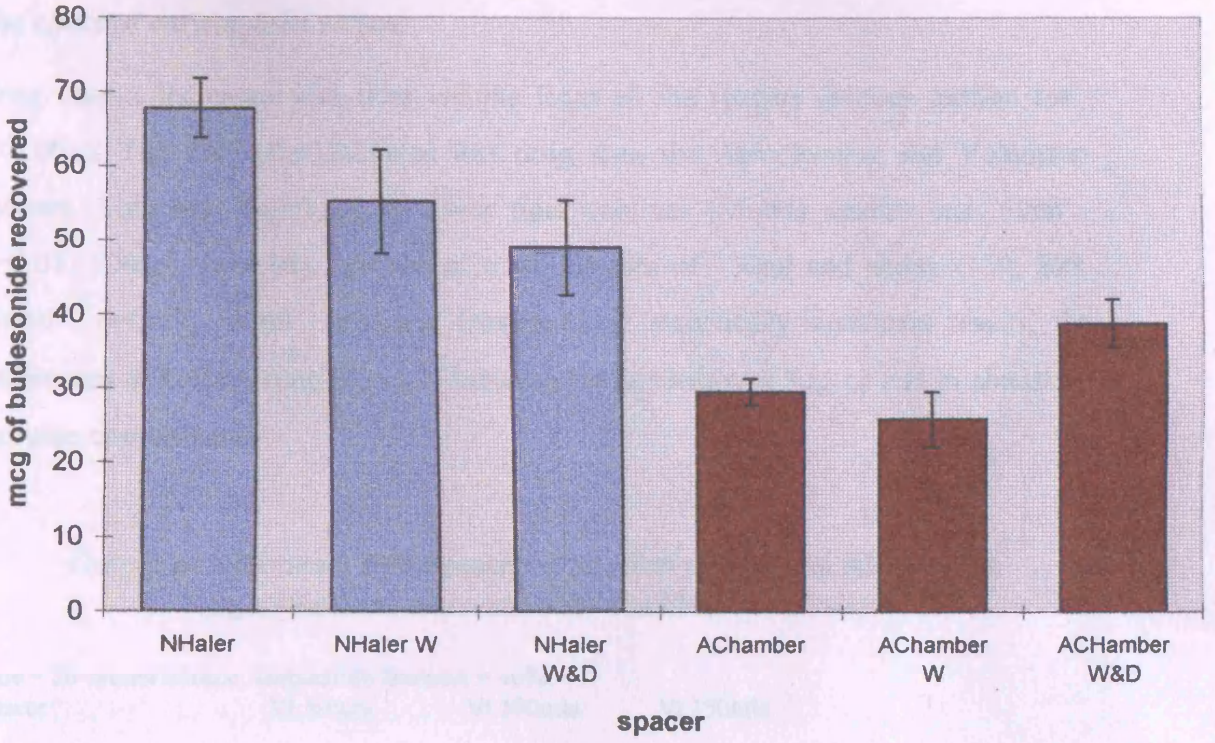
Tidal Volume 50ml:



Tidal Volume 150ml:



Tidal Volume 300ml:



There was no consistent effect of washing the spacer briefly in 0.1% Cetrimide solution compared to washing in water, nor of wiping the washed spacer dry compared with air drying on the laboratory bench.

Salbutamol results

The effect of varying tidal volume

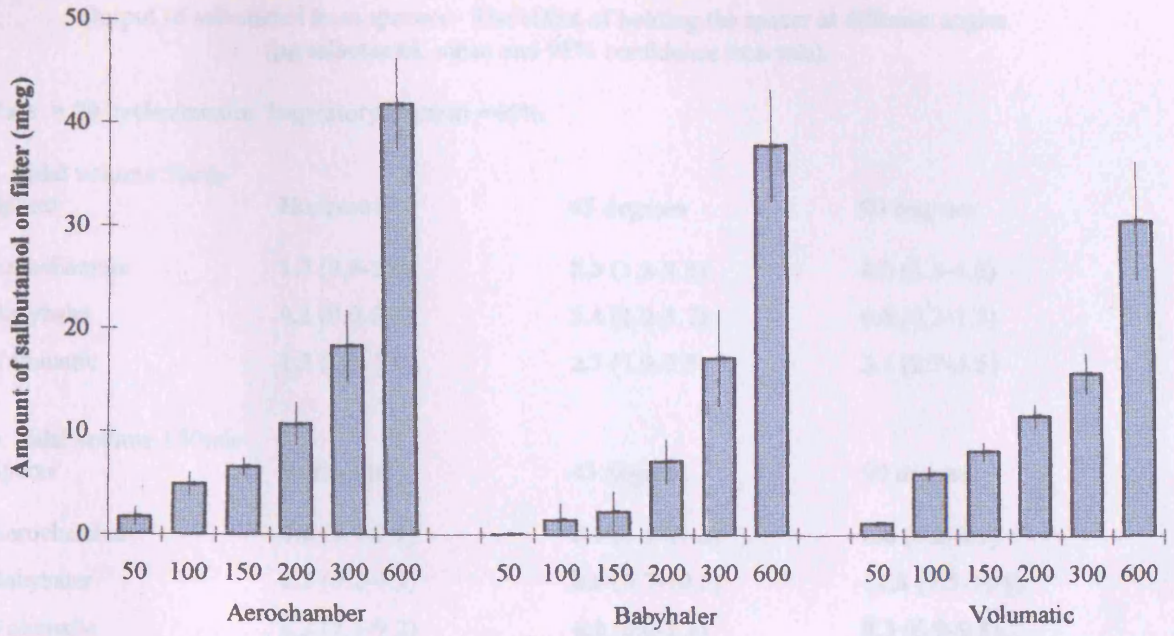
Drug output increased with tidal volume from all the spacers (Moods median test, $p<0.001$). The Babyhaler delivered less drug than the Aerochamber and Volumatic spacers. This was significant at lower tidal volumes (Moods median test, 50ml - $p=0.01$, 100ml - $p=0.02$), but not at tidal volumes of 150ml and above (150, 200, 600ml - $p=0.07$, 300ml - $p=0.14$). Despite these statistically significant results, the differences in median drug recovery between the spacers was 5μg or less in almost all pairwise comparisons.

Output of salbutamol from spacers - The effect of changing tidal volume
(μg salbutamol, mean and 95% confidence intervals).

Rate = 20 cycles/minute, Inspiratory fraction = 40%.

Spacer	Vt 50mls	Vt 100mls	Vt 150mls
Aerochamber	**1.7** (0.8-2.6)	**4.9** (3.8-6.0)	**6.6** (5.7-7.5)
Babyhaler	**0.1** (0.0-0.1)	**1.5** (-0.1-3.1)	**2.3** (0.2-4.3)
Volumatic	**1.2** (1.0-1.4)	**6.0** (5.3-6.7)	**8.2** (7.3-9.2)

Spacer	Vt 200mls	Vt 300mls	Vt 600mls
Aerochamber	**10.7** (8.6-12.8)	**18.3** (14.9-21.7)	**41.7** (37.2-46.2)
Babyhaler	**7.2** (5.1-9.3)	**17.2** (12.5-22.0)	**38.0** (32.5-43.0)
Volumatic	**11.6** (10.4-12.8)	**15.8** (13.8-17.8)	**30.6** (24.8-36.4)



The effect of varying spacer position

Taking into account the different spacers and tidal volumes assessed, there was no overall effect of varying spacer position on drug output (Multivariate ANOVA, p=0.07). However, using the spacers at an angle did increase the drug output at the 50ml tidal volume (Moods median test; Aerochamber - p=0.01, Babyhaler p=0.003, Volumatic p=0.003). Angling the Babyhaler also increased the output of salbutamol at the 150ml tidal volume (p=0.01).

Output of salbutamol from spacers - The effect of holding the spacer at different angles.
(µg salbutamol, mean and 95% confidence intervals).

Rate = 20 cycles/minute. Inspiratory fraction =40%.

a. Tidal volume 50mls

Spacer	Horizontal	45 degrees	90 degrees
Aerochamber	**1.7** (0.8-2.6)	**2.3** (1.8-2.8)	**4.0** (3.3-4.8)
Babyhaler	**0.1** (0.0-0.1)	**3.4** (1.2-5.7)	**0.8** (0.3-1.3)
Volumatic	**1.2** (1.0-1.4)	**2.7** (1.9-3.5)	**3.1** (2.7-3.5)

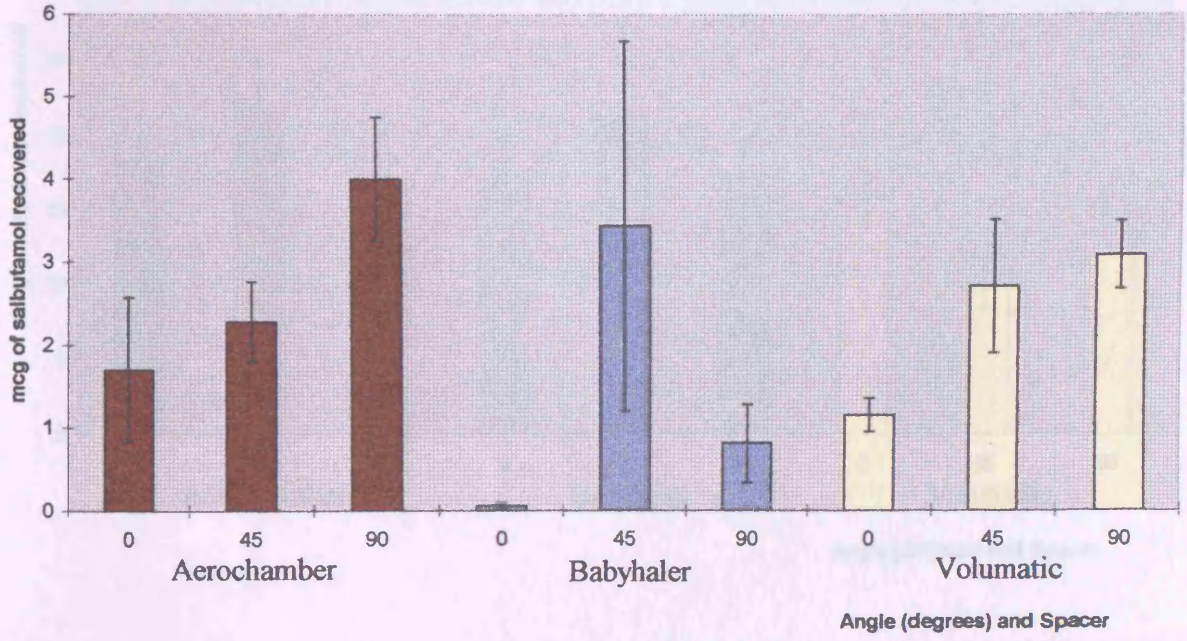
b. Tidal volume 150mls

Spacer	Horizontal	45 degrees	90 degrees
Aerochamber	**6.6** (5.7-7.5)	**8.7** (6.1-11.2)	**6.6** (4.2-8.9)
Babyhaler	**2.3** (0.2-4.3)	**8.6** (6.7-10.5)	**11.8** (7.5-16.0)
Volumatic	**8.2** (7.3-9.2)	**6.9** (6.0-7.7)	**8.3** (6.9-9.8)

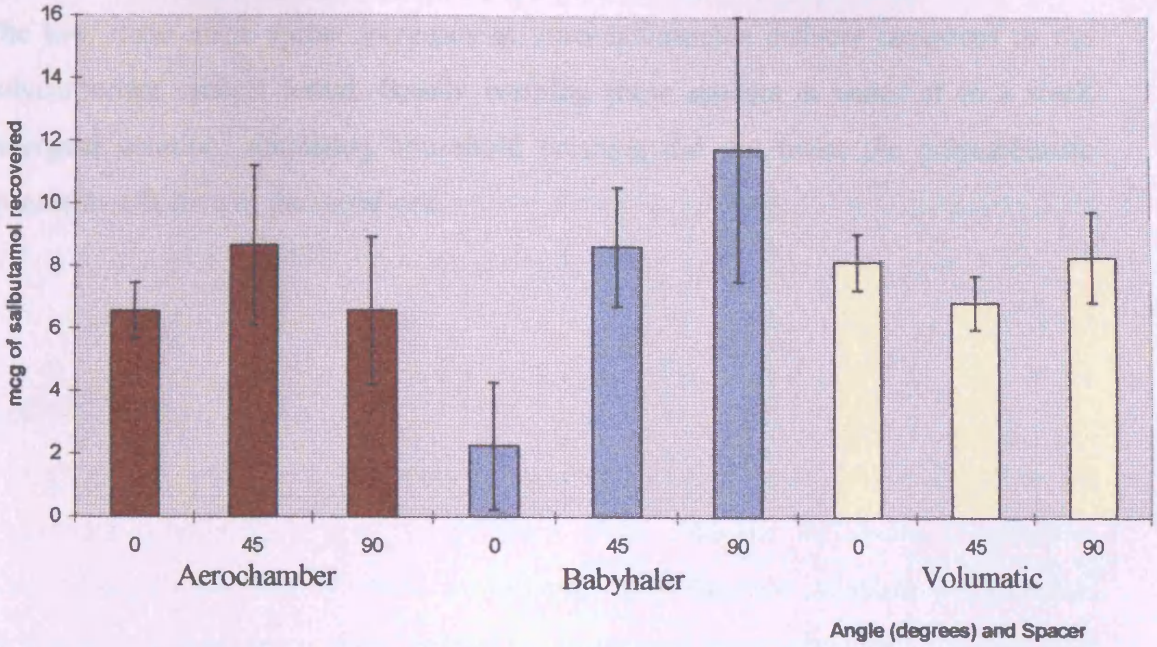
c. Tidal volume 300mls

Spacer	Horizontal	45 degrees	90 degrees
Aerochamber	**18.3** (14.9-21.7)	**21.9** (18.7-25.1)	**17.3** (14.5-20.1)
Babyhaler	**17.2** (12.5-22.0)	**21.5** (10.9-32.0)	**17.2** (14.6-19.9)
Volumatic	**15.8** (13.8-17.8)	**10.1** (8.4-11.8)	**13.4** (11.8-14.9)

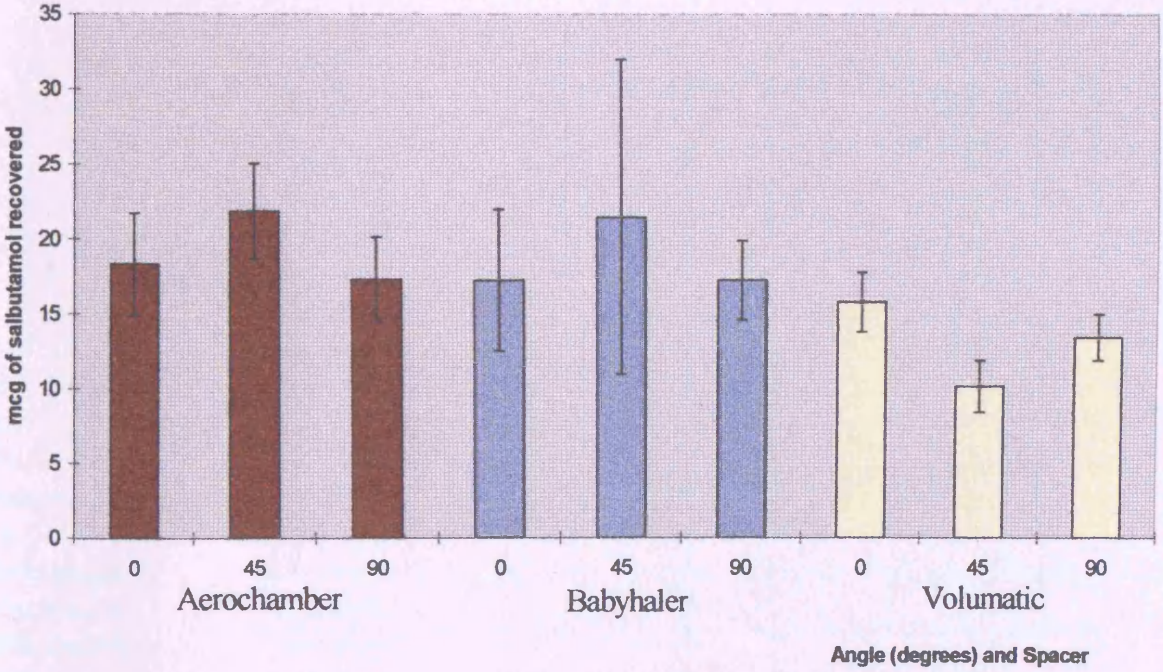
Tidal Volume 50ml:



Tidal Volume 150ml:



Tidal Volume 300ml:



Summary

The low static metal spacer increases in vitro budesonide delivery compared to the polycarbonate spacers tested. Briefly washing these spacers in water or in a weak detergent solution, simulating household washing did not make the polycarbonate spacers as effective as the metal one.

Assessment of Budesonide Nebuliser Suspension

Aim

To measure the particle size output of budesonide suspension from various nebulisers and compressors under different conditions of use.

Methods

The glass multistage liquid impinger was used to determine the output of budesonide from various nebulisers and compressors. Five different nebulisers (Sidestream, Ventstream, Cirrus, Pari LC Plus, Acorn) and three different compressors (Medicaid Portaneb, Pari Inhalierboy, Pari Master) were assessed for a nebulisation time of three and five minutes. Each nebuliser/compressor combination was assessed on up to four occasions (Table). The Easimist Ultrasonic Nebuliser (Medix, Lutterworth, UK) was also tested at high power and low power.

Nebuliser	Compressor	Nebulisation Time (min)	Repeats
Acorn	Portaneb	5	4
Acorn	Portaneb	3	2
Cirrus	Portaneb	5	4
Cirrus	Portaneb	3	4
Cirrus	Pari Boy	5	3
Pari LC Plus	Portaneb	5	4
Pari LC Plus	Portaneb	3	4
Pari LC Plus	Pari Master	5	4
Pari LC Plus	Pari Master	3	4
Pari LC Plus	Pari Boy	5	4
Pari LC Plus	Pari Boy	3	4
Sidestream	Portaneb	5	4
Sidestream	Portaneb	3	4
Sidestream	Pari Boy	5	4
Sidestream	Pari Boy	3	4
Ventstream	Portaneb	5	4
Ventstream	Portaneb	3	4
Ventstream	Pari Boy	5	1

Table: Nebuliser/compressor combinations tested

The nebulisers were prepared by washing ten times in soap and water, according to manufacturers instructions.

Two millilitres of budesonide nebuliser suspension (500μg in 2ml, Pulmicort, Astra, Kings Langley, UK) was added to the nebuliser, which was attached to the MSLI via a connector with adjustable entrainment holes, allowing the flow through the nebuliser to equal 20 l/min, while maintaining 60l/min through the MSLI. This device was used with all chambers except the Sidestream, where the supplied mouthpiece already has an entrainment hole, allowing the required flow (20l/min) to be drawn through the chamber.

At the end of the prescribed time, the amount of budesonide captured on each stage of the experimental apparatus was measured using high pressure liquid chromatography as described in Chapter 5.6. (Budesonide, second method).

Results

The total recovery of budesonide from the experimental apparatus, the recovery from the MSLI (the 'dose to patient'), the percentage of the mass of budesonide placed in the nebuliser that was emitted, the rate of nebulisation, in μg per minute, the mass of drug recovered in particles smaller than five and three μm, and the MMAD and GSD for each nebuliser/compressor combination tested are given in the accompanying Table (mean values and 95% confidence intervals).

The greatest delivery of budesonide was from the Pari LC Plus nebuliser, although this also produced the largest particles, MMAD 5-7μm. In contrast, the Ventstream and Sidestream nebulisers delivered a lower total dose, but a similar dose of budesonide in particles smaller than 5μm. The conventional nebulisers delivered the least drug.

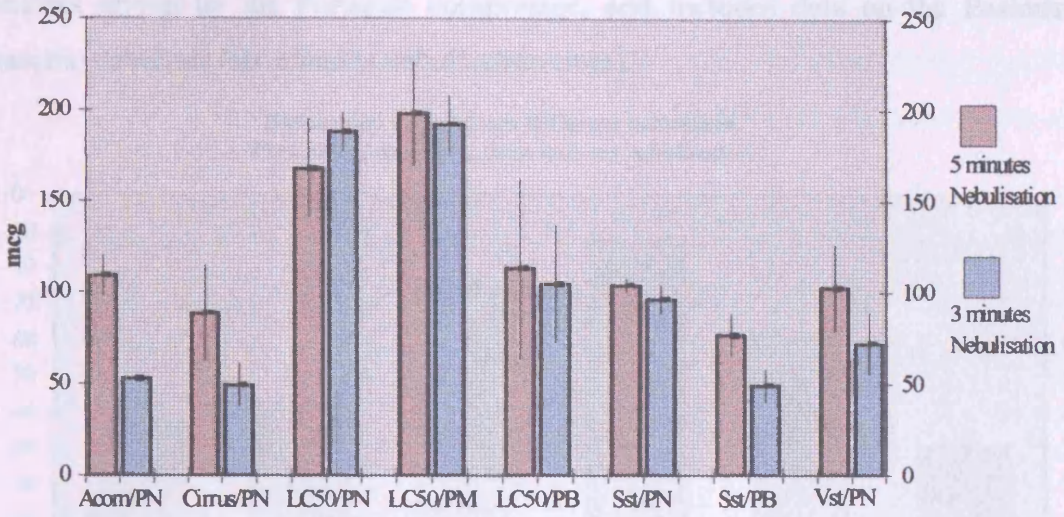
With any one nebuliser, the Portaneb and Pari Master compressors delivered the most budesonide, and the Pariboy compressor the least. The Easimist ultrasonic nebuliser delivered the least budesonide, less than seven percent of the nominal dose, perhaps representing the amount of budesonide in held solution.

Nebuliser	Comp	Vol. Fill	T	n	Total Recovery	Dose to Patient	% mass emitted	Rate of Nebulisation	Dose <5mcm	Dose <3mcm	MMAD	GSD
		(ml)	(min.)		(μg)	(μg)	(%)	(μg/min.)	(μg)	(μg)	(μm)	
Acorn	Portaneb	2	5	4	438.2 424.7-451.7	109.0 97.69-120.25	24.9 22.73-26.97	21.8 19.54-24.05	75.5 71.82-79.26	55.3 52.43-58.18	3.1 2.72-3.40	2.6 2.5-2.8
Acorn	Portaneb	2	3	2	478.4 471.1-485.8	52.8 51.7-53.9	11.0 11.0-11.1	17.6 17.2-17.98	36.7 36.26-37.19	28.48 28.05-28.92	2.86 2.70-3.02	3.0 2.9-3.2
Cirrus	Portaneb	2	5	4	524.0 480.7-567.3	88.44 62.24-114.63	16.72 12.95-20.48	17.69 12.45-22.93	70.40 53.74-87.06	52.64 41.59-63.69	2.45 2.20-2.69	2.3 2.2-2.4
Cirrus	Portaneb	2	3	4	437.2 399.5-474.9	49.43 37.50-61.37	11.40 8.22-14.58	16.48 12.50-20.46	42.16 34.89-49.44	32.84 27.93-37.75	2.25 2.04-2.46	2.1 1.8-2.4
Cirrus	Pari boy	2	5	3	458.4 449.1-467.7	38.93 27.09-50.78	8.53 5.75-11.32	7.79 5.42-10.16	29.91 22.02-37.80	21.56 15.23-27.88	2.77 2.19-3.35	2.2 1.9-2.4
Pari LC50	Portaneb	2	5	4	442.0 405.0-479.0	167.88 141.20-194.57	37.80 34.85-40.75	47.33 40.39-54.27	80.99 69.21-92.77	52.39 41.85-62.93	5.25 4.33-6.17	3.05 2.8-3.3
Pari LC50	Portaneb	2	3	4	438.5 409.9-467.1	187.73 177.14-198.33	42.85 41.63-44.06	62.58 59.05-66.11	75.10 70.89-79.31	45.85 41.09-50.62	6.68 6.51-6.86	3.1 2.8-3.4
Pari LC50	Pari Master	2	5	4	473.7 432.1-515.2	197.74 169.48-226.00	41.63 38.82-44.43	51.06 37.45-64.68	89.53 80.18-98.89	58.35 53.15-63.55	5.71 5.33-6.08	2.9 2.7-3.2
Pari LC50	Pari Master	2	3	4	483.3 451.2-515.4	191.95 176.27-207.63	39.68 39.01-40.36	63.98 58.76-69.21	79.45 72.22-86.68	46.75 41.33-52.16	6.29 6.01-6.57	2.9 2. 7-3.1
Pari LC50	Pari boy	2	5	4	335.0 193.5-476.6	113.25 63.65-162.85	33.81 30.27-37.35	23.73 12.89-34.57	48.02 25.97-70.07	19.03 18.67-19.40	6.82 5.13-8.50	3.5 2. 9-4.1
Pari LC50	Pari boy	2	3	4	461.20 449.05-473.35	104.54 72.55-136.53	22.61 15.86-29.36	34.85 24.18-45.51	48.68 37.69-59.66	36.42	5.66 4.26-7.06	3.8 3.1-4.5

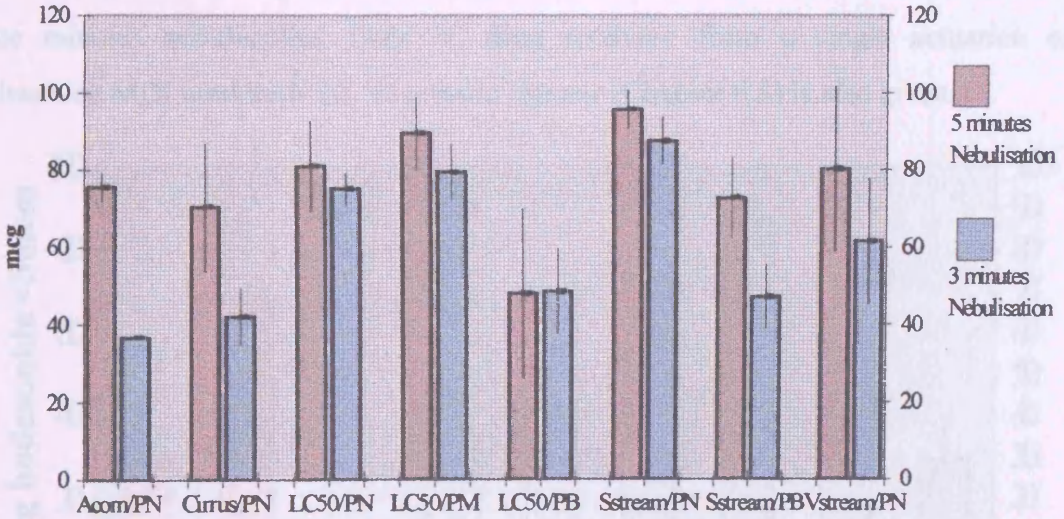
Nebuliser	Comp	Vol. Fill	T	n	Total Recovery	Dose to Patient	% mass emitted	Rate of Nebulisation	Dose <5mcm	Dose <3mcm	MMAD	GSD
		(ml)	(min.)		(μg)	(μg)	(%)	(μg/min.)	(μg)	(μg)	(μm)	
Sidestream	Portaneb	2	5	4	478.7 459.5-497.8	103.9 100.4-107.5	21.7 20.7-22.8	22.7 19.7-25.7	95.7 90.8-100.6	76.3 69.7-82.8	2.0 1.9-2.2	1.9 1.9-1.9
Sidestream	Portaneb	2	3	4	488.70 472.8-504.6	96.37 88.5-104.3	19.71 18.4-21.0	32.12 29.5-34.8	87.39 81.4-93.4	66.4 62.9-69.9	2.25 2.1-2.5	1.8 1.8-1.9
Sidestream	Pari boy	2	5	4	462.2 432.3 -492.2	76.9 65.0-88.7	16.6 14.4-18.9	15.4 13.0-17.7	72.6 62.0-83.3	60.0 51.1-68.8	1.9 1.8-2.1	1.8 1.7-1.9
Sidestream	Pari boy	2	3	4	477.1 461.6-492.5	49.2 40.3-58.0	10.3 8.4-12.2	16.4 13.4-19.3	47.1 38.8-55.3	40.2 33.7-46.7	1.8 1.7-1.9	1.8 1.8 -1.8
Ventstream	Portaneb	2	5	4	430.3 378.8-481.7	102.7 79.0-126.3	23.9 18.8-29.1	20.5 15.8-25.3	80.2 58.8-101.6	61.1 44.0-78.1	2.6 2.4-2.8	2.4 2.3-2.5
Ventstream	Portaneb	2	3	4	452.3 397.8-506.8	72.0 54.80-89.18	16.2 11.4-21.0	24.0 18.3-29.7	61.5 45.1-77.8	50.1 36.5-63.8	2.1 1.8-2.4	2.4 1.9-2.8
Ventstream	Pari boy	2	5	1	457.9	70.1	15.3	14.0	53.4	40.3	2.6	2.5
Easimist	(High power)	2	5	3	--	33,9 18.6-49.3	--	6.8 3.7-9.9	30.0 17.2-42.7	18.8 12.6-25.5	2.9 2.8-3.1	1.5 1.5-1.6
Easimist	(low power)	2	5	4	--	23.4 17.9-28.8	--	4.7 3.6-5.8	21.0 15.9-26.0	14.0 10.6-17.4	2.8 2.8-2.9	1.6 1.5-1.6

The following graphs show the effect of three or five minutes nebulisation on the output from the different nebuliser/compressor combinations.
(PN=Portaneb, PB= Pariboy)

Nebulisation times. 5 vs 3 minutes.
Total dose to patient



Nebulisation times. 5 vs 3 minutes.
Dose less than 5mcm

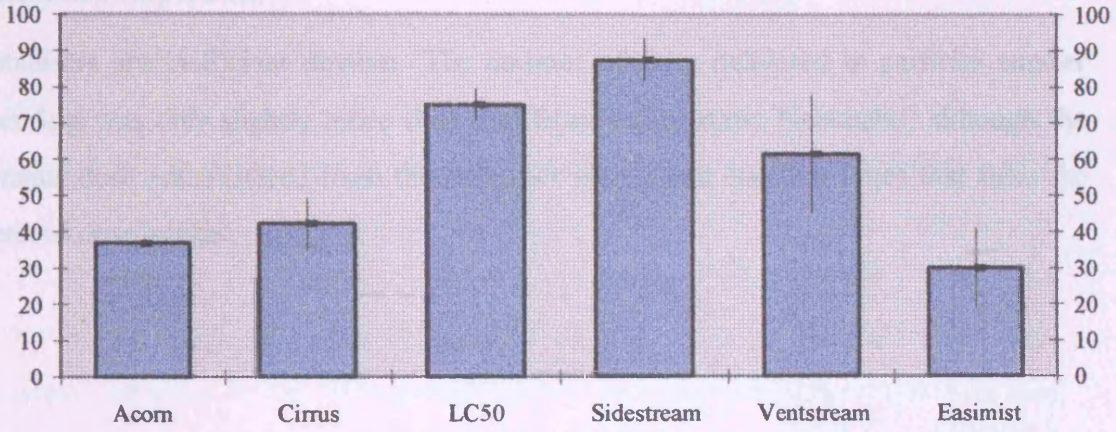


There was little increase in drug delivery from the LC Plus nebuliser (noted in the

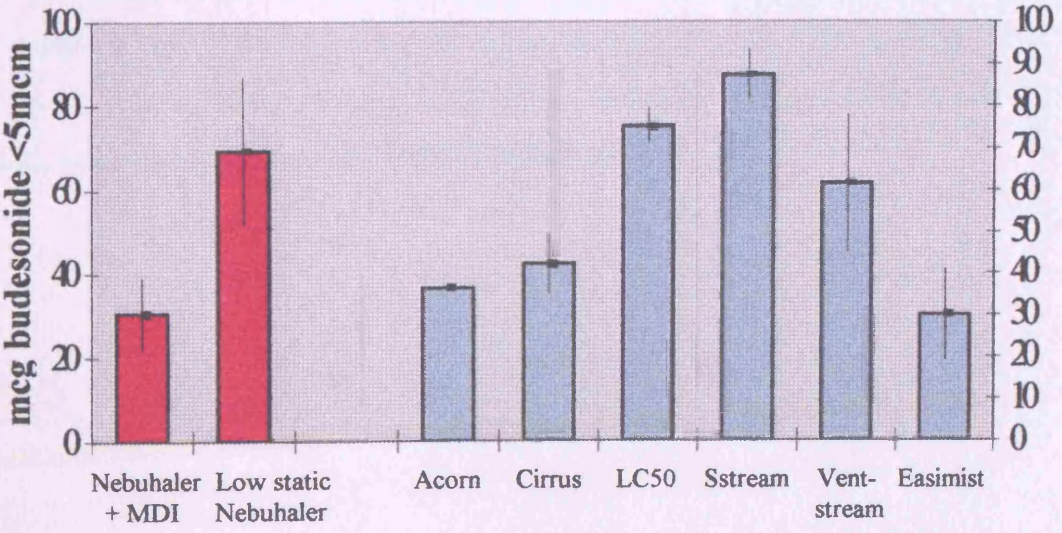
graphs as the 'LC50') after three minutes, in contrast to the conventional nebulisers. The Sidestream nebuliser had finished effective nebulisation when used with the Portaneb Compressor, but not when used with the less powerful Pariboy compressor.

The following graphs show the output of particles smaller than 5μm for different chambers driven by the Portaneb compressor, and includes data on the Easimist ultrasonic nebuliser (six minutes nebulisation time).

Budesonide output from different nebulisers.
Portaneb compressor, three minutes nebulisation.



For comparison, the recovery of budesonide from a single actuation of a MDI used with the Nebuhaler (Chapter 6.1) is compared with that from different nebulisers after three minutes nebulisation. Data on drug recovery from a single actuation of budesonide MDI used with the 'low static' spacer (Chapter 6.8) is also given.



Summary

Six nebulisers were tested in this experiment, and delivered widely different masses of budesonide, both in total and as small particles of drug. The nebuliser with the largest particle size delivered the most drug, but the Sidestream nebuliser was more selective, delivering a slightly greater mass of small particles, without the larger particles delivered by the LC Plus. The ultrasonic nebulier performed poorly, supporting Astra's recommendation that ultrasonic nebulisers should not be used for the nebulisation of budesonide suspension.

Nebulisers are inefficient devices. The amount of drug delivered in particles smaller than 5μg was only slightly more than that from a low static Nebuhaler, although the nominal dose administered from the nebuliser is two and one half times that from the metered dose inhaler.

A comparison of methods of measuring nebuliser output

Aim

The purpose of this study was to compare the accuracy of different methods of measuring nebuliser output, weighing the nebuliser before and after nebulisation (the gravimetric method) and measuring the osmolality of drug remaining in the nebuliser (the osmolality method), compared with direct measurement of the drug remaining in the nebuliser (the direct method).

Methods

A Novair II compressor and Cirrus nebuliser (Intersurgical Ltd, Wokingham, UK) were used to nebulise either sodium cromoglycate solution 10mg/ml (Intal, Fisons PLC, Loughborough, UK) or gentamicin solution 40mg/ml (DBL Ltd, Warwick, UK). The nebuliser was weighed, two millilitres of drug solution added, and the nebuliser reweighed. Nebulisation was then undertaken for five minutes. At the end of this time, the nebuliser was weighed, and an aliquot removed from the nebuliser reservoir for determination of osmolality (Freezing point method, 3MO micro-osmometer, Vitech Scientific Ltd, West Sussex). The nebuliser was rinsed and dried, and the experiment repeated. At the end of nebulisation on the second occasion, the nebuliser was washed with a known volume of water, and the amount of drug present was determined by UV spectrophotometry (sodium cromoglycate) or fluorescence polarisation (gentamicin). Each experiment was repeated four times for each drug. Analysis of variance was used to compare the three experimental methods. Laboratory temperature and relative humidity were constant during each of the experiments.

Results

The results are given in the table.

Mass of drug emitted from the nebuliser when determined by three different methods.

Method of Measurement	Mass of drug emitted (mg; mean and 95% confidence intervals) Sodium cromoglycate (20mg dose)	Gentamicin (80mg dose)
Gravimetric	7.6 (7.1-8.1)	33.4 (30.1-36.6)
Osmolality	5.9 (5.0-6.9)	28.3 (21.7-34.8)
Drug Assay	3.9 (3.3-4.4)	14.1 (11.8-16.4)

Both the gravimetric and the osmolality techniques overestimated drug output by 95 and 51% (sodium cromoglycate, $p<0.001$) and by 135 and 100% (gentamicin, $p=0.002$) respectively compared to formal laboratory measurement of drug concentration.

Summary

The results of this study confirm that the gravimetric method vastly overestimates drug output from nebulisers and also show that the modified gravimetric method, incorporating osmolality measurements, is inaccurate. These methods have been widely used in previous studies, and our findings suggest that the results of such studies may have greatly overestimated nebuliser drug output.

Breathing simulation - Nebulisers

Aim

To determine the effect of different breathing patterns on the delivery of drug from different nebulisers, and to compare this with delivery under constant flow conditions.

Methods

The Pari Sinus Breathing Simulator, described in Chapter 5.4, was used to produce tidal airflow through different nebulisers. Drug released from the nebulisers was captured on in line filters and assayed.

The following nebuliser-compressor combinations were evaluated; Cirrus with Novair II Compressor (Intersurgical Ltd, Wokingham, UK); Sidestream with Portaneb 50 compressor (Medicaid, Pagham, UK); Pari LC Plus with Juniorboy Compressor (Pari Medical, Surrey, UK).

The nebulisers were tested using either a constant flow from a vacuum pump (Edwards ECB8, Edwards, Surrey, UK) of 20l/min or 60l/min, or using a breathing simulator, (Sinus Breathing Simulator, Pari GmbH, Starnberg, Germany).

Electrostatic filter pads were used, held in a plastic filter assembly (dead space 11 ml). Nebulisers were connected to the filter assembly by the T-piece (Cirrus and LC Plus) or mouth-piece (Sidestream) supplied by the nebuliser manufacturer. This mouthpiece did not occlude the Sidestream open vent. Waste aerosol released to the atmosphere during 'expiration' was scavenged onto a second filter placed near the end of the T-piece (Cirrus) or entrainment hole of the Sidestream mouthpiece using a household vacuum cleaner. The 'expiratory' filter was connected to the second limb of the T-piece supplied with the LC Plus, and contained a one way valve so that airflow would be directed through the nebuliser during 'inspiration', imitating patient use.

The rate, tidal volume and inspiratory to expiratory ratio of the breathing simulator was varied to represent the breathing patterns of children at various ages, as shown in the Table:

Representative Age	6 months	1 year	3 years	5 years	10 years	16 years
Tidal Volume (Vt, ml)	50	75	125	150	225	600
Rate (breaths/min)	30	28	24	20	16	16
I:E ratio	40:60	40:60	40:60	40:60	40:60	40:60

TABLE. Breathing Simulator Settings Used.

Two millilitres of budesonide suspension (Pulmicort Nebuliser Suspension 250μg/ml, Astra, Kings Langley, UK) was added to the nebuliser. This was operated for five minutes, after which the 'inspiratory' filter was removed from the housing and the amount of budesonide deposited on the filter was assayed by high performance liquid chromatography. In later experiments, the nebuliser was operated 'to dryness', defined as the time at which there has been no visible nebuliser output for thirty seconds. Each nebuliser was assessed at each breathing pattern and flow a minimum of six times.

In the main experiments, the amount of budesonide on the 'inspiratory' filter only was assayed, but in pilot studies using the same experimental apparatus, the amount of drug deposited in all parts of the experimental apparatus was measured. 94.1% (95% confidence intervals (CI) 88.7-99.4) of the nominal 500μg of budesonide placed in the nebuliser was recovered. Less drug was recovered from the Sidestream nebuliser compared to the other nebulisers studied, as some losses occurred from the entrainment ports.

To determine the amount of drug passively collected on the inspiratory filter, the experiments were also undertaken with the breathing simulator switched off (tidal volume of 0ml). 8μg (95% CI 6.6-9.4) of budesonide was recovered from the inspiratory filter when the LC Plus was assessed, and only 2.3μg (95% CI 2.0-2.6) of budesonide with the other nebulisers.

The recovery of budesonide from the 'inspiratory' filter was compared between flows with each nebuliser and between nebulisers at the same flow using analysis of variance. Statistical significance was assumed at $p=0.05$.

RESULTS

The amount of budesonide recovered from the filter in the different experiments is given in the Table. The drug output from all the nebulisers was greatest when they were assessed under constant flow, and was reduced by the use of a simulated breathing pattern. It can be seen from the table that the measured output of the nebulisers may vary by over 700%, depending on the method of measurement.

Nebuliser/ compressor	Cirrus/ Novair II	Sidestream/ Portaneb	Ventstream/ Portaneb	Pari LC Plus/ Pariboy
Experimental method	μg	μg	μg	μg
Constant flow, 20l/min	102.9 (88.3-117.5)	150.4 (130.1-170.8)	110.8 (104.9-116.6)	176.2 (158.5-194.0)
Constant flow, 60l/min	122.7 (106.8-138.5)	130.0 (114.4-145.5)	38.6 (21.7-55.4)	93.3 (89.5-97.2)
Tidal volume 600ml, Rate 16 breaths/minute	35.5 (33.5-37.4)	19.3 (14.6-24.1)	42.1 (40.4-43.9)	82.4 (79.0-85.7)
Tidal volume 225ml, Rate 16 breaths/minute	39.4 (33.9-45.0)	17.4 (13.0-21.8)	30.1 (25.8-34.4)	70.9 (65.3-76.5)
Tidal volume 150ml, Rate 20 breaths/minute	34.1 (28.6-39.7)	18.2 (13.1-23.4)	26.5 (23.5-29.5)	73.5 (64.1-82.9)
Tidal volume 125ml, Rate 24 breaths/minute	37.1 (32.4-41.7)	18.5 (17.0-20.0)	26.5 (23.2-29.9)	69.3 (61.1-77.4)
Tidal volume 75ml, Rate 28 breaths/minute	27.4 (24.5-30.3)	13.5 (12.0-15.0)	19.6 (18.1-21.1)	71.3 (65.7-76.9)
Tidal volume 50ml, Rate 30 breaths/minute	4.4 (3.4-5.6)	4.5 (3.4-5.6)	12.3 (10.8-13.8)	25.0 (14.1-35.8)

Table: Amount of Budesonide Recovered from the Filters with Different Nebulisers and Breathing Patterns. (Values are the mean and 95% confidence intervals for the recovery of budesonide in μg).

The Cirrus nebuliser shows tidal volume related increase in filter drug deposition up to 125ml tidal volume (minute volume 3l), with constant output above this. Note the low output at 225ml Vt. These experiments were undertaken at a different time from the others and need validating.

The Sidestream nebuliser deposits less drug than the other nebuliser/compressor combinations and also shows constant output above 125ml tidal volume (minute volume 3l). Much aerosol appeared to be lost from around the entrainment holes of the nebuliser during these experiments.

The LC+/Pariboy deposits more drug on the filter than the other nebuliser/compressor combinations at all breathing patterns assessed. The nebuliser is not breath dependent or breath enhanced at tidal volumes of 75ml and above (minute volumes above 2.1l).

The Ventstream deposited less drug at lower tidal volumes (except 50ml) than the Cirrus, but more drug at higher tidal volumes, demonstrating a minute volume dependent increase in drug delivery.

The output of the LC Plus was markedly reduced at higher flow, from 176μg at 20l/min to 93.3μg at 60l/min ($p<0.001$). The output from the Sidestream was also reduced at the higher flow, and that from the Cirrus increased, but these did not reach statistical significance.

Budesonide output was lowest at a tidal volume of 50ml. All the nebulisers except the Ventstream gave constant output at tidal volumes above 125mls, and at any one breathing pattern the LC Plus deposited more budesonide on the inspiratory filter than the other nebulisers ($p<0.001$).

- Nebulisation to dryness

Differences between the nebulisers may have been inadvertantly emphasised by the decision to evaluate the nebuliers for five minutes. To assess this possibility, further experiments were undertaken operating the nebulisers 'to dryness', defined as the time when there had been no visible nebuliser output for thirty seconds. These experiments were undertaken with three tidal volumes, 50, 150 and 300mls, all with a breathing frequency of 20b/min and an inspiratory fraction of 0.4. Note that these values are different from some of the parameters used above.

Six repeats at each breathing pattern were made

Time to 'dryness' is given below (minutes; mean & 95% confidence intervals):

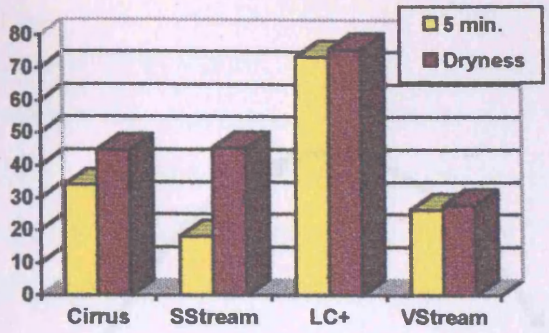
	Tidal Volume (mls)		
Nebuliser	50	150	300
Cirrus	**7.6** (6.5-8.0)	**9.4** (8.9-10.0)	**8.6** (8.5-8.8)
Sidestream	--	--	--
LC+	**5.3** (5.2-5.5)	**5.5** (5.3-5.8)	**5.5** (5.3-5.7)
Ventstream	**12.1** (12.1-12.2)	**12.1** (12.0-12.1)	**12.7** (12.6-12.8)

Time to dryness was not recorded for the Sidestream, which has only been assessed at 150ml tidal volume.

The amount of budesonide deposited on the filter is given below (μg; mean & 95% confidence intervals):

	Tidal Volume (mls)		
Nebuliser	50	150	300
Cirrus	**12.4** (9.3-15.5)	**44.9** (41.6-48.2)	**43.8** (39.8-47.8)
Sidestream	--	**45.4** (38.5-52.4)	--
LC+	**30.2** (19.6-40.7)	**75.8** (70.8-80.8)	**100.6** (91.5-109.6)
Ventstream	**11.9** (10.4-13.4)	**27.8** (26.2-29.4)	**43.4** (41.9-44.9)

Comparing the results at 5 minutes and to dryness at 150mls tidal volume (3l minute volume):

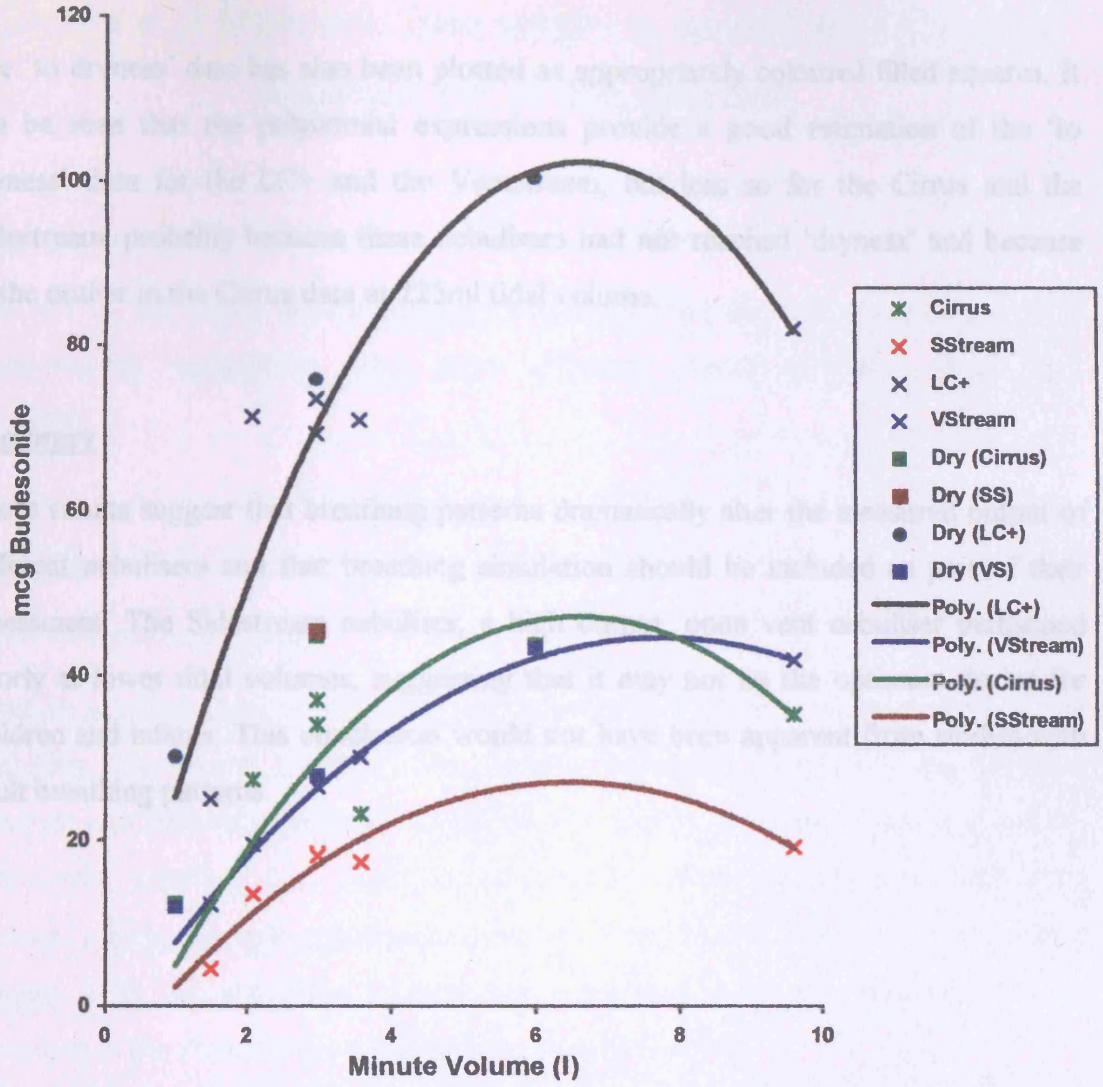


Thus the LC+ and Ventstream appear to have finished releasing drug by five minutes, whereas the Cirrus and Sidestream are still producing drug containing aerosol.

- Output at five minutes as a function of minute ventilation

The five minute data can be used to construct a graph of output against minute volume (as above), from which trend lines can be derived.

This is shown below:



A second power polynomial expression (solid lines) has been fitted to the five minute data (coloured 'x' marks).

The polynomial expressions are as follows, where 'y' is the recovery of budesonide from the filter, and x is the minute volume:

Cirrus	$y = -1.3508x^2 + 17.839x - 11.62$	$R^2 = 0.56$
Sidestream	$y = -0.8219x^2 + 10.68x - 7.573$	$R^2 = 0.87$
LC+	$y = -2.3987x^2 + 32.031x - 4.4337$	$R^2 = 0.70$
Ventstream	$y = -0.8071x^2 + 12.556x - 4.0694$	$R^2 = 0.997$

The 'to dryness' data has also been plotted as appropriately coloured filled squares. It can be seen that the polynomial expressions provide a good estimation of the 'to dryness' data for the LC+ and the Ventstream, but less so for the Cirrus and the Sidestream, probably because these nebulisers had not reached 'dryness' and because of the outlier in the Cirrus data at 225ml tidal volume.

Summary

These results suggest that breathing patterns dramatically alter the measured output of different nebulisers and that breathing simulation should be included as part of their assessment. The Sidestream nebuliser, a high output, open vent nebuliser performed poorly at lower tidal volumes, suggesting that it may not be the optimum device for children and infants. This conclusion would not have been apparent from studies with adult breathing patterns.

The output of budesonide from nebulisers assessed using breathing simulation and inertial impaction

Aim

The aim of this study was to determine the particle size and mass output of a corticosteroid, budesonide, from three different 'breath enhanced, open vent' nebulisers.

Methods

Nebulisers and medication

Three jet nebuliser/compressor combinations were assessed: The LC Plus nebuliser and Turboboy compressor (38G00, Pari GmbH, Starnberg, Germany), the LC Star nebuliser and Turboboy compressor (38G00, Pari GmbH, Starnberg, Germany) and the Ventstream nebuliser and Portaneb compressor (Medicaid, Pagham, UK) were assessed with budesonide nebuliser suspension (500μg in 2ml, Pulmicort, Astra, Kings Langley, UK). Six nebulisers of each type were used. Each was washed ten times according to the manufacturers instructions prior to first use.

Budesonide particle size measurements

Particle size distribution of the nebuliser output was measured using a glass multistage liquid impinger, as described in Chapter 5.1. Flow through the MSLI and nebuliser was constant at 30l/min.

The nebulisers were charged with 2ml (500μg) of budesonide suspension, and connected to the MSLI inlet by a short plastic sock. 10ml of ethanol was placed in each stage of the MSLI to dissolve any impacted budesonide and prevent re-entrainment of drug particles. The MSLI and nebuliser were switched on and operated for five minutes.

After each experiment, the stages were washed with ethanol, and the amount of drug collected in each stage was assayed by high performance liquid chromatography, described below. Each nebuliser/compressor combination was tested four times.

Breathing simulation

Total drug output from the nebulisers was also measured using a breathing simulator, the Pari Sinus Breathing Simulator (Pari GmbH, Starnberg, Germany), as described in Chapter 5.4. Two ml (500μg) of budesonide nebuliser suspension were added to the nebuliser, which was attached to the breathing simulator. Nebulisers were connected to the filter assembly by the T-piece or mouth-piece supplied by the nebuliser manufacturer. Waste aerosol released during 'expiration' was collected on the expiratory filter. Nebulisers were operated for up to fifteen minutes, and nebulisation was interrupted briefly after each minute up to five minutes, and again after ten and fifteen minutes, to allow the inspiratory filter to be changed. In this way, the drug output at different times could be determined. The process of changing the filter took less than ten seconds. In a separate series of experiments, nebulisation continued for five minutes and the amount of drug collected on the filter compared with the main experiments.

Two different breathing patterns were used, one to represent a child and noted hereafter as the 'paediatric' breathing pattern, and one to represent an adult, noted hereafter as the 'adult' breathing pattern. The tidal volume, respiratory rate and inspiratory time were 150ml, 20breaths/min, 40% and 600ml, 12breaths/min, 40% respectively. These settings gave a minute volume, maximum inspiratory flow and mean inspiratory flow of 3l, 11.8l/min, 7.5l/min and 7.2l, 28.3l/min, 18l/min for the two breathing patterns respectively.

Each nebuliser was assessed at each breathing pattern six times up to five minutes, and three times up to fifteen minutes. At the end of each experiment the inspiratory filter was removed from the housing and washed with an appropriate solvent. The amount of drug collected on the filter was determined by high performance liquid chromatography. In pilot studies, drug depositing on all parts of the experimental

apparatus was measured, allowing the total recovery of drug to be estimated as a percentage of that added. A mean of 88% of the budesonide added was recovered (95% confidence intervals 86.8-89.8%). Total drug recovery was not dependent upon the nebuliser type.

Running the nebuliser continuously for five minutes without changing the filter increased the filter deposition by a mean of 16%, consistent with a ten second interruption of nebulisation each minute while the filter was changed.

To determine the passive deposition of drug particles on the inspiratory filter, experiments were repeated with budesonide and the nebulisers attached to the breathing simulator as shown in figure 1, but with the breathing simulator switched off. Eight experiments were undertaken and a mean (SD) of 8.7μg (12.0) of budesonide was recovered from the inspiratory filter, representing less than 2% of the budesonide added to the nebuliser.

First minute and total output of budesonide from the different nebulisers measured with the breathing simulator, and of that contained in particles smaller than 6.1μm (representing drug collected on stage 4 and the filter of the MSLI) was compared using analysis of variance. The time taken for the nebuliser to deliver 90% of the total output was calculated by interpolation from a graph of drug recovery plotted against time. Analysis was undertaken using Minitab statistical software (Clecom Ltd., Birmingham, UK).

Results

Particle size

The mass median aerodynamic diameter, the geometric standard deviation, the mass of drug and the percentage of the nebuliser budesonide output contained in particles smaller than 6.1μg, are given in the Table.

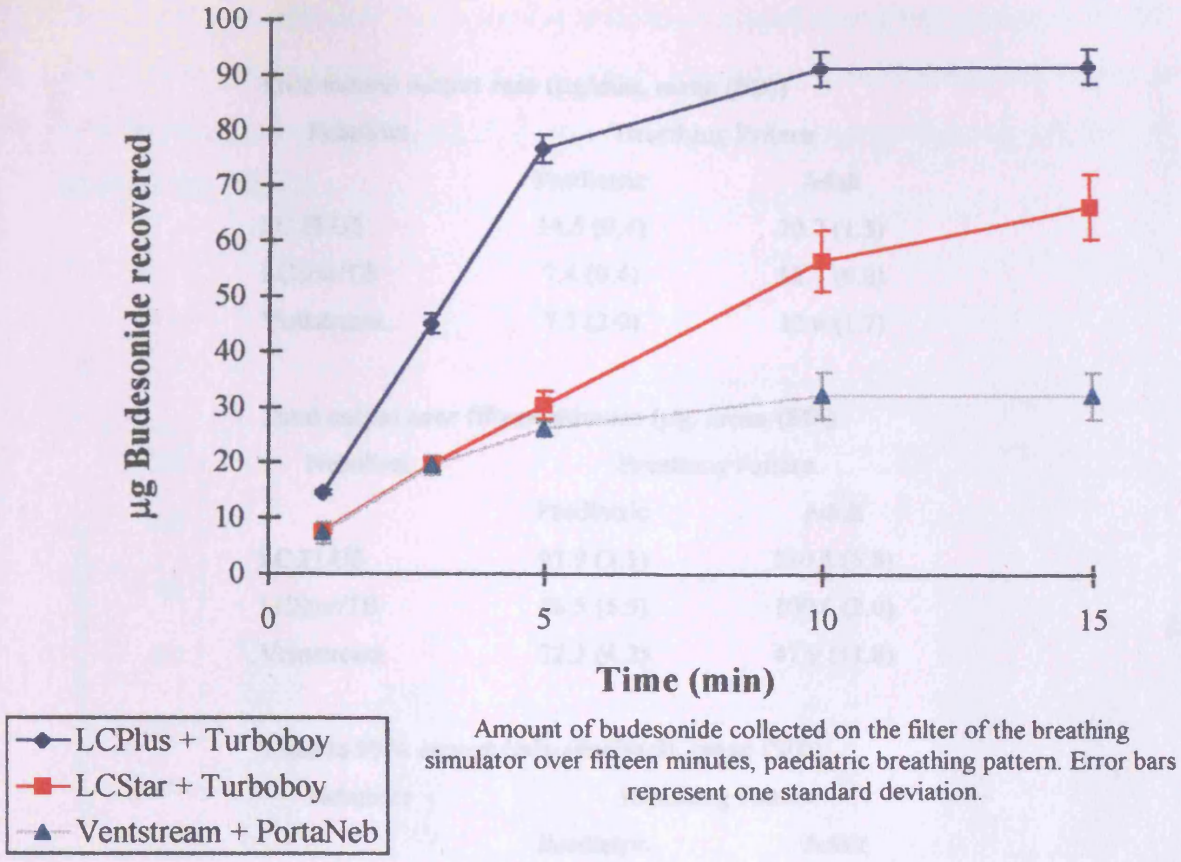
	MMAD μm	GSD –	Drug <6.1μm μg	% <6.1μm %
LC Plus/Turboboy	4.1 (3.9-4.2)	2.1 (2.0-2.2)	95.0 (87.0-103.1)	69.8 (67.6-72.0)
LC Star/Turboboy	3.8 (3.5-4.0)	1.9 (1.8-1.9)	118.1 (113.1-123.1)	77.2 (71.3-83.1)
Ventstream/Portaneb	3.1 (2.8-3.3)	2.0 (1.9-2.1)	101.8 (91.8-111.8)	87.0 (85.3-88.6)

The mass median aerodynamic diameter, the geometric standard deviation, the mass of drug and the percentage nebuliser output collected on stages four and the MSLI filter, representing drug particles smaller than 6.1μg, are given.

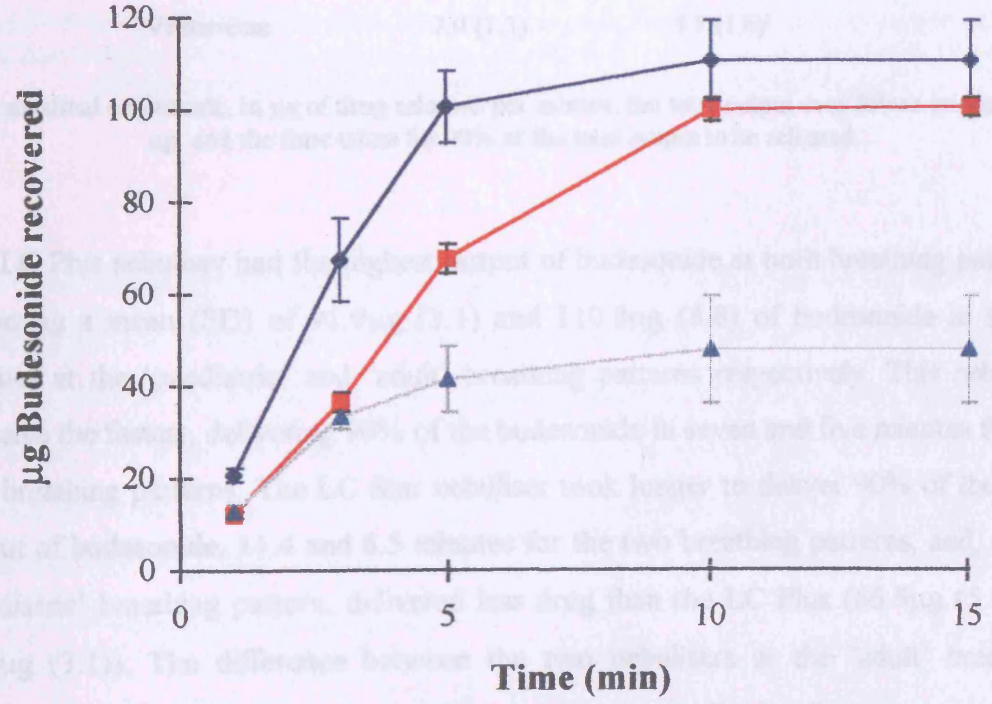
Under the constant flow used by the MSLI, the LC Star delivers the most drug, and deposits the most drug on stages 4 and filter. The LC Plus nebuliser has the largest particle size output, MMAD 4.1μm. The Ventstream, with the smallest particle size output (MMAD 3.1μm), delivers a smaller mass of budesonide to stage 4 and filter than the LC Star, but a higher percentage, delivering less drug in large particles to the upper stages of the impinger.

Breathing simulation

All nebulisers had a constant initial output of drug, which declined after a variable time (Figures). The maximal output rate, in μg of drug released per minute, the total output over fifteen minutes, in μg, and the time taken for 90% of the total output to be released are given in the Table.



Amount of budesonide collected on the filter of the breathing simulator over fifteen minutes, paediatric breathing pattern. Error bars represent one standard deviation.



Amount of budesonide collected on the filter of the breathing simulator over fifteen minutes, adult breathing pattern. Error bars represent one standard deviation.

First minute output rate (μg/min, mean (SD))

Nebuliser	Breathing Pattern	
	Paediatric	Adult
LC PLUS	14.5 (0.4)	20.7 (1.5)
LCStar/TB	7.4 (0.4)	12.1 (0.8)
Ventstream	7.3 (2.0)	12.6 (1.7)

Total output over fifteen minutes (μg, mean (SD))

Nebuliser	Breathing Pattern	
	Paediatric	Adult
LC PLUS	91.9 (3.1)	110.8 (8.8)
LCStar/TB	66.5 (5.9)	100.8 (2.0)
Ventstream	32.3 (4.2)	47.9 (11.8)

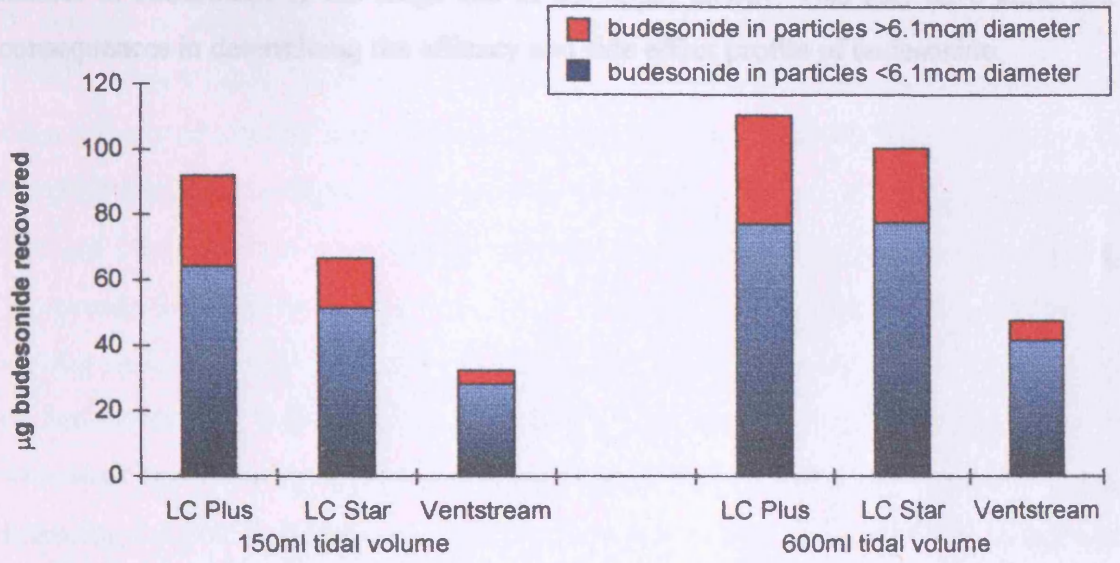
Time to 90% output (min (decimal), mean (SD))

Nebuliser	Breathing Pattern	
	Paediatric	Adult
LC PLUS	7.03 (0.2)	4.9 (0.1)
LCStar/TB	11.4 (0.9)	8.5 (0.1)
Ventstream	7.0 (1.3)	5.7 (1.6)

The maximal output rate, in μg of drug released per minute, the total output over fifteen minutes, in μg, and the time taken for 90% of the total output to be released.

The LC Plus nebuliser had the highest output of budesonide at both breathing patterns, delivering a mean (SD) of 91.9μg (3.1) and 110.8μg (8.8) of budesonide in fifteen minutes at the 'paediatric' and 'adult' breathing patterns respectively. This nebuliser was also the fastest, delivering 90% of the budesonide in seven and five minutes for the two breathing patterns. The LC Star nebuliser took longer to deliver 90% of the total output of budesonide, 11.4 and 8.5 minutes for the two breathing patterns, and, at the 'paediatric' breathing pattern, delivered less drug than the LC Plus (66.5μg (5.9) vs. 91.9μg (3.1)). The difference between the two nebulisers at the 'adult' breathing pattern (100.8μg (2.0) vs. 110.8μg (8.8)) was not statistically significant.

The Ventstream nebuliser had a similar maximum output rate of budesonide to the LC Star, but stopped releasing drug after a much shorter time, so that the total output at both breathing patterns (32.3μg (4.2) and 47.9μg (11.8) respectively) was only half as much as the LC Star.



Calculated amount of budesonide collected on the filter of the breathing simulator in particles smaller that 6.1μm and larger than 6.1μm, assuming that the particle size output of the nebulisers is the same as that measured by the MSLI, and does not change during the breathing cycle.

The Figure shows the calculated amount of budesonide collected on the filter of the breathing simulator in particles smaller and larger than 6.1μm aerodynamic diameter, assuming that the particle size output of the nebulisers is the same as that measured by the MSLI, and does not change during the breathing cycle. The LC Star nebuliser delivers the same amount of budesonide in small particles as the LC Plus, but less in larger particles that may impact in the upper airway. The Ventstream delivers less drug in both small and large particles.

Summary

This study has identified differences between the nebulisers that would not have been apparent with current standards for nebuliser assessment. Incorporation of breathing simulation in the study imitates patient use and allows effective nebulisation times to be predicted. The results also suggest that the nebulisers studied would deliver different masses of budesonide to the lungs and to the upper airway. This may have important consequences in determining the efficacy and side effect profile of budesonide.

Discussion on Chapter 6.1 - Delay between MDI actuation and sampling

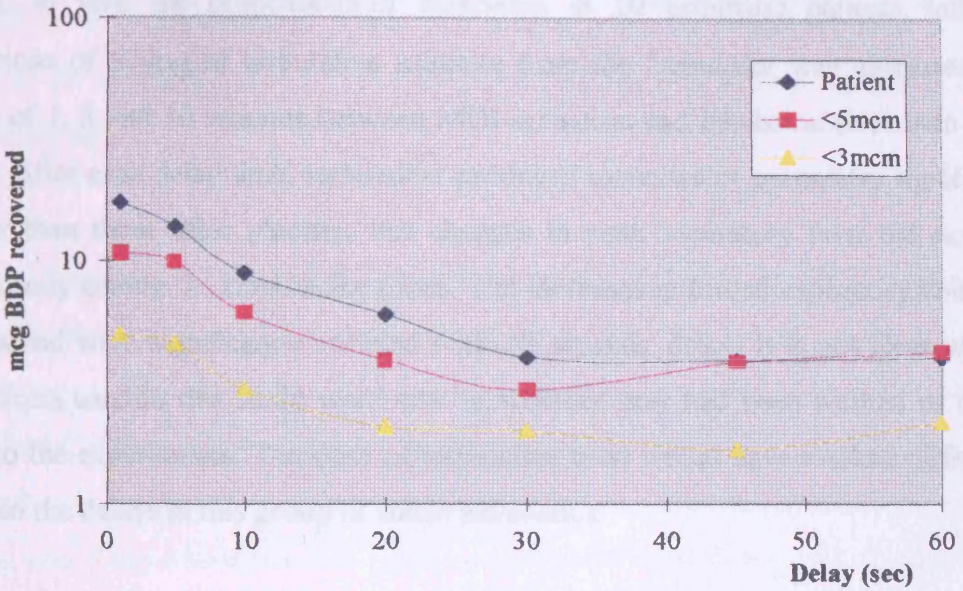
In Chapter 6.1, it was shown that the amount of drug recovered in small particles from different spacers decreased with increasing delay between metered dose inhaler actuation and sampling. The magnitude of this reduction with delay was dependent upon the drug and spacer being used, and even, in one case, the condition of the spacer - old or new.

A number of other authors have also investigated this phenomenon. O'Callaghan (1993), using a similar methodology to that described in this thesis, investigated the in vitro delivery of sodium cromoglycate from the Fisonair. Delay between actuation and sampling significantly reduced the recovery of sodium cromoglycate from the Fisonair. The half life of sodium cromoglycate particles smaller than 5μm was 11 seconds, and 16 seconds for particles smaller than 3μm. The effect was almost totally abolished by coating the spacer with an anti-static lining. The same author investigated the effects on beclomethasone delivery of delay between MDI actuation and sampling from the Volumatic spacer, using a similar methodology to that described in Chapter 6.1, but extending the period of delay up to 60 seconds, and testing MDIs of 50μg, 100μg and 250μg strengths (O'Callaghan *et al.*, 1994). The results are summarised in the table, which gives the mass of drug recovered in particles smaller than 5μm (presented as μg per actuation) from the different MDIs with up to 20 seconds delay, and the results from Chapter 6.1 for comparison.

	O'Callaghan Data			Chapter 6.1
Delay (sec)	**50μg**	**100μg**	**250μg**	**100μg**
1	10.8	21.7	45.6	17.3
5	9.9	14.9	41.6	13.4
10	6.1	10.0	40.4	12.9
20	3.9	5.8	31.2	8.9

The plot of drug recovery against time for O'Callaghan's data appears to show two phases, particularly for the 50μg MDI, with an initial rapid reduction in drug recovery over the first twenty seconds, then an almost constant recovery over the next forty

seconds (Figure). While it may be that the sensitivity of the assay may be insufficient to discriminate between small differences in drug recovery, it is interesting to speculate that these results represent two groups of particles, with one, perhaps charged, group being rapidly attracted to the inner surface of the spacer, and the other, perhaps uncharged group of particles remaining suspended for longer.



Recovery of beclomethasone dipropionate from 50µg per actuation MDI after delay between MDI actuation and sampling. 'Patient' refers to recovery from MSLI, '<5mcm' and '<3mcm' refers to recovery in particles smaller than 3µm and 5µm aerodynamic diameter respectively. Data from O'Callaghan *et al.*, 1994

The Perth Medical Aerosol Research Group investigated the effect of delay between MDI actuation and sampling on salbutamol recovery from the Babyhaler in vitro, comparing artificially charged spacers with those that had been treated to reduce their surface charge (Wildhaber *et al.*, 1996). Over twenty seconds, drug recovery (in particles smaller than 6.8µm MMAD) fell by 74% from 32.9µg to 8.6µg per 100µg actuation from the highly charged spacer. This effect was not seen in the 'static reduced' Babyhaler, where the recovery was 56.3µg per 100µg actuation with one second delay, and 53.7µg per 100µg actuation with twenty seconds delay.

These in vitro observations were confirmed by a pharmacokinetic study from Dundee (Clark and Lipworth, 1996). Ten healthy volunteers inhaled 1,200µg of salbutamol from MDI and Volumatic spacer either immediately or twenty seconds after MDI

actuation. Peak plasma salbutamol levels were higher following immediate inhalation, with a two fold greater lung bioavailability. The spacers used in this study were 'washed in warm water and left to drip dry', which was thought to reduce spacer static charge, although this was not measured. Thus, in contrast to in vitro studies, washing the spacer did not abolish the effect of delay on drug delivery.

Finally, in vivo the bronchodilator responses in 10 asthmatic patients following inhalations of 500μg of terbutaline sulphate from the Nebuhaler was measured after delays of 1, 5 and 30 seconds between MDI actuation and inhalation (Newman *et al.*, 1988). After each delay time, terbutaline produced increases in spirometry significantly greater than those after placebo, but changes in peak expiratory flow did not vary significantly among the three delay times. The increases in forced expiratory volume in one second were significantly reduced with 30 seconds' delay. It is not clear whether the spacers used in this study were new or whether they had been washed or treated prior to the experiments. The dose of terbutaline used would have masked differences between the delays in this group of stable asthmatics.

From this information it may be concluded that delay between MDI actuation and inhalation from a spacer device reduces delivery of medications from spacers. The rate of this reduction is dependent on the drug and spacer studied, and is at least in part related to spacer static charge. The effect may not be significant for stable patients delivering inhaled β_2 agonists, but is likely to be so for patients with acute severe asthma, and for inhaled steroids. When administering medications from spacer devices, inhalation should take place as soon as possible after MDI actuation.

Discussion on Chapter 6.2 - Multiple actuations of the MDI into the Spacer

In Chapter 6.2, it was shown that the amount of drug recovered (per MDI actuation) in small particles from different spacers decreased when multiple actuations of the MDI were placed in the spacer prior to inhalation. In this circumstance, dose delivered is not the same as dose administered. This is important where the dose delivered needs to be high, as in the treatment of an exacerbation of asthma, and where the dose delivered needs to be precisely known, for instance in comparative studies of the effects of different drugs.

Many of the studies described in the discussion of delay between MDI actuation and sampling also investigated the effect of multiple MDI actuations on drug delivery. O'Callaghan (1993) demonstrated a reduction in the recovery of sodium cromoglycate from the Fisonair when multiple actuations were used. The amount of sodium cromoglycate recovered in particles smaller than 5μm aerodynamic diameter fell from 0.59mg (per 5 mg actuation) with one actuation prior to sampling, to 0.41mg with two actuations, and 0.26mg with three actuations. Mean MMAD increased with multiple actuations, supporting the theory that particle agglomeration occurs in the spacer. It should also be remembered that multiple actuations introduce a time delay between MDI actuation and sampling, which will also reduce drug recovery. O'Callaghan also investigated the effects on beclomethasone delivery of multiple actuations into the Volumatic spacer prior to sampling (O'Callaghan *et al.*, 1994). The results are summarised in the table, which gives the mass of drug (μg per actuation) recovered in particles smaller than 5μm from the different MDIs.

Number of Actuations	O'Callaghan Data		
	50μg	100μg	250μg
1	11.0	22.4	47.2
2	8.1	11.8	20.8
3	5.2	11.2	12.0
4	5.2	8.0	11.2
5	4.2	7.0	10.4
10	3.9	3.8	6.4

The Perth Medical Aerosol Research Group investigated the effect of multiple actuations into the spacer prior to sampling on salbutamol recovery from the Babyhaler in vitro, comparing artificially charged spacers with those that had been treated to reduce their surface charge (Wildhaber *et al.*, 1996). The spacer was continually attached to the MSLI, and the vacuum pump running while each of ten actuations were fired into the spacer, or with it switched on after every second or every fifth actuation. From the highly charged spacer, drug recovery (in particles smaller than 6.8μm aerodynamic diameter) fell by 50% from 32.9μg to 16.3μg per 100μg actuation with two actuations, and by 66% from 32.9μg to 11.2μg per 100μg actuation with five actuations. This effect was reduced in the 'static reduced' Babyhaler and the metal Nebuchamber.

These in vitro observations were confirmed by a pharmacokinetic study from Dundee (Clark and Lipworth, 1996). Ten healthy volunteers inhaled 1,200μg of salbutamol from MDI and Volumatic spacer either as single actuations prior to inhalation, or as 3 times four actuations prior to inhalation. Peak plasma salbutamol levels were higher following single actuations, with a two fold greater lung bioavailability (C_{max} falling from 5.11ng/ml to 2.75ng/ml).

The effect on aerosol deposition from a MDI and Nebuhaler was also assessed by means of an in vivo radiotracer technique (Newman *et al.*, 1984). Nine patients with obstructive lung disease took part in the study. The pattern of deposition associated with inhalation of single actuations of aerosol was compared with four puffs actuated in rapid succession and then inhaled. With single actuations 20.9 % of the dose reached the lungs. With four actuations 15.2 % reached the lungs.

Current British Thoracic Society guidelines suggest the administration of two puffs of bronchodilator into a spacer device prior to inhalation in the emergency treatment of asthma in adults, where a nebuliser is not available (British Thoracic Society, 1993). Administering each puff separately would increase the amount of drug available for inhalation by over 20%. Previous guidelines recommended inhalation after multiples of

five puffs, reducing respirable drug delivery by almost two thirds (British Thoracic Society, 1990).

If spacers are used in studies of dosimetry, conclusions on dose-equivalence and dose response may be made from an incorrect assumption of dose delivered. In a recent study, the dose equivalence of salmeterol and salbutamol in patients with asthma was determined (Smyth *et al.*, 1993). Salbutamol was delivered by multiples of five actuations into a Volumatic spacer prior to inhalation, and salmeterol by metered dose inhaler without a spacer. It was estimated that 50μg of salmeterol was equivalent to up to 500μg of salbutamol. Our study suggests that administering 500μg of salbutamol in the way described only delivers the same amount of respirable drug as two separate 100μg actuations. Other clinical studies have used multiple actuations into the spacer (Campbell *et al.*, 1995; Noble *et al.*, 1992), without considering the effect of this on the drug dose delivered.

Multiple actuations of the MDI may decrease the amount of respirable drug recovered because of agglomeration of particles increasing their size, displacement of aerosol out of the spacer or onto the spacer walls, or the electrostatic attraction of particles to the spacer and each other. As different drug formulations and spacer devices have different physical and electrostatic properties, findings of one study may not be applicable to other drugs and devices. However, in general, it may be concluded that multiple actuations of the MDI into the spacer device prior to inhalation reduces drug delivery, and where possible, drug should be administered by repeated single actuations of the metered dose inhaler into the spacer, each followed by inhalation;

Discussion on Chapter 6.3 - The use of different spacer devices

The data described in chapter 6.3 demonstrates large variations in the amount of drug delivered from different spacer devices, and variations in the relative efficacy of a spacer to deliver different drugs. The Aerochamber, for instance, delivers only 36% of the fine particle dose of sodium cromoglycate obtained when the MDI is used without a spacer, but 78% of the equivalent dose from the budesonide MDI. Both the Aerosol Cloud Enhancer and the Dynahaler delivered very small amounts of medication (less than 33% of the MDI fine particle dose). It is clear from these results that experiments with one spacer device or drug cannot be extrapolated to others.

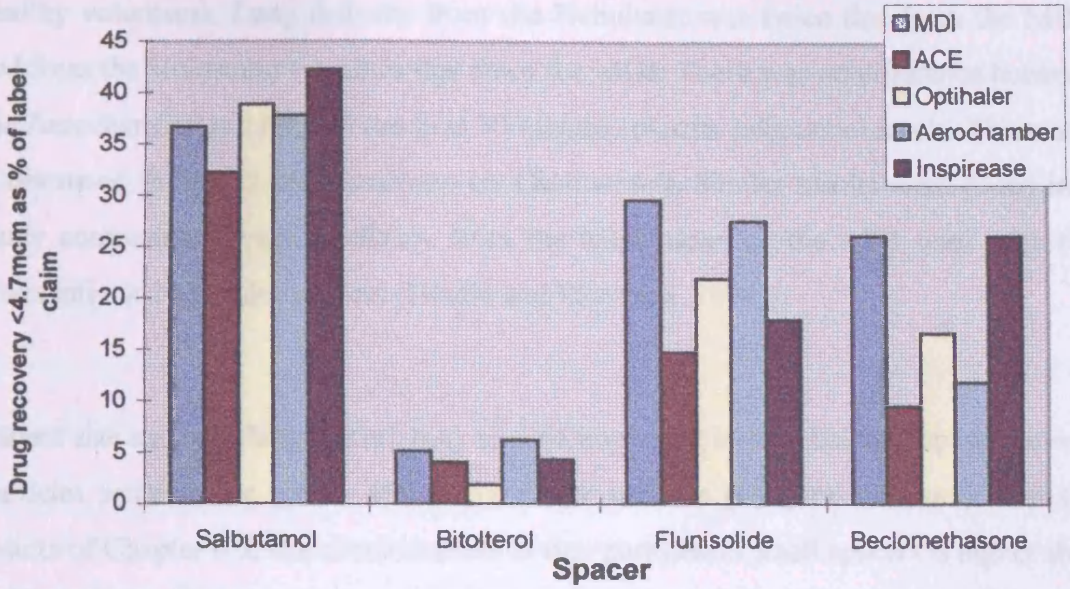
Others have examined the effect of different size and shaped spacer devices on drug delivery in vitro, but have not investigated the particle size distribution of the spacer output, one of the main determinants of the site of drug deposition. Moren (1978) measured oral and spacer deposition of terbutaline using spacers of different length, diameter and shape, showing that increasing the spacer length from 5 to 10cm decreased oral deposition of drug, but did not affect total drug recovery or, by inference, drug delivery to the patient. Maximum drug delivery was achieved using a pear shaped spacer of 25cm length.

Kim et al (Kim *et al.*, 1987) compared the in vitro delivery of a variety of inhaled steroids and bronchodilators used with the Nebuhaler, Aerochamber and Inspirease. They found that the large volume Nebuhaler substantially increased the aerosol delivery to the lung of their model (by up to 38% compared to the MDI), but that the smaller Aerochamber and Inspirease did not. Delivery of drug from the spacers was estimated by weighing filters placed at the spacer outlet, and deposition in the oropharyngeal part of the model estimated by subtraction. The methodology of the study was subsequently criticised (Gonda, 1988), as delivery of surfactants and other MDI excipients was also estimated by the method, rather than 'drug delivery' itself. Kim (1987) also suggested that there was increased delivery to the model lung when steroid suspensions were compared with bronchodilator solutions delivered through the smaller spacers, similar to our finding of less difference between spacers when used with the budesonide MDI.

Holzner and Muller (1994) studied the delivery of two formulations of sodium cromoglycate MDI from 9 spacers. Four were non valved spacers - the Aru Spacer, Ankerpharm, Rudolstadt, Germany; the Inhacort Spacer (two types) Boehringer Ingleheim, Ingleheim, Germany; and the Viarox Spacer, Byk Gulden, Konstanz, Germany. The others were valved spacers - the Nebuhaler; the Rondo Spacer; the Fisonair; the Beclomet Spacer, Orion Pharmaceuticals, Finland; and the Volumatic Spacer. Spacers were assessed continually attached with the impinger vacuum pump turned on throughout. For both MDIs, all spacers had similar stage 2 recoveries of 25-30% (Aerosol A) and 20-40% (Aerosol B), except for the Volumatic and Beclomet, which had inexplicably low stage 2 recoveries of 4% (Aerosol A) and 7-8% (Aerosol B). The authors attributed this to the design of the spacer valve, but the discrepancy between their results with the Volumatic and other studies makes it difficult to interpret their work.

Another large study (Ahrens *et al.*, 1995) measured the output of salbutamol (Proventil), bitolterol (Tornalate), flunisolide (Aerobid) and beclomethasone (Beclovent) from four spacers; the Optihaler, the Aerochamber, the Inspirease, and the ACE spacer. Drug output and particle size was measured using the Andersen cascade impactor. The Optihaler was tested continually attached to the impactor, the other spacers were attached one second after MDI actuation, and held in place for eight seconds. In the case of the Inspirease, the spacer was removed from the impactor when 'approximately three quarters empty' (just over one second at the Andersen flow rate of 28.3L/min), re expanded and attached again. To provide sufficient drug for the drug assay, ten individual actuations were administered to the Andersen before analysis, and thirty in the case of beclomethasone, raising the possibility of drug re entrainment in the impactor. No estimate of the variability of the experimental method is given in the paper, and spacer deposition of drug was not measured, so the possibility that the MDI did not deliver the prescribed mass of drug, or that drug losses occurred, cannot be excluded. The amount of drug recovered in particles smaller than 4.7μm, expressed as a percentage of the MDI label claim, is given in the figure.

be excluded. The amount of drug recovered in particles smaller than 4.7μm, expressed as a percentage of the MDI label claim, is given in the figure.



Data from (Ahrens *et al.*, 1995)

The results for salbutamol from this study and from Chapter 6.3 are given in the table for comparison:

	Mass of drug in particles smaller than 4.7μm (Ahrens *et al*), or 5μm (Chapter 6.3), as % of MDI label claim.	
Spacer	**Ahrens *et al***	**Chapter 6.3**
MDI	37	46.3 (36.3-56.3)
Aerochamber	38	20.0 (15.6-24.5)
Inspirease	42	25.6 (21.5-29.7)
Optihaler (Dynahaler)	39	3.1

There is good agreement between the MDI recoveries, where the experimental methods are the same, and the differences between recoveries of drug when the spacer is used may be attributable to differences in the sampling method, particularly with the Dynahaler/Optihaler. The rank order of the other two spacers tested in both studies is, however, the same, and Ahrens work supports the conclusion that MDIs should not be used interchangeably with spacers just because the adapter fits.

Lipworth and Clark (1998) investigated the lung absorption profile of CFC free salbutamol, Airomir, from the Aerochamber, Volumatic spacer and Nebuhaler in ten healthy volunteers. Lung delivery from the Nebuhaler was twice that from the MDI, and from the Volumatic 1.5 times that from the MDI. There was no difference between the Aerochamber and MDI in the first 30 minutes plasma salbutamol levels. This study is discussed further in the discussion on Chapter 6.9. Similar results were found in a study comparing Ventolin delivery from the MDI alone or the MDI used with the Volumatic or Nebuhaler spacers (Hindle and Chrystyn, 1994).

Patient size and breathing pattern may also be important in the choice of spacer device. Particles settle in the spacer after only a few seconds (Chapter 6.1) and, from the results of Chapter 6.3, the concentration of fine particles in small spacers is higher than in larger ones. This may be important in small children, for whom a few small breaths from concentrated aerosol in a small spacer may deliver more drug than would be obtained from a large spacer. This is supported by an in vitro study evaluating the total dose of sodium cromoglycate aerosol obtained at different tidal volumes through spacer devices (Everard *et al.*, 1992) which showed that the Aerochamber delivered more drug than the Nebuhaler at tidal volumes below 50ml, but that this was reversed at a tidal volume of 150ml.

In radiolabelled and clinical studies of adults and older children, large cone spacers are more effective than the MDI used alone (Levison *et al.*, 1985; Cushley *et al.*, 1983; Newman *et al.*, 1984), whereas small volume spacers are only as effective as the optimally used MDI (Dolovich *et al.*, 1983; Gurwitz *et al.*, 1983). In a group of adult asthmatics, the Inspirease provided greater bronchodilatation than the MDI alone (Tobin *et al.*, 1982), measured by respiratory impedance plethysmography, or than the Aerochamber (Crimi *et al.*, 1987), measured by spirometry. The differences between the devices were small, but were greater in those who had poor MDI technique. In 13 children with asthma, aged 2-5 years old, the Inspirease and the Aerochamber were tested in a double-blind crossover trial with metaproterenol (Konig *et al.*, 1988). Respiratory resistance was measured by the forced oscillation method. Thirty minutes after metaproterenol administration there was a significant decrease in resistance with

both spacers, while no significant change occurred after placebo administration. There was no difference in degree of bronchodilation between the two spacers.. Differences in clinical response to bronchodilators delivered via different spacer devices have been described in some studies (Chapman and Crompton, 1990; Dhand and Sethi, 1990; Crimi *et al.*, 1987; Lee and Evans, 1987), but not all (Lulling *et al.*, 1983; Fuller, 1986), perhaps because the dose of drug delivered tends to fall on the flat part of the dose response curve (Sackner and Kim, 1985). The clinical relevance of small differences in bronchodilation with different spacers is not clear.

Matthys (1990) has reported lung deposition of various drugs from different spacers, although exact details of the experimental method are not given.

Spacer & drug	Deposition at different sites (as % of total deposition (SD)) in 5 normal subjects		
	Spacer	Oropharynx	Lungs
Nebuhaler & Terbutaline	58.5 (3.5)	8.6 (3.7)	32.6 (5.7)
Volumatic & Salbutamol	69.3 (5.3)	2.6 (1.1)	28.3 (4.9)
Aerochamber & Fenoterol	78.0 (7.2)	1.6 (0.9)	20.5 (6.5)
Aerochamber & Salbutamol	86.3 (3.9)	2.1 (0.8)	11.6 (3.3)
Aerochamber & Terbutaline	92.1 (2.6)	1.3 (0.5)	6.6 (2.1)

All the spacers tested in Chapter 6.3 reduced the total amount of drug delivered from the spacer compared to the MDI alone. This is one advantage of spacers, as larger drug particles are retained in the spacer, rather than being deposited in the oropharynx, where they may contribute to drug related side effects. Both the small and large volume spacers reduce oropharyngeal deposition and side effects of inhaled steroid therapy (Selroos and Halme, 1991). There is little clinical data comparing different spacers in the delivery of inhaled steroids.

Both the Dynahaler and Aerosol Cloud Enhancer spacers gave very poor output of fine particle drug. However, these spacers are designed for adults who will be able to commence inhalation just before MDI actuation. Even when sampling is commenced before MDI actuation, mimicking the optimum clinical use, the spacers deliver small amounts of sodium cromoglycate relative to a large volume spacer or the MDI alone.

Up to 70% of adults are not able to co-ordinate the hand-breath manoeuvres of MDI use (Sackner and Kim, 1985). Our study implies that the Dynahaler and Aerosol Cloud Enhancer may not be useful for patients with poor inhaler technique.

Although some spacers are physically suitable for many different brands of MDI, and have been recommended for a range of inhalers (Hodges *et al.*, 1981), it may not be possible to use the results of an experiment with one particular MDI and spacer to predict the performance of the spacer with another drug. Data presented here suggests that differences in the delivery of budesonide from different spacers are not as great as those observed with sodium cromoglycate and salbutamol. This may be due to a number of factors, such as decreased aerosol density with budesonide (one actuation of budesonide weighs 60-70mg, compared with 140mg for sodium cromoglycate), differences in aerosol cloud morphology or interaction with the spacer walls due to static charge. The imminent phasing out of chlorofluorocarbon use in MDIs may change the aerosol cloud characteristics and necessitate a re-evaluation of MDIs and spacer devices.

The data presented in chapter 6.3 suggests that the in vitro delivery of drug in small particles varies greatly between different spacer devices, and that the efficacy of a particular spacer with one drug cannot be assumed for another drug. The factors underlying this and the design of the optimum spacer are yet to be determined, and confirmed in clinical trials, but our data suggests it is inappropriate to uncritically use any drug with any device just because the MDI adapter fits.

Discussion on Chapter 6.4 - The length of spacer devices

Many different spacer devices are currently available to aid inhalational therapy, ranging from 750ml pear shaped spacers to 60ml cylinders. There have been few comparative studies of the merits of different designs and the various factors that may affect the output of drug from spacers have not been fully investigated. Data presented in chapter 6.4 suggests that the optimum spacer volume for the delivery of budesonide is 300ml or more, but that sodium cromoglycate is best delivered from larger spacers of 1,000mls. Furthermore, where there is a delay between metered dose inhaler actuation and inhalation, a larger volume spacer may be preferable to a smaller one. These results are consistent with studies in Chapter 6.3, and the discussion of Chapter 6.3 suggesting that spacer output is determined in part by the drug being evaluated.

Aerosol particles are produced by MDIs at speeds of over 100km/hr, and rapidly decelerate once released (Dhand *et al.*, 1988). Spacers allow this deceleration to take place before the drug impacts on the patient's oropharynx. Different MDI formulations may produce aerosols of different speeds. In Chapter 6.5, a high speed video system was used to demonstrate significant differences in aerosol cloud morphology and volume between MDIs. It may be that a MDI that produces a small volume, aerosol cloud, such as budesonide, or one of a lower velocity, such as some hydrofluoroalkane containing MDIs (Chapter 6.9), will be optimally used with a smaller spacer.

Children and those with significant airways disease may breathe in much smaller volumes than the spacer volume, taking a number of breaths to clear the spacer of aerosol. The amount of drug available for inhalation from a spacer falls off markedly only a few seconds after MDI actuation (Chapter 6.2). Thus a small volume spacer, containing a higher concentration of aerosol, may be more appropriate for patients with low tidal volumes than a larger aerosol which theoretically contains more drug, but takes longer to clear. However, the half life of budesonide (in particles smaller than 5μm) was approximately six seconds for the 10cm length spacer, and fifteen seconds for the 20cm length spacer. This compares with the Nebuhaler spacer (23cm length) in

which it is thirteen seconds (Chapter 6.1). Thus the choice of spacer for patients with low tidal volumes will be a compromise between a smaller spacer, which can be cleared quickly, and a larger one, in which the aerosol will remain suspended for longer.

Discussion on Chapter 6.5 - Analysis of the Aerosol Cloud Produced by Metered Dose Inhalers

The metered dose inhaler (MDI) is a popular and convenient method of delivering medication to the lungs. However, only 10-15% of medication from each actuation of a MDI reaches the lungs (Dolovich *et al.*, 1981), the rest depositing in the patients oropharynx, reducing the effectiveness of the medication, and contributing to local and systemic side effects. The main mechanism of extrathoracic drug deposition is inertial impaction, where high velocity particles are unable to turn down into the throat, and land on the back of the pharynx instead. Reducing aerosol speed may reduce oropharyngeal deposition, and some inhalational aids are specifically designed to do this (Newman and Clarke, 1993; Gunawardena *et al.*, 1997). A major determinant of aerosol speed is the formulation used in the MDI. New propellants are currently being developed to replace chlorofluourocarbons, and these may have different aerosol characteristics from current formulations.

Data presented in Chapter 6.5 demonstrates how high speed video photography may be used to evaluate aerosol cloud morphology and speed from a MDI, and the differences between formulations. Previous workers have also used high speed photography to evaluate MDIs (Dhand *et al.*, 1988), though at a much slower frame speed of 200 frames per second.

The system described does not measure aerosol particle size, a major determinant of aerosol particle deposition, nor does it distinguish between aerosol containing drug particles and aerosol containing additives such as surfactants. The analysis of aerosol drug formulations should therefore include methods such as cascade impactors which allow the calculation of the particle size of the aerosol, as described in this thesis. High speed video analysis should be used as an adjunct to other measures of particle characteristics to compare MDIs and study their suitability for different inhalational delivery aids.

Drug particles are deposited in spacers by inertial impaction, electrostatic attraction between charged particles and the spacer wall, and gravitational settling. The aerosol produced by the Ventolin MDI was moving faster than that produced by the Airomir MDI. Others have also measured the speed of aerosols from metered dose inhalers (Dhand *et al.*, 1988), producing similar results to ours for a Ventolin MDI. Particles from the Ventolin MDI would have reached the end of the Aerochamber (11cm length) some 8msec after MDI actuation, compared with 23msec for Airomir. Thus, when they reached the end of the spacer the particles from the Ventolin MDI would have been travelling faster, and would have been bigger, as less time had elapsed for evaporation of propellants surrounding the drug. Increased inertial impaction of the Ventolin aerosol on the spacer wall may occur and be a reason for the difference in drug output observed. The Ventolin aerosol also occupied a larger volume than the Airomir, and at 60ms after MDI actuation would have completely filled the Nebuhaler (700ml volume), whereas the Airomir would have occupied just over a third of the spacer volume. Drug particles from the Ventolin aerosol would therefore have been closer to the spacer walls at any given time after MDI actuation. Mechanisms for drug deposition in spacer devices such as electrostatic attraction, diffusion and interception are all greater for particles close to the spacer walls.

Discussion on Chapter 6.6 - Generic formulations of metered dose inhalers

In Chapter 6.6, significant differences were demonstrated in the fine particle output of drug between different metered dose inhalers of salbutamol and beclomethasone when used with the Volumatic spacer device, and in the total output of salbutamol between metered dose inhalers when used alone. The results of this study may suggest that different metered dose inhalers cannot be assumed to be equivalent when used with spacer devices, even if equivalence is suggested when they are used alone.

Generic drugs may be released at the end of a protected marketing period which provides a monopoly for the innovator of a new medication (Wong and Hargreave, 1993). This period allows the innovator to make a return on the investment in time, money and expertise made to produce the original product. At the end of this period, other manufacturers may release products based on the original. These 'second entry' or 'generic' products are often substantially cheaper than the innovator product, as the manufacturers do not have to bear the development and research costs of the original product. This makes generic substitution of medications attractive to health care providers.

Generic substitutes are assumed to be equivalent to the innovator product, and for orally administered drugs, bioavailability data for the generic drug may be all that is required by regulatory authorities. This data is more difficult to obtain for inhaled medications, where the drug is intended for local, rather than systemic effect, and where blood concentrations of drug may be too low to detect reliably. In any case blood levels may not reflect drug levels within the lung, or drug efficacy. Furthermore the delivery of inhaled medications is highly dependent upon the drug delivery device used, as this thesis has demonstrated.

Because of these difficulties, generic substitutes have been licensed in the past if they have similar in vitro properties to the innovator product, and are efficacious compared with placebo. Demonstrations of efficacy when used with a particular drug delivery device have not been universally required.

Cyr (1991) and Blake (1992) have demonstrated differences in the first spray content between generic salbutamol preparations and the innovator product, Ventolin (Glaxo Wellcome). Differences in total output between different brands of metered dose inhaler have subsequently been demonstrated for other medications (LeBelle *et al.*, 1996; Cyr *et al.*, 1997). These differences have been attributed to a number of factors (Byron, 1994). Suspension formulations are liable to creaming (Chapter 4.2) and the rate of creaming is dependent upon the suspended particle size and rate of flocculation. Rapid creaming may lead to low dose emissions from MDIs. Thus inadequate micronisation, poor particle desegregation and subsequent particle re-aggregation may all contribute to variability in the emitted dose between different MDIs. Differences in MDI valves may also be important, as the salbutamol MDI innovator product uses a valve (Bespak 300) not available to the generic manufacturer (Byron, 1994). Finally differences in the MDI adapter may lead to variable adapter retention of drug between different brands (Phillips *et al.*, 1990).

In Vitro, Lee (1993) found a significant (at $p=0.05$) but small difference in salbutamol delivery to the lower chamber of a twin impinger from eleven generic inhalers and the innovator product. In vivo studies have also found small differences between the innovator and generic products. Saarelainen (1991) compared changes in spirometry following the inhalation of 200μg of salbutamol from the Ventolin and Salbuvent (Leiras, Torku, Finland) formulations, in a double blind, cross over study. 33 adult asthmatics with at least a 15% improvement in baseline FEV_1 following 200μg of salbutamol were enrolled. Over a six hour period, all lung function parameters were significantly higher following Ventolin inhalation. In a similar study (Chhabra, 1987), two brands of salbutamol MDI were given to 31 adult asthmatics. Differences were apparent in the change in specific airways conductance between the two MDIs. Parkkali (1983) studied the bronchodilator effect of Ventolin and Salbuvent following methacholine challenge. It is not entirely clear from the paper whether this was a cross over study, or a two group study. No placebo group was included in the trial. Patients inhaled increasing concentrations of methacholine until a greater than 15% fall in FEV_1 was observed. They then inhaled 200μg of salbutamol as either the Ventolin or Salbuvent formulations. Over the next twenty minutes, PEF was greater following

Ventolin inhalation (mean PEF 400 (SD124) L/min) than the Salbuvent (mean PEF 374 (SD120) L/min). The difference in the improvement of PEF between the two formulations was statistically significant. In a randomised, double blind, double dummy trial, Ruffin (1989) compared the bronchodilator effect of 200μg of salbutamol delivered from the innovator product, Ventolin, or a generic MDI manufactured by Riker Laboratories. Two generic MDIs were tested, with a 25μl and 50μl metering valve respectively. Over four hours, no differences were seen between the formulations in change in FEV_1 from baseline, although the increase following the Ventolin MDI was consistently greater than that following the Riker products, and the statistical analysis was subsequently criticised (Curran, 1989). The study had only a 75% power of detecting a 25% difference between the MDIs, and so may have missed a smaller difference, although the authors question whether a smaller difference would be clinically important. A smaller trial compared the efficacy of Ventolin and a generic salbutamol (Orion Pharmaceutica, Finland) in eight adult asthmatics (Vidgren *et al.*, 1991). No difference in maximal peak expiratory flow was seen following inhalation of 200μg of salbutamol from the MDIs in this randomised cross over trial, although the peak expiratory flow was higher following the Ventolin than the generic preparation at one and two hours.

In contrast, in 29 adults with moderately severe asthma, Williamson (1997) could not find a difference in spirometry or salbutamol MDI usage over a two week period of using (blinded) Ventolin or Salamol, a generic salbutamol MDI from Norton Health Care. These MDIs have similar in vitro properties in Chapter 6.6. Interestingly, 45% of the patients in Williamson's study felt that there was a difference (either greater or lesser efficacy) between the Ventolin used in the study and their usual Ventolin inhaler. In ten healthy volunteers, Clark (Clark *et al.*, 1996) found no difference in lung bioavailability between two generic preparations (Salamol, Baker Norton, and Salbulin, 3M Healthcare) and Ventolin, although the dosing schedule used in this study (twelve sequential actuations of the MDI) has been criticised (Chrystyn *et al.*, 1996).

The Volumatic spacer is marketed by Allen and Hanburys, and is recommended for use with their products. There has been much recent debate concerning the use of generic MDIs with the 'innovator' spacer device (Bell, 1993; Snell, 1993; Bell, 1993; Snell, 1993), as although the generic MDI adapters physically fit the spacer, this does not necessarily mean that the generic MDI will have the same output.

As shown in Chapter 6.6, most generic metered dose inhalers deliver similar amounts of salbutamol to the innovator product through the Volumatic spacer. Chege and Chrystyn (1994) measured the urinary salbutamol excretion to compare the relative lung deposition of Salamol (Baker Norton) and Ventolin via the Volumatic. Eleven healthy volunteers inhaled four separate actuations of salbutamol from one of the MDIs plus Volumatic on separate days. There was no difference in either the thirty minute urinary excretion, representing lung deposition, or the 24 hour excretion, representing total systemic absorption. These results, however, only apply to the generic product tested, in this case Salamol, and significant differences may exist with other generic inhalers, as suggested by the in vitro study of Chapter 6.6..

Based on the urinary excretion method, two studies allow computation of the relative effect of the Volumatic spacer on lung deposition of a generic and innovator salbutamol (Chege and Chrystyn, 1994; Clark *et al.*, 1996). The ratio of urinary salbutamol following 400μg of salbutamol by MDI and Volumatic, or 1200μg by MDI alone is 1:2.1 (21.3 to 45.1μg/30min) for Ventolin, and 1:2.5 (22.2 to 57.0μg/30min) for Salamol, suggesting that the Volumatic increases lung deposition of salbutamol, and that this increase is greater for Ventolin than Salamol. This analysis assumes that the dose response curve for urinary salbutamol is linear across the range described here, but suggests that different formulations of the same drug may interact differently with a spacer device.

Generic formulations of beclomethasone diproprionate were compared in an unpublished study from Dr M Gerard Lee of the Mersey Quality Control Service (Lee, 1994). Using the twin impinger, the author found comparable amounts of beclomethasone delivered to the lower impingement chamber from metered dose

inhalers of Becotide (Allen and Hanburys, the innovator product), and Beclazone (Baker Norton). Initial testing of the Filair MDI (3M Health Care) demonstrated reduced delivery to the lower impingement chamber. Lee repeated the experiments with this brand only after modifying the twin impinger inlet, and found increased delivery to the lower impingement chamber compared to the Becotide and Beclazone products with the 50μg and 100μg preparations, and equal deposition with the 250μg formulation. Mean values of five tests are given in the report, with no indication of the variability of the measurement.

For the 100μg inhaler, as tested in Chapter 6.6, Lee's results suggested slightly greater delivery to the lower impingement chamber with the Baker Norton formulation (36%) as opposed to Becotide (30%).

Beclomethasone metered dose inhalers 250μg per actuation were also tested by Lee with the Volumatic Spacer. Again, mean results of five experiments were presented, with no indication of the variability of the results, but the author suggested that there were statistically significant differences between the lower impingement chamber delivery from the Becloforte (66.1μg/250μg dose) and the Beclazone (58.4μg/250μg dose) and the Filair MDIs (32.2μg/250μg dose). The methodology for these experiments is not described in the report, and no indication is given of pre-treatment, washing or conditioning of the spacers prior to use.

Miller (1995) washed Volumatic spacers in soapy water, and rinsed them with benzalkonium chloride solution to remove any spacer static charge before measuring the total output of different beclomethasone formulations (Becloforte, Beclazone and Filair, 250μg per actuation). Two actuations were discharged into a Volumatic spacer, each actuation aspirated after a two second delay through a filter which was subsequently analysed. Significant differences were found, with the Filair formulation delivering 36% less beclomethasone and the Beclazone formulation 10% less than the innovator product. Kenyon (1995) also measured the output of beclomethasone from innovator and generic 250μg metered dose inhalers plus Volumatic in vitro, using the high performance multi stage liquid impinger. This study suggested that more drug was delivered from the Becloforte than the generic formulations, and that although the

amount of beclomethasone in particles smaller than 6.8μm, the cut off diameter of the twin impinger, was similar between the Beclazone and Filair formulations, significant differences existed in the amount of drug contained in smaller particles. The ranking of formulations in delivery of drug in small particles was Becloforte > Beclazone > Filair (Table).

HPMLI Stage	Becloforte (% on each stage)	Beclazone (% on each stage)	Filair (% on each stage)
Actuator	5.2	4.8	5.4
Spacer	63.2	70.3	69.3
Throat	1.5	1.7	1.5
Stage 1	1.1	1.0	4.9
Stage 2	2.1	2.6	2.1
Stage 3	10.1	7.6	7.3
Stage 4 + Filter	17.1	12.1	9.8
MMAD (μm)	2.4	2.9	4.6
% < 5μm	23.2	16.7	13.7
% < 3μm	18.8	13.1	9.4

The bimodal distribution of the Filair aerosol, with high stage 1 recovery, may suggest re entrainment of particles from the HPMLI throat, and the use of forty MDI actuations may have altered the measured particle size, but this study supports the conclusions of Chapter 6.6, that significant differences may exist between generic and innovator MDIs when used with the Volumatic spacer.

Discussion on Chapter 6.7 - The emptying pattern of spacers

In Chapter 6.7, it was shown that the emptying pattern of carbon dioxide was different between variously shaped spacers. In general, the cylindrical spacers appeared to empty progressively along their length, whereas there was considerable mixing of the CO_2 in the diamond or pear shaped spacers. There were also obvious losses of CO_2 from all the spacers during simulated exhalation, despite the presence of an expiratory valve.

The importance of these findings has not been investigated in this thesis. Although a number of studies compare different spacers using either simulated or actual patient breathing (Agertoft and Pedersen, 1994; Everard *et al*, 1992; Bisgaard *et al*, 1995), none have compared spacers of the same volume, materials, and valve design, but different shapes. Two studies investigating different ways of breathing from large volume spacers suggested that tidal breathing was as effective as one or two slow, steady, vital capacity breaths (Gleeson and Price, 1988; James and Masters, 1990). Inhaled volume was controlled for the two manoeuvres in the study by James and Masters, although the inspiratory volume achieved is not stated in the paper.

Everard (1992) used a mathematical model to estimate the fraction of the maximum obtainable dose delivered from a spacer as a function of the breath number and tidal volume. If the aerosol concentration in the spacer is given by the term C_0, and the tidal volume is V_t, then the mass of drug inhaled on the first breath, denoted by M_0 is:

$$M_0 = V_t \times C_0$$

(Note that this expression assumes that the spacer has no 'dead space' of aerosol free air between the patient and the holding chamber).

Subsequently a volume of clear air equal to V_t will be drawn into the spacer, and *assuming that there is complete mixing of the 'new' air and the aerosol* the concentration will fall to:

$$C_1 = C_0 \times ((V-V_t)/V) \times \exp(-\lambda T)$$

where V is the spacer volume, and the time decay of the aerosol in the spacer is given by the exponent of $(-\lambda T)$, λ being the concentration half life and T being the time between inhalations. The dose delivered on the second breath is thus:

between inhalations. The dose delivered on the second breath is thus:

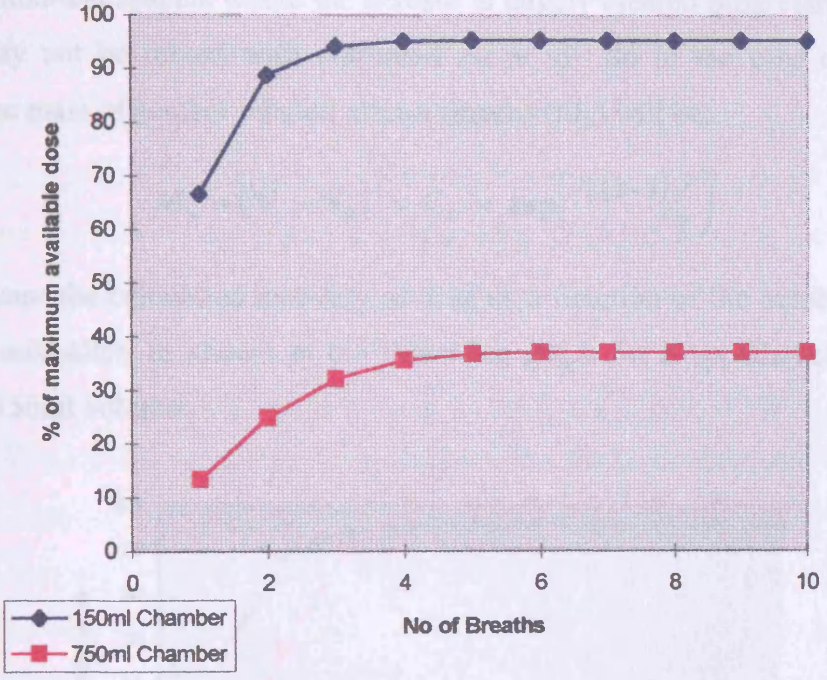
$$M_1 = V_t \times C_0 \times ((V-V_t)/V) \times \exp(-\lambda T)$$

T is related to the inverse of the breathing frequency, and is given by I - 1/γ.

Everard concluded that the dose delivered after *n* breaths would be equal to:

$$M = V_t \times C \times \sum_{i=1}^{i=n} \left(1 - \left[V_t/V\right]\right)^{i-1} \times \exp\left(-\lambda(i-1)/\gamma\right)$$

This equation was used to compute the total dose available from two different spacers as a function of the number of breaths inhaled. (Graph)



The graph compares the relative efficiency of a 150ml and 750ml chamber at a tidal volume of 100ml and respiratory frequency of 32 breathes per minute, derived from the mathematical model of Everard et al (1992). A drug half life of 15 seconds is assumed. Comparison may be made with the experimental results of chapters 6.3 and 6.10. The Nebuhaler spacer delivered 47.5μg of budesonide under a constant flow of 60l/min, and 14.9μg after five breaths with the breathing simulator at a tidal volume of 100ml and 20 breaths per minute, 31% of the total delivery. Everard's model

suggests that 33% of the dose would be delivered. In contrast with the Aerochamber (145ml) the experimental results demonstrate 22% of the total delivered with breathing simulation, whereas Everard's model predicts 95% delivery.

The results of Chapter 6.7 also suggest that this model may not be accurate, as this expression does not include terms for loss of aerosol on exhalation, nor the effect of spacer dead space. Correcting for dead space gives:

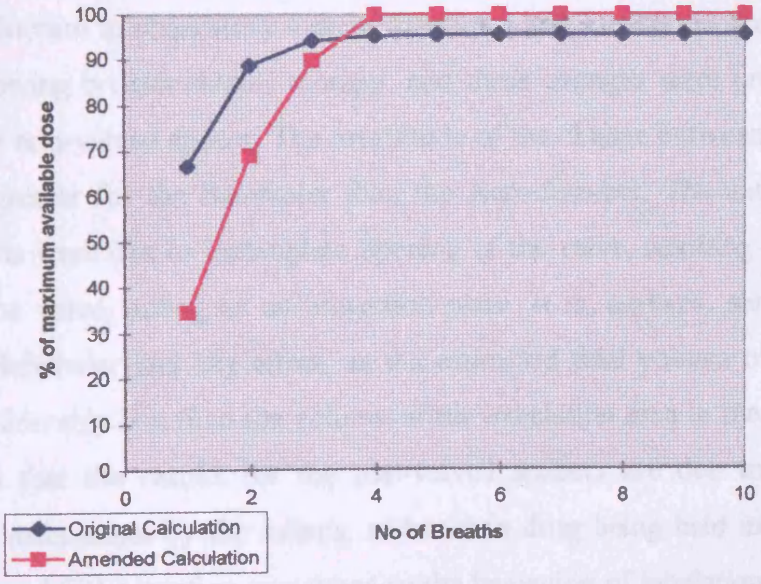
$$M = (V_t - V_d) \times C \times \sum_{i=1}^{i=n} \left(1 - \left[\left(V_t / V\right)\right]^{i-1} \times \exp\left(-\lambda(i-1)/\gamma\right)\right)$$

where V_d is the dead space of the holding chamber.

Furthermore, the aerosol will not be completely mixed by the entrained air, and in the case of cylindrical spacers where the aerosol is largely cleared progressively along its length, may not be mixed with entrained air at all. So in the case of cylindrical spacers, the mass of aerosol inhaled after *n* breaths (M_n) will be:

$$M_n = (V_t - V_d) \times C_0 \times \exp\left(-\lambda(i-1)/\gamma\right)$$

In which case the calculated recovery of drug as a function of the number of breaths inhaled would alter, as shown in the following graph for a hypothetical cylindrical spacer of 150ml volume.



with budesonide. Exhalation of air into the chamber may partly explain this.

Most holding chambers incorporate a one way valve to prevent exhalation into the chamber and displacement of aerosol during tidal breathing. In practice, valves are not totally effective, allowing exhalate into the chamber, and as shown in Chapter 6.7, the loss of aerosol from the spacer during exhalation. Also, it may be difficult for the patient to open (Cox *et al.*, 1984; Bucknall, 1984), or to close (Beasley and O'Donnel, 1985). Although the pressures required to open and close the valves are generally very low (less than 0.1 kPa), and inspiratory flow requirements are within the physiological limits for infants' normal tidal breathing, expiratory flow requirements may be higher, and the flow required to prevent rebreathing from the chamber may exceed the physiological flow limits for normal tidal breathing (Sennhauser and Sly, 1989). This is of particular importance in drug administration to infants, in whom the spacer should be positioned to keep the valve open.

Fok *et al* (1997) compared the response of preterm infants to salbutamol inhaled from an unmodified spacer, or a spacer with the valve removed. Two groups of ten infants were studied at between one a four months of age, weighing between 1.4kg and 2.8kg. Each infant was given two treatments of 200μg of salbutamol four hours apart from either the unmodified, or the valveless spacer, in random order. The first group used the Aerochamber, the second the Babyhaler. In both groups, use of the valveless spacer led to significantly increased heart rate compared to the unmodified device. All infants showed a reduction in respiratory system resistance and increase in functional residual capacity following bronchodilator therapy, and these changes were greatest following the use of the non-valved spacer. The magnitude of the change between valved and non valved was greater for the Babyhaler than the Aerochamber. The authors postulated that the results were due to incomplete opening of the valve, resulting in deposition of aerosol on the valve, acting as an impaction plate. It is, perhaps, surprising that the unmodified Babyhaler had any effect, as the estimated tidal volume of the infants (7-14ml) is considerably less than the volume of the exhalation area in the Babyhaler. It is also possible that the results for the non-valved spacers are due to the immediate inhalation of salbutamol by the infants, rather than drug being held in the spacer and then inhaled, as MDI actuation was timed to the beginning of inhalation, and the spacer

was held firmly in place by a researcher.

From the work of Fok and the results of Chapter 6.7, one may conclude that spacer valves are unnecessary for small infants, when good technique is assured by a devoted researcher, and that, even when they are used, valves do not prevent exhalation into a spacer, and the loss of aerosol from it.

Discussion on Chapter 6.8 - Spacer Static Charge

The effect of static charge on spacer devices was first described by O'Callaghan at the April 1992 meeting of the British Paediatric Association in Warwick, England. In his presentation, Dr O'Callaghan presented the results of in vitro work measuring the particle size output of sodium cromoglycate from the Fisonair spacer. Compared to the new spacer, coating the spacer with an anti-static spray, normally used on ladies undergarments, more than doubled the output of drug in particles smaller than 5μm aerodynamic diameter. Particle size measurement was undertaken using the glass multistage liquid impinger and the drug was assayed by UV spectrophotometry. Formal measurements of static charge were not made. Unfortunately, the spray used was toxic on inhalation and not suitable for human use. Searching the laboratory for a safer alternative, and noting that the spray made the inside of the spacer feel 'sticky', Dr O'Callaghan's team found a jar of honey in a laboratory refrigerator. Smearing the inside of the spacer with honey had a similar effect on the drug output as the anti-static spray, suggesting that there may be more than one way of affecting spacer static charge to improve aerosolise drug output.

This study was subsequently published in part (O'Callaghan *et al.*, 1993). The amount of sodium cromoglycate contained in particles of various size available for inhalation (per 5 mg actuation) from a 750 ml polycarbonate spacer was determined by impinger measurement and spectrophotometric assay. Lining the spacer with an anti-static spray increased the mean amount of sodium cromoglycate in particles < 5μm available for inhalation by 244%, from 0.59 to 1.44mg. When there was a 20 second interval between actuation into the spacer device and inhalation, small particle sodium cromoglycate recovered decreased by 67% (from 0.59mg to 0.2mg).

In a presentation to the Paediatric Research Society in 1993 (Barry and O'Callaghan, 1995), we described the use of an electrostatic locator to assess the electric field within different spacer devices, and the effect of washing, storage and repeated use of the spacer on the field strength. We determined the amount of Sodium Cromoglycate (SCG) delivered by spacers with different field strengths.

Measurements were made at the edge of a spacer split in the middle, 2cm above the

spacer surface. New and used spacers were assessed, and the effect of lining the spacer with from one to ten actuations of SCG MDI assessed. Results are expressed in arbitrary units of field strength.

Spacer	Nebuhaler	Fisonair	Volumatic	Fisonair with Anti-static
New spacer	1055	5200	3000	60
Repeated use	262.5	200	-335	-

Actuation of SCG into Fisonair	1 actuation	2 actuations	5 actuations	10 actuations
	300	150	120	120

For SCG 5mg metered dose inhalers, the dose recovered in particles smaller than 5μm diameter was increased by 61% using a spacer with low static charge (0.288mg (0.022) to 0.465mg (0.107)).

In a separate study, presented at the British Thoracic Society meeting in December 1994 (Barry and O'Callaghan, 1994), we measured the field strength arising from the static charge on the inside of a previously unused Volumatic spacer, and confirmed that this was more than five times that measured in one which had been used and washed ten times (2800 arbitrary units in the new spacer, 550 units in the old). The field strength of the new spacer was reduced to 90 units by wiping with an anti-static cloth.

A multistage liquid impinger was used to measure the amount of salbutamol available for inhalation from the new and used spacers, and to determine the effect of delay between metered dose inhaler actuation and sampling. The table shows the amount of salbutamol recovered in particles smaller than 5μm per 100μg actuation:

Spacer type	Field Strength	No Delay	20 Sec. Delay
NEW	2800	19.3μg	3.3μg
USED	500	54.2μg	40.8μg

The output of salbutamol from the Volumatic spacer is reduced by high static charge carried on the spacer wall. This charge may be reduced by wiping the spacer with an anti-static cloth.

In a Chapter 6.8, the mean amount of budesonide (SD) recovered from the Nebuhaler spacer (in particles smaller than 5μm, per 200μg actuation) increased from 30.5μg (8.8) to 69.3μg (17.9) with a spacer coated with a low static paint. A twenty second delay between actuation and inhalation reduced the amount recovered to 10.9μg (3.2), but no reduction was seen when using a low static spacer after the same delay.

Subsequent work with salbutamol, salmeterol, beclomethasone and fluticasone failed to show an increase in drug delivery from the Babyhaler, Aerochamber or Volumatic spacers after brief washing in detergent and rinsing with water (Barry and O'Callaghan, 1999). Static charge was not reliably measured in this study, but where it was determined, there appeared to be only a weak relationship between charge and drug delivery. Although small numbers preclude a proper analysis, this relationship appeared to be drug specific, as it was strongest with salbutamol, and not detected with beclomethasone. Furthermore, washing the spacers actually *decreased* the recovery of salmeterol.

Others have also looked at the effect of different washing regimes on spacer static charge. Dewsbury compared the output of salbutamol from Volumatic spacers handled in one of five different ways (Dewsbury *et al.*, 1996). Compared to the untreated, new spacer, handling the spacer with latex gloves reduced the output of salbutamol in particles smaller than 6.8μm; washing the spacer in tap water did not alter the drug recovery; there was a small increase in the salbutamol output after soaking the spacer

in soapy water for 15 minutes; the largest increase was seen when a 'static eliminator' was used on the spacer during metered dose inhaler actuation. No statistical analysis was performed in this paper, and the differences are probably not statistically significant except perhaps the last two. The greatest variability in drug recovery occurred with the spacers soaked in soapy water.

Wildhaber has undertaken a number of studies on the effect of spacer static charge on drug delivery (Wildhaber *et al.*, 1996; Wildhaber *et al.*, 1996). He measured the charge on new Volumatic spacers; on patients' old spacers; on spacers rubbed to increase their charge; on spacers coated with aluminium foil or sprayed with anti static spray; and on artificially charged spacers subsequently washed in a variety of ionic and non-ionic detergents. He confirmed the previous work that new spacers are highly charged, and that this charge diminishes with patient use. He demonstrated that rinsing spacers in tap water reduced the charge, but not predictably, and that the delivery of salbutamol from rinsed spacers is the same as from highly charged new spacers. Soaking the spacers in detergent for an hour, without subsequent rinsing, reduces charge considerably and increases the delivery of salbutamol. This effect is smallest for the non-ionic detergent used, and lasts for more than one but less than two weeks. However, a questionnaire in the same study, and our own experience, suggests that parents wash their spacers by briefly immersing them in water, with or without detergent, and then rinse them. Furthermore, the effect of subsequent handling on spacers is unknown.

The same group undertook a subsequent study to assess the effect of reducing static charge on in vivo lung deposition (Pierart *et al.*, 1998). Eight healthy adults inhaled salbutamol through a new Volumatic spacer, and one that had been coated in the detergent 'Palmolive' (dilution 1/5000) and allowed to drip-dry for 12-24 hours. Quantitative measurement of electrostatic charge on each spacer was performed using an electrometer. Salbutamol was labelled with 99m technetium. Static charge was high (> 5 $\mu C / m^2$) in all new spacers, and low (< 1.2 $\mu C / m^2$) in all detergent coated spacers. The mean (range) lung deposition of radiolabelled salbutamol was 45.6 % (43.4 - 49.5) through a detergent coated spacer compared to 11.5 % (7.6 - 17.9) through a static spacer. ($p < 0.001$).

Clark and Lipworth undertook a pharmacokinetic study of the lung bioavailability of

salbutamol from the Volumatic spacer (Clark and Lipworth, 1996). Ten healthy volunteers inhaled 1200μg of salbutamol via a Volumatic spacer that had been pre-washed and allowed to drip dry. It is not clear from the paper whether detergent was used. Comparisons were made between the peak and average salbutamol levels after inhalation from these spacers or ones that had been coated in a static dissipative paint. No differences were found between the washed spacers and those coated with the anti-static paint, apart from a small increase in C_{max} with the washed spacers. As the study also confirmed a number of other in vitro findings with respect to spacer use (such as the effect of multiple actuations prior to inhalation and delay between metered dose actuation and inhalation) the authors concluded that there probably was a static effect, and that this had been abolished by washing the spacer before use.

In contrast to this work, the Perth group measured the output of salbutamol in vitro from a number of spacers both before and after coating them in detergent solution (Wildhaber *et al.*, 1996). They examined the effect of multiple actuations prior to sampling and delay between metered dose actuation and sampling, drawing comparisons between new spacers and those with reduced static charge. They found that the delivery of salbutamol in particles smaller than 6.8μm was increased by between 46.5 and 71% by the use of spacers with reduced static charge. They confirmed the reduction in drug delivery with delay and multiple actuations from new spacers, as the recovery was reduced to 26.1% of the immediate value by a twenty second delay, and to 34% by 5 actuations of the metered dose inhaler into the spacer before sampling. However, the effect of delay was abolished, and the effect of multiple actuations reduced in the low static spacers.

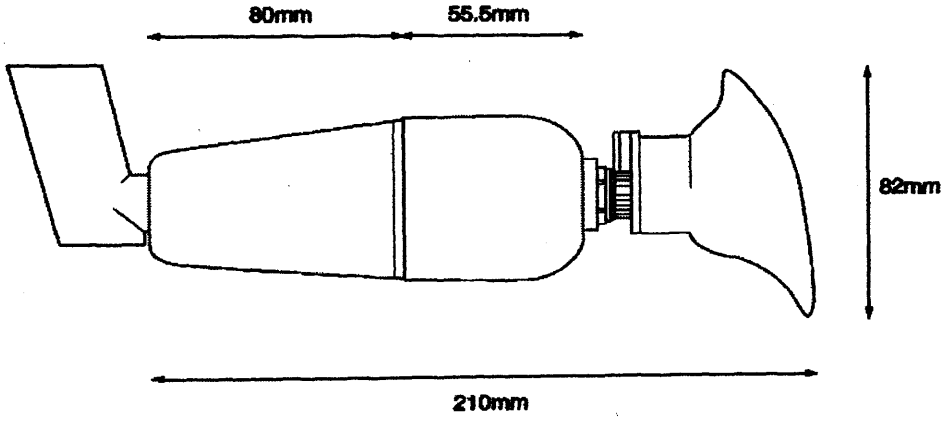
It is not clear why the authors of this paper give their results in terms of the amount of drug on stages 3 and 4, representing drug less than 6.8μm aerodynamic diameter, rather than also giving the amount of drug deposited on stage 4 only, representing drug less than 3.1μm aerodynamic diameter, or calculating the amount of drug in particles smaller than 5μm, for instance.

It should also be appreciated that the in vitro testing method used by the Perth group is poorly representative of patient use, as the spacer is attached to the impinger, and air is flowing through the device at 60l/min when the metered dose inhaler is actuated into it,

rather than, as is our practice, actuating the metered dose inhaler into the spacer, and then after a delay of approximately one second, sampling from it. Differences in the analytical procedure should be borne in mind when comparing the results of different research groups.

Thus we have evidence that reducing spacer static charge greatly improves the delivery of salbutamol and budesonide in vitro, and the lung deposition in vivo by both radiolabelled and pharmacokinetic techniques. It is clear from these studies that the fine detail of washing and handling the spacer affects the output of certain drugs. Further research is needed to provide definitive guidance to patients on the optimal washing regime for their spacer and to determine the potential for dose reduction of inhaled salbutamol, and possibly other medications.

A metal spacer has been developed to overcome problems with static charge on spacers, and is intended specifically for aerosol administration to young children. This device is considerably more expensive than the polycarbonate spacers currently available, but gives a more predictable lung deposition.



In vitro, the half life of budesonide in the metal spacer was greater than 30 seconds, compared to less than 9 seconds in a new polycarbonate spacer (Nebuhaler) (Bisgaard *et al.*, 1995). Coating the Nebuhaler spacer with benzalkonium chloride to reduce static charge (although this was not measured) increased the half life to a similar value as the metal spacer. Most interestingly, the metal spacer delivered a mean of 38% of

the nominal dose to a filter placed between the spacer and the patient, and this amount was not related to age or size. A coated Nebuhaler delivered between 19% and 42%, and this amount *was* related to age, suggesting that another factor, such as volume or shape or valve design, determines the age related delivery of drug from spacers, independent of static charge.

In a similar study (Bisgaard *et al.*, 1995), the same authors compared the amount of budesonide delivered from the metal spacer compared with three polycarbonate spacers (the Nebuhaler, Babyhaler and Aerochamber) that were primed by actuating the metered dose inhaler into the spacer fifteen times over five minutes in an attempt to reduce spacer static charge, although this was not measured. Children respired through the spacer for sixty seconds, and drug was again collected on a filter between the patient and the spacer. Expressed as a percentage of the nominal dose, a mean of 39% was delivered from the metal spacer, significantly more than from the Babyhaler (28%), Nebuhaler (21%) or the Aerochamber (19%). There was some discordance between the doses collected on the filter in vitro, under constant flow conditions, and that in vivo under tidal breathing.

It should be noted that the endpoint of these studies was delivery of drug onto a filter, which may not correlate with lung deposition, especially as in vitro and in vivo studies have suggested that the metal spacer delivers many large droplets of medication, which impact in the oropharynx.

In a scintigraphic lung deposition study, Kenyon (1998) determined the effect of spacer static charge on lung deposition of radiolabelled budesonide via the metal spacer and two polycarbonate spacers, the Volumatic and the Nebuhaler. Ten adults with mild to moderate asthma participated in a randomised, six way cross over study, receiving budesonide from a Nebuhaler, Volumatic or Nebuchamber spacer, either a new spacer, or one that had been 'primed' by actuating a placebo inhaler into the spacer twenty times, lining the inside of the spacer with surfactant. No direct measurements of spacer static charge were made. It is not clear how the authors managed to get the budesonide actuator to fit the Volumatic spacer without distorting the actuator. Priming both the Nebuhaler and Volumatic spacers increased lung deposition of radiolabel, whereas priming the metal Nebuchamber had no effect. Whole lung deposition increased with

priming from 26.7% (SD 6.2) to 37.7% (12.3) with the Nebuhaler, and from 22.1% (SD 10.1) to 32.0% (10.8) with the Volumatic. There was no change in the pattern of deposition of radiolabel within the lung. The oropharyngeal deposition recorded in the study was high - 27% of the total deposition with the metal spacer, and priming the polycarbonate spacer increased the oropharyngeal deposition to 23.7% with the Nebuhaler and 12.8% with the Volumatic. The authors concluded that the metal spacer would give more consistent drug delivery, as it was not dependent on the state of priming.

From this information, it may be concluded that drug output from polycarbonate spacers is inversely related to spacer static charge, at least for sodium cromoglycate, budesonide and salbutamol; that charge is highest on a new spacer, and falls with actuating the metered dose inhaler into the spacer; that spraying or wiping the spacer with an anti static spray or cloth, painting it with a static dissipative paint or coating it with a detergent solution are effective in reducing the charge, but that washing it in water may not be. A metal spacer reduces the variable effect of spacer charge, but the spacer design is important to optimise drug delivery, minimising extrathoracic drug deposition. Finally, the effect of spacer static charge may be drug and spacer specific.

Discussion on Chapter 6.9 - Comparison of CFC and CFC-free Salbutamol formulations.

The data presented in Chapter 6.9 demonstrates large differences in the amount of drug obtained in small particles when the conventional and CFC free formulations of salbutamol MDIs are used with different spacer devices, and in the aerosol cloud geometry and speed of the two different formulations. Airomir has been formulated to be the same as the CFC containing innovator product, Ventolin, and in clinical trials the two formulations are equally effective when used without a spacer (Dockhorn *et al.*, 1995; Kleerup *et al.*, 1996).

The is, however, no doubt that Airomir is different from the CFC containing inhalers, as discussed in Chapter 4.3. The aerosol produced by the Airomir MDI is slower than that produced by the Ventolin MDI. In the study described in Chapter 6.9, the Ventolin aerosol would have reached the end of the Aerochamber (11cm length) some 8msec after MDI actuation, compared with 23msec for Airomir. Not only would the particles from the Ventolin MDI have been travelling faster, they would have been bigger, as less time had elapsed for evaporation of propellants surrounding the drug. Thus increased inertial impaction of the Ventolin aerosol may have been a reason for the difference in drug recovery observed. The low static Nebuhaler increased the recovery from both Airomir and Ventolin MDI. Static charge of the aerosol particles was not measured, but differences in either the charge or the static dissipative properties of the aerosol constituents may also have contributed to the differences observed.

This work confirms the principle that that the efficacy of a particular spacer with one formulation cannot be assumed for another formulation, even of the same drug. Thus it is inappropriate to uncritically use any drug with any device just because the MDI adapter fits.

The Nebuhaler is not recommended for use with either Ventolin or Airomir MDIs. Ventolin is normally prescribed with the Volumatic spacer, but the Airomir MDI actuator does not fit the Volumatic, and the Nebuhaler was therefore assessed as an

example of a large volume spacer. It may be that the poor fit of the Ventolin MDI adapter affected our results. However, the work presented in Chapter 6.9 compares well with the study in Chapter 6.1 in which Ventolin was used with the Volumatic spacer; 19.9μg of salbutamol was recovered in particles smaller than five microns, a similar amount to that obtained from the Nebuhaler in this study. Chapter 6.3 gives data on recovery of Ventolin from Volumatic spacers that had been washed to reduce their static charge, obtaining similar recoveries of salbutamol to this study from the low static Nebuhaler (54.3μg vs. 68.5μg in particles smaller than five μm). Lipworth and Clark (1998) investigated the lung absorption profile of Airomir from the Aerochamber, Volumatic spacer and Nebuhaler in ten healthy volunteers. The results are summarised in the table below. The spacers were washed in warm water and drip dried prior to each use in an attempt to minimise static charge, in contrast to the new, unwashed spacers used in Chapter 6.9. Spacer static charge was not measured in the study by Lipworth (1998), but the same washing technique was used in a prior study in which washed Volumatic spacers were found to be equivalent to spacers treated with the same anti-static paint used in Chapter 6.9.

Experiment	Aerochamber	New Nebuhaler	'Low static' Nebuhaler
In Vitro (μg <5μm) (Chapter 6.9)	**40.4** (31.2-49.6)	**42.1** (36.3-47.9)	**74.8** (64.0-85.6)
In Vivo (Lipworth and Clark, 1998):			
C_{max} (ng/ml)	**5.51** (4.65-6.55)		**8.87** (7.48-10.47)
C_{ave} (ng/ml)	**4.98** (4.17-5.94)		**8.09** (6.78-9.66)

The ratio of the results from the Aerochamber to that from the 'low static' Nebuhaler is 1:1.85 for in vitro recovery of particles smaller than 5μm aerodynamic diameter; 1:1.61 for maximum salbutamol concentration (C_{max}) in vivo; and 1:1.62 for average salbutamol concentration (C_{ave}) in vivo, demonstrating good agreement between in vitro and in vivo testing.

Chapter 7.9

The data presented in chapter 6.9 suggests that the CFC free formulation of salbutamol interacts differently with different spacer devices than the CFC containing formulation. These differences may be due to the different speed and aerosol plume geometry of the CFC free formulation, or to differences in aerosol charge. This study suggests that it is incorrect to assume that data obtained with the CFC containing formulations will also apply to the CFC free one.

Discussion on Chapter 6.10 - Breathing simulation and spacers

In Chapter 6.10, it was shown that the amount of drug recovered (per MDI actuation) from different spacers increased with increasing simulated tidal volume, except for the low static metal spacer (the Nebuchamber), which had a constant output at tidal volumes above 150mls. The highest recovery was from the Nebuchamber at all tidal volumes assessed, and the lowest recoveries from the small volume Aerochamber with budesonide, and from the Babyhaler with salbutamol. These results are different from those undertaken using constant flow (Chapter 6.3), where the total dose of budesonide emitted from the Aerochamber was only slightly less than that from the Nebuhaler. This divergence of results between the two methods may represent differences in spacer charge between the devices used in the experiments, or differences in spacer design, such as emptying pattern, valve function and the 'dead space' of the exhalation area, that only become apparent in experiments utilising simulated breathing. Agertoft (1994) also found that the filter dose of budesonide was higher from the Nebuhaler than the Aerochamber, using children aged between ten and twenty-five months to generate the breathing pattern. A mean of 39.4μg (SD 13.6, range 19μg to 67μg) of budesonide per 200g actuation was deposited on the filter from the Aerochamber (19.7% of the nominal dose), compared with 53.5μg (SD 10.4, range 34μg to 88μg) from the Nebuhaler (26.7% of the nominal dose). Patient breathing parameters were recorded in Agertoft's study (Agertoft and Pedersen, 1994). Tidal volume varied from 60 to 260ml, with a mean of 192ml, and breath frequency from 16 to 44, with a mean of 26.2 breaths per minute. These parameters are similar to those used in Chapter 6.10.

Everard (1992) used a Starling ventilator to estimate the output of sodium cromoglycate from a Nebuhaler or Aerochamber. Tidal volumes of 25mls, 50mls and 150mls were compared, with respiratory rate of 32 breaths per minute and an inspiratory fraction of 40%. The output from both spacers increased with increasing tidal volume, with greater delivery from the Aerochamber at a tidal volumes of 25ml and 50ml (3.3% of the nominal dose vs. 2.9% respectively at 25ml tidal volume, 11.5% vs. 9.3% at 50ml tidal volume), and greater delivery from the Nebuhaler than the Aerochamber at a tidal volume of 150ml (15.5% of the nominal dose vs. 14.1%

respectively at 150ml tidal volume). In Chapter 6.10, the Nebuchamber and Antistatic Nebuhaler delivered the same amount of budesonide at the highest tidal volume, but significantly different amounts at lower tidal volumes. Thus experiments undertaken with older children and adults may not be extrapolated to younger children and infants.

In Chapter 6.10, in contrast to the results of Chapter 6.8, there was no difference in the recovery between the ordinary Nebuhaler and the Nebuhaler treated with antistatic paint. However, the 'ordinary Nebuhalers' used in Chapter 6.10 had been previously used in other experiments, and had been washed and rinsed a number of times. As shown in Chapter 6.8 this has a unpredictable effect on spacer static charge, but in general reduces it. Spacer static charge was not measured in these experiments, but it may be that the charge on the 'ordinary Nebuhaler' was low, minimising any difference between this device and the anti static painted one.

With salbutamol, brief washing with 0.1% cetrimide confers no advantage over rinsing with water, and drying the spacer with a paper towel after washing does not decrease the amount of budesonide released from the spacer. Given the studies that have shown a relationship between drug output and static charge, one explanation of the results is that spacer static charge was not altered by these washing and drying manoeuvres. This is supported by the data of Chapter 6.8.

Measurement of drug delivery using filter deposition is a useful non-invasive method of estimating drug delivery from different devices. The breathing simulator allows parameters such as tidal volume and breathing frequency to be controlled, overcoming the large intrasubject variability seen when patients are used to 'inhale' from the drug delivery device (Bisgaard, 1995; Devadason *et al.*, 1997; Lodrup Carlsen *et al.*, 1992), and which may make it difficult to determine if there is a relationship between parameters such as age or size and drug delivery (Devadason *et al.*, 1997). However, there should be some caution in inferring clinical effect from experiments with the breathing simulator, which produces a sine wave flow pattern and may not reflect 'real' breathing patterns (Bisgaard, 1997). Also the mass of drug deposited on filters

represents all the medication that may be 'inhaled' by the patient, and is not the same as lung deposition which will depend upon a number of factors, such as the mode of inhalation and the aerosol particle size. In this study, particle size was greatest when the budesonide MDI was used with the Nebuchamber, largely because of high deposition on the inlet of the MSLI (43% of the drug emitted from the spacer), so that only 27% of the budesonide was contained in particles smaller than 4.7μm. Using the Anderson impactor, Bisgaard found that 60% of the budesonide from the Nebuchamber was contained in small droplets, compared to 69% from the Nebuhaler and 80% from the Aerochamber (Bisgaard, 1995), the latter spacers having been primed with 15 actuations of the budesonide MDI. The differences between these figures and our study may be due to this priming, to the exact method of particle sizing, or to the relatively high flow of the MSLI (60l/min), compared to the Anderson impactor (28l/min). The greatest mean inspiratory flow in Bisgaard's group of children (Bisgaard, 1995), assuming an inspiratory fraction of 40%, was 17l/min, although the peak flow may have been much higher than this, and there is great variability in typical breathing patterns in children (Bisgaard, 1997). In a study of fifteen children aged 6.7 to 18 years using the Volumatic spacer (James and Masters, 1990), the average inspiratory flow rate was 70l/min. Also, other findings using the MSLI at 60l/min have been confirmed by pharmacokinetic studies in vivo (Clark and Lipworth, 1996), suggesting that the MSLI method does have some clinical relevance.

Lipworth (1998) compared the lung delivery of salbutamol (as the CFC free formulation Airomir) from a Volumatic spacer and a Nebuchamber in twelve healthy adult volunteers. There were no significant differences in either salbutamol concentration or extrapulmonary beta$_2$-responses between the Volumatic and Nebuchamber spacers. In a randomised crossover trial (Lipworth and Clark, 1998) the lung dose of salbutamol from the Airomir formulation was compared when administered via the Nebuhaler, Volumatic or Aerochamber spacers. Ten healthy adult volunteers were studied. Both of the large volume spacers, the Nebuhaler and the Volumatic, delivered significantly more salbutamol than the MDI alone. The Nebuhaler also produced greater deposition than either the Volumatic or the Aerochamber

spacers; Nebuhaler vs Volumatic: 1.39-fold difference (95% CI 1.09-1.76), Nebuhaler vs Aerochamber: 1.63-fold difference (95% CI 1.20-2.21). There were no significant differences between the Aerochamber and the MDI alone.

Wildhaber (1997) compared aerosol delivery to wheezy infants from two small volume spacers. Twenty wheezy infants (aged 4-12 months) inhaled salbutamol (Ventolin) from a low static Babyhaler, and from a Nebuchamber. Salbutamol was collected on a filter placed between the spacer and the patients. The mean drug deposition on the filter was 40.2% (150 micrograms) of the total actuated dose for the detergent- coated Babyhaler and 40.7% (154 micrograms) of the total actuated dose for the Nebuchamber. There was no significant difference in drug deposition on the filter between the two spacers. There was no weight dependence in drug deposition on the filter for the two spacers. This lack of weight dependence with a low static spacer was also seen in a study by Bisgaard (1995) using budesonide, and means that smaller infants will receive a larger dose of drug per kilogram body weight than older children.

These studies with simulated or actual breathing demonstrate that the effect of spacer static charge is important, changing both the delivered dose of drug and the weight dependence of the spacer output, possibly because of changes in aerosol half life within the spacer. In general terms, the low volume spacers perform less well than larger spacers, although again this difference may be eliminated (at least for the Babyhaler) by reducing spacer static charge.

Discussion on Chapter 6.12 - The assessment of Nebulisers

It has been previously shown that the gravimetric method and the osmolality method vastly overestimates drug output from nebulisers (O'Callaghan *et al.*, 1989). The results of chapter 6.12 confirm this and also show that the modified gravimetric method, incorporating osmolality measurements, is inaccurate. These methods have been widely used in previous studies, and the findings suggest that the results of such studies may have greatly overestimated nebuliser drug output.

The mass of aerosol released during nebulisation has been measured in the past by simply weighing the nebuliser before and after nebulisation (the gravimetric technique, (Smith *et al.*, 1995; Kradjan and Lakshminarayan, 1985)). This method overestimates drug output (O'Callaghan *et al.*, 1989), as evaporation of solvent occurs during nebulisation, and weight loss due to this is not taken into account.

To overcome this, other studies (Newman *et al.*, 1985; Newman *et al.*, 1986; O'Callaghan *et al.*, 1989) have measured the osmolality of the nebuliser solution before and after nebulisation, having determined that drug concentration is linearly related to osmolality over the experimental range. By multiplying the concentration of drug in the nebuliser at the end of nebulisation by the weight of solution remaining, the mass of drug released as aerosol may be calculated as:

$$\frac{M_i C_i}{D_i} - \frac{M_r C_r}{D_r}$$

Where M_i and M_r are the mass of drug solution initially placed in the nebuliser reservoir and the residual mass of drug solution after nebulisation respectively, C_i and C_r are the initial and final drug concentrations and D_i and D_r are the initial and final solution densities.

Studies using this method have measured the osmolality of an aliquot of drug solution from the nebuliser reservoir. It was hypothesised that this approach may still greatly

overestimate drug output from the nebuliser, as concentrated drug may be deposited on the nebuliser baffles and internal walls, leaving a coating of residual drug in these areas. Measurement of the osmolality of the reservoir contents would not include this drug. This hypothesis is supported by the findings in Chapter 6.12, where osmolality measurements overestimated drug output by fifty percent or more.

In this study nebulisation was stopped after five minutes. It is likely that if nebulisation had continued until visible aerosol output ceased, more drug would have been deposited on the nebuliser internal structures, and the difference between osmolality measurements and laboratory assay of drug concentration would have been even greater.

Nebuliser therapy is becoming increasingly popular as new preparations are made available. Successful therapy depends upon using a nebuliser that will deliver sufficient drug to the site of action, and many different devices are currently marketed. In order to compare different nebulisers, clinicians need to know the amount of drug released from the nebuliser, using reliable and accurate methods of measurement. The recently published British Standard 7711 part 3 for jet nebulisers uses a chemical tracer to assess nebuliser output. Sodium fluoride is added to the nebuliser solution, collected onto a filter during nebulisation, and assayed (Dennis *et al.*, 1990). For this method to be used, it must be certain that the tracer behaves in the same way as the drug to be nebulised, which may not be true for suspensions, such as nebulised steroids, or preparations of different viscosity or surface tension. This method also ignores the effect of breathing pattern on the delivered dose of drug. Others have shown that the breathing pattern of children affects drug delivery from nebulisers (Collis *et al.*, 1990), and this is supported by the results of Chapter 6.13. It is proposed that, in a laboratory situation, collection of aerosol leaving the nebuliser and assay of the amount of drug present is to be recommended as a more accurate method for determining nebuliser drug output than the gravimetric or osmolality methods. Particle size estimation and breathing replication will further enhance the applicability of output data to the clinical situation.

Discussion on Chapters 6.11, 6.13 and 6.14 - The output of Budesonide from Nebulisers

Despite recent reports of side effects from inhaled steroid therapy (Cumming *et al.*, 1997), no information is currently required by regulatory authorities on the dose of steroid actually reaching the patient from different nebulisers. As new, more efficient nebuliser designs are marketed, this information is essential to allow both therapeutic effects and side effects to be evaluated.

Others have measured the particle size and drug output with some of the nebulisers evaluated in this thesis. Leflein et al (1995) studied the output of Triamcinolone Acetonide from the LC Plus and MB5 nebulisers, driven by a Pulmo-Aide compressor, output 6.3l/min (output measurement method not specified). Particle size was determined using both a laser particle sizer and (for the MB5 nebuliser) a cascade impactor. Fill volume was 3mls. The nebulisers were run for different lengths of time to allow the output rate to be estimated. Drug output was calculated by adding a known amount of drug to the nebuliser, and subtracting the amount left in the nebuliser after nebulisation. The Pari nebuliser released 190µg of drug after one minute, and 370µg after two minutes. The MMD for the Pari nebuliser was 4.6µm, GSD 1.4, and the percentage of drug in particles between 1 and 5µm was 47%.

Elna Berg (1988) measured the output of budesonide from the MAD2 and the Pariboy nebulisers, using subtraction of drug remaining after nebulisation to estimate drug output, and a Malvern Mastersizer and a liquid impinger to measure particle size. The output of the compressor used with the Pari nebuliser was 3.3l/min. Experimental details are somewhat sketchy in the paper, and the volume fill is not stated. The nebulisation time may have been ten minutes, although this is not consistent with some of the results.

The output of budesonide from the Pariboy was 123µg/min, of which 30% was said to be in particles smaller than 5.5µm. As far as one can tell from the figures in the paper,

the particle size distribution was approximately:

Budesonide in particles >10.5μm: 40%
Budesonide in particles 5.5-10.5μm: 35%
Budesonide in particles 3.3-5.5μm: 15%
Budesonide in particles <3.3μm: 10%

The MMD given for the Pariboy nebuliser is 7.0μm measured with the Malvern, and 8.0μm measured with the impinger. The paper contains some interesting comparative data between the Mastersizer and an impinger, and the measurement of 'wet' nebuliser particles and those that have been dried by evaporation prior to measurement.

Loffert (1994) compared 15 different nebulisers, measuring total output be the gravimetric technique, and particle size by laser diffraction. Nebulisers were filled with 2.5ml of salbutamol/saline, and studied with constant flow of 20l/min. The compressor used was not given. MMD (given as Volume median diameter), % respirable (not defined) and time to 'sputtering' were:

Cirrus	6.15μm.	35.6%	8.77min.
Pari LC Jet	4.38μm.	52.3%	2.54min.
Sidestream	3.77μm.	71.9%	7.14min.

Genentech have undertaken extensive particle sizing with nebulisers and DNAse (Cipolla *et al.*, 1994, and Product information: 'Pulmozyme and aerosol delivery systems'. Aerosol Delivery Group, Genentech, San Francisco). The 'respirable fraction' (1-6μm), nebuliser efficiency (% of nominal dosage released as aerosol), and the MMAD (measured by cascade impaction) for different nebulisers were:

LC Plus/Pariboy	47.8%	51.4%	5.2μm
LC Plus/Parimaster	73.5%	29.7%	3.3μm
Sidestream/Portaneb	80.4%	50.6%	2.8μm
Sidestream/CR50	82.3%	55.3%	3.1μm
Ventstream/Portaneb	82.6%	41.9%	2.5μm
Ventstream/CR50	79.9%	48.8%	3.1μm

Medicaid have released data on the Ventstream/CR50, giving a MMD (measured on a Malvern 2600, with a nebulised solution) of 3.12μm, with only a small decrease in particle size with increasing inspiratory flow from 6-20l/min (Product information: 'Introducing the Ventstream from Medicaid'. Medicaid Ltd, Pagham, UK).

Knoch and Wunderlich presented data to Respiratory Drug Delivery IV in 1994 (Knoch and Wunderlich, 1994) looking at the output of the LC Plus measured by measuring deposition of saline onto filters, and particle size using the Malvern 2600.

DGF (l/min):	3	4	5	6
Pressure (bar):	0.55	0.9	1.35	1.75
Output (mg/min):	265	330	415	430
MMD (μm):	5.0	4.0	3.2	2.8
% <5μm:	50	62	70	74

The output of solution was also expressed as a function of inspiratory flow (driving gas flow kept constant at 5l/min):

Insp. Flow (l/min):	0	10	15	20	25	30
Output (mg/min):	140	275	365	415	430	435
MMD (μm):	3.7			3.4		2.9

Thus output (of the solution) increased with increasing inspiratory flow up to 20l/min, but was unchanged above this. Output declines at higher flows (above 40l/min). As the droplet size remains almost the same between 0 and 20l/min inspiratory flow, the suggestion is that the increased flow evaporates particles produced by primary generation, allowing more to be released from the nebuliser. At higher flows, the effective cut off of the nebuliser baffles is reduced due to impaction of droplets within the nebuliser. Knoch and Wunderlich also assessed the LC Plus and Pariboy compressor (3.5l/min DGF; MMD 4.3μm) with a breathing simulator set to Vt -500ml, *f*-15 breaths/min, Ti 0.5, volume fill 3mls, nebulisation to dryness. 31% of the nominal

dose was delivered to the inspiratory filter, 14% to the expiratory filter, and 55% remained in the nebuliser. Nebulisation time to dryness was six minutes.

With budesonide, using the same breathing simulator parameters, volume fill 2ml of 500µg/ml budesonide suspension, Pari have studied a number of nebulisers:

Nebuliser/ compressor	Time to Dryness	Filter Inspiratory	Filter Expiratory	Nebuliser
LC Plus/Boy:	5min	167µg (21%)	72µg (9%)	566µg (70%)
LC Plus/Master:	4.25min	190µg (22%)	117µg (14%)	544µg (64%)
Ventstream/ Freeway Lite:	13.3min	134µg (16%)	107µg (13%)	602µg (71%)

Nikander has also studied the output of budesonide from various nebulisers (Nikander, 1994), with a breathing simulator set to Vt -440ml, *f*-19 breaths/min, Ti 0.38, volume fill 2mls, nebulisation to dryness using a CR60 compressor with all nebulisers. MMD was measured for the Ventstream and Pari nebulisers using the Malvern Mastersizer, but it is not clear if this is the MMD of budesonide nebuliser suspension or a solution such as saline.

Nebuliser	MMD µm	µg budesonide on filter Inspiratory	µg budesonide on filter Expiratory	Ratio I:E
Aiolos	----	142	195	42:58
De Vilbiss 646	----	59	79	42:58
Hudson	----	130	204	39:61
Spira	----	125	170	42:58
Turret	----	64	96	33:67
Pari LL:	4.5	192	96	67:33
Ventstream:	3.4	139	78	64:36

Nikander and Wunderlich (1996) studied the output of budesonide from the LC Plus and LL nebulisers using different compressors, the Pariboy 37.00 (3.6l/min), the

Pariboy 38G00 (4.3l/min) and the Pari Walkboy (2.8l/min). Nebulisers were filled with 2ml (500μg/ml) of budesonide suspension, and run to dryness, attached to a breathing simulator set to Vt -440ml, *f*-19 breaths/min, Ti 0.38. Drug on the filters was analysed by HPLC. Particle size was assessed using the Malvern Mastersizer X at 20l/min simulated inspiratory flow.

Nebuliser/ compressor	Filter Inspiratory	Expiratory	MMD	DGF (l/min)
LL/37	225.4μg	69.0μg	5.7μm	3.6
LL/38	192.1μg	81.2μg	5.2μm	4.3
LC Plus/38	232.3μg	107.5μg	5.0μm	4.3
LC Plus/Walkboy	196.1μg	80.0μg	6.0μm	2.8

Thus reducing the driving gas flow increases particle size, and has a small affect on budesonide output.

Denyer and Dyche (1993) studied the Ventstream nebuliser with a tracer, with salbutamol and with budesonide. The nebuliser output at different constant inspiratory flows was first determined at a range of flows from 0-25l/min. Particle size was measured using a Malvern Mastersizer. Volume output increased with increasing inspiratory flow, up to 21l/min, and was constant above this, although it was not tested above 25l/min. The volume % smaller than 5μm was constant at around 80%.

With the tracer, the Ventstream was compared to a System 22 Acorn with a breathing simulator set to V_t -630ml, *f*-20 breaths/min, T_i 0.33, volume fill 4mls of sodium fluoride solution 1% w/v. Driving gas flow was 7l/min. Volume output to the inspiratory filter was 18% of the nominal dose with the Acorn, and 32% with the Ventstream.

With the budesonide, the Ventstream was operated with the Portaneb 50 Compressor, and attached to a breathing simulator set to V_t -440ml, *f*-26 breaths/min, (v 11.4l), T_i 0.38, volume fill 2mls of budesonide suspension (500μg/ml). 144μg of budesonide was

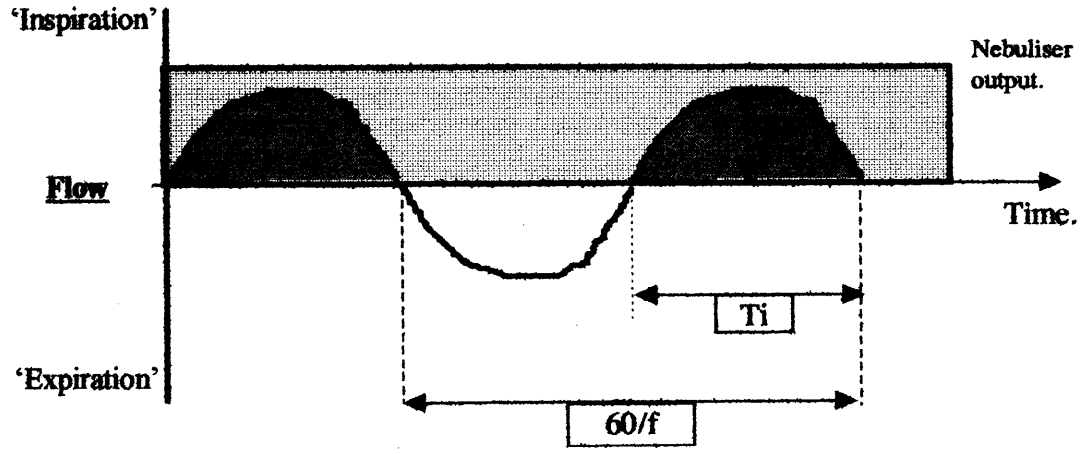
recovered from the inspiratory filter, and 81μg from the expiratory filter.

Salbutamol was subsequently studied with the Ventstream *in vivo* (Newnham and Lipworth, 1994), showing a two fold increase in the delivery of salbutamol to the lung compared with a conventional nebuliser.

Given differences in the breathing simulator settings and the assessment methods, these data are largely in agreement with the results of Chapter 6.13. One could infer, on theoretical grounds, what the effect on filter deposition of different respiratory patterns should be.

If one takes, first of all, the constant output Cirrus nebuliser. Assume that nebulisation rate is constant throughout the five minute study period.

The simplest case is where the inspiratory flow is less than the nebuliser output:



In this case, the volume of drug containing aerosol inspired is approximately equal to the mean inspiratory flow multiplied by the inspiratory fraction.

The mean flow is given by the expression 1/Ti x Vt x f, and is given below in l/min for various different values of these three parameters:

1. Ti = 0.3						2. Ti = 0.4					
Vt	**50**	**100**	**200**	**300**	**600**	**Vt**	**50**	**100**	**200**	**300**	**600**
f						**f**					
20	3.3	6.7	13.3	20	40	**20**	2.5	5	10	15	30
30	5	10	20	30	60	**30**	3.75	7.5	15	22.5	45
40	6.7	13.3	26.7	40	80	**40**	5	10	20	30	60
50	8.3	16.7	33.3	50	100	**50**	6.25	12.5	25	37.5	75

3. Ti = 0.5					
Vt	**50**	**100**	**200**	**300**	**600**
f					
20	2	4	8	12	24
30	3	6	12	18	36
40	4	8	16	24	48
50	5	10	20	30	60

Where the inspiratory flow is less than the nebuliser output, the volume of aerosol inspired (drug delivery; DD) should be proportional to the minute volume,

*(DD $\propto$ mean flow x Ti; mean flow $\propto$ 1/Ti x Vt x f. Therefore DD $\propto$ Vt * f)*

and changing the inspiratory time should not alter the drug delivery. Similarly, changing the frequency or tidal volume should only affect drug delivery if they alter minute volume.

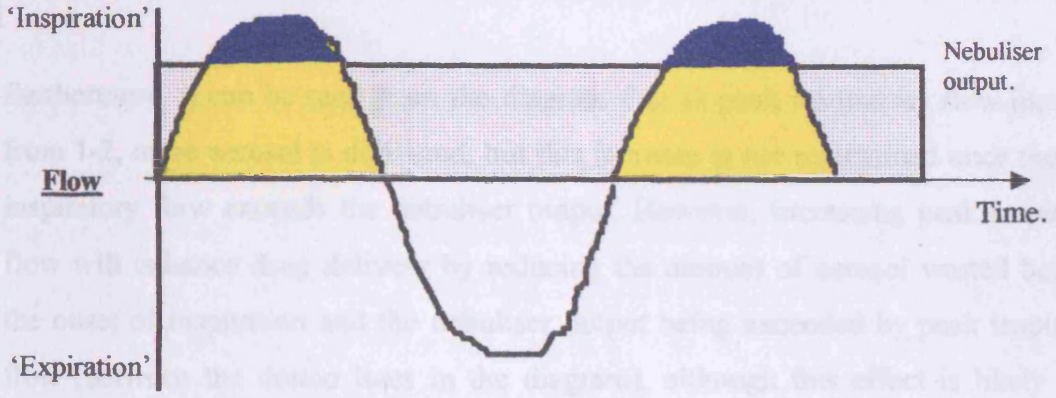
This relationship will be affected by the dead space between the nebuliser and the filter, as the first part of the inspired air (equal in volume to the dead space, DS) will not

not contain any drug. Thus

$$DD \propto (Vt - DS) * f.$$

The dead space will have a proportionally greater effect where it is large relative to the tidal volume.

The above equation also breaks down to $DD \propto (Vt * f) - (DS * f)$ suggesting that for a given minute volume, drug delivery will be enhanced at lower frequencies.



At a certain point, the peak inspiratory flow will become greater than the nebuliser output, as shown in the above diagram, and air which does not contain aerosol will be entrained.

The peak inspiratory flow, V_{max}, in litres, is given by the equation:

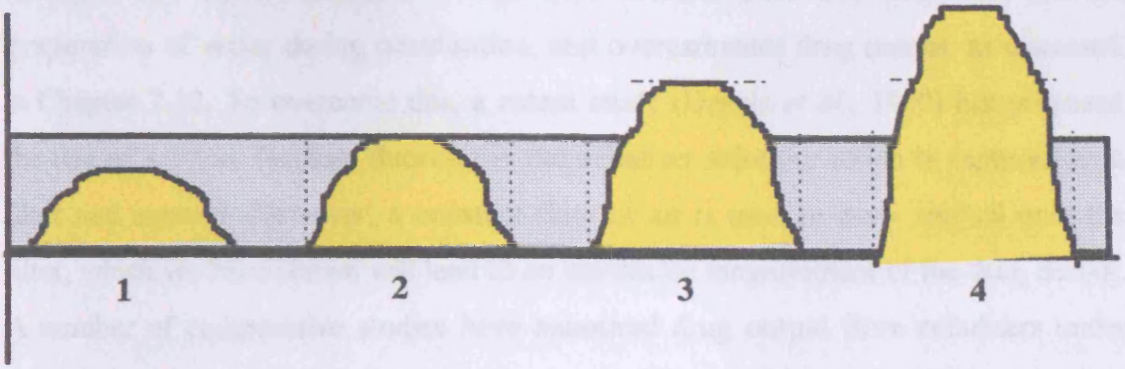
$$\Pi * (Vt / 1000) * f / (2 * Ti)$$

Mean and peak inspiratory flows for various combinations of breathing parameters are given in the accompanying tables, in l/min.

The inspiratory flow at any given point in the breathing cycle is given by the peak flow multiplied by the sine of the point of interest (0° - 180° for inspiration, 180° - 360° for expiration; see accompanying table).

Once the peak inspiratory flow is greater than the nebuliser output, the drug delivery

should be independent of minute volume, although the dead space will still affect the delivery, as described above.



Furthermore, it can be seen from the diagram that as peak inspiratory flow increases from 1-2, more aerosol is delivered, but this increase is not maintained once the peak inspiratory flow exceeds the nebuliser output. However, increasing peak inspiratory flow **will** enhance drug delivery by reducing the amount of aerosol wasted between the onset of inspiration and the nebuliser output being exceeded by peak inspiratory flow (between the dotted lines in the diagram), although this effect is likely to be small.

Thus, for a conventional nebuliser, drug delivery will be related to minute volume, up to the point where peak inspiratory flow is greater than nebuliser output. After this point, drug delivery will increase more slowly (and progressively less) as minute volume increases. Due to dead space effects, drug delivery will fall off exponentially at smaller tidal volumes, and will be inversely proportional to frequency for a given minute volume. Where the nebuliser output is greater than the inspiratory flow and nebulisation is complete before the time allotted (five minutes in the experiments above), drug delivery will be reduced compared to slower constant output devices as more drug is lost both during inspiration and expiration.

Assuming that breath enhanced nebulisers such as the Ventstream and LC+ increase the volume of aerosol released during inspiration with a commensurate increase in drug mass delivered, i.e. the drug concentration in the aerosol is constant throughout breathing, the same principles apply to these nebulisers as to the constant output

In the past, nebulisers have been tested by weighing before and after operation for a certain time, without any additional airflow to represent inhalation by the patient (Kradjan and Lakshminarayan, 1985). This method does not take into account evaporation of water during nebulisation, and overestimates drug output, as discussed in Chapter 7.12. To overcome this, a recent study (Dennis *et al.*, 1990) has proposed the use of a tracer (sodium fluoride) in the nebuliser solution, which is captured on a filter and assayed. However, a constant flow of air is used to draw aerosol onto the filter, which we have shown will lead to an inaccurate measurement of the drug output. A number of comparative studies have measured drug output from nebulisers under constant or no entraining flow (Hung *et al.*, 1994; Smith *et al.*, 1995). Our data suggests that, for some nebulisers, these methods may give very misleading results

The recently published British Standard Institutes' specification for nebulisers (British Standards Institute, 1994), which specifies the information about nebulisers that should be provided by manufacturers and the test methodologies that should be employed also ignores the importance of simulated breathing pattern. This should be taken into account when European standards are formulated.

Nebulised drug deposition in the lungs is affected by the size of drug particles produced. In Chapter 6.13, the total amount of drug released from the nebuliser and deposited on the inspiratory filter was measured, without measuring particle size, and our results cannot therefore be used to suggest drug delivery to the lungs. Aerosol particle size may be measured by inertial impaction devices, which commonly sample aerosol at a high flow rate. For instance, an Andersen Impactor samples at 28.3l/min, whereas a multistage liquid impinger samples at 60l/min. The results of Chapter 6.13 suggest that connecting a Pari LC Plus nebuliser to these two devices would give very different results. Assessing nebulisers at high flows may be detrimental, possibly because the high flow through the nebuliser encourages impaction of particles on the internal baffles. Breath enhanced, open vent nebulisers such as the LC Plus and Ventstream nebulisers (Medicaid, Pagham, UK) are designed to use the patient's inspiratory flow to increase nebuliser output (Nikander, 1994), and particle size may also change with different flows through these nebulisers. Clearly assessing these nebulisers under constant flow conditions will not reliably measure their output during

use by patients.

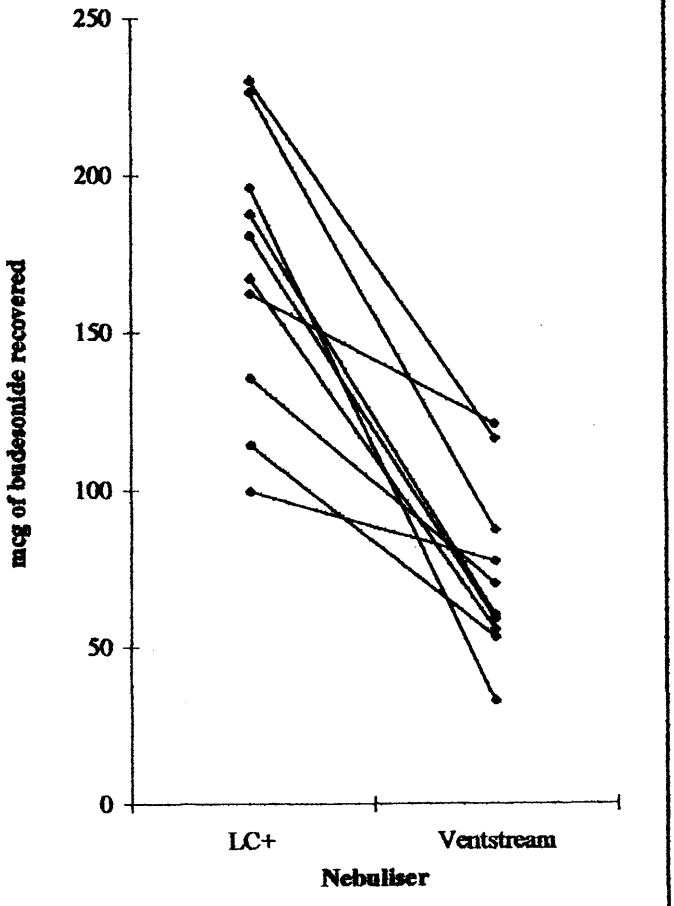
Many factors, such as driving gas flow rate and nebuliser design will determine the output of drug from a nebuliser (O'Callaghan and Barry, 1997). The actual dose reaching the lungs of the patient will also be influenced by the anatomy of the upper airway and the degree of airways obstruction, and the breathing pattern of the patient. Others have demonstrated the effect of entrained air diluting the inspired aerosol (Collis *et al.*, 1990; Everard *et al.*, 1992), so that, from conventional nebulisers, the amount of drug inhaled is independent of tidal volume once inspiratory flow is greater than the nebuliser driving gas flow. Results for the Cirrus nebuliser, where deposition on the filter is constant at tidal volumes above 125ml, support this. The output from this nebuliser when assessed with the breathing simulator was just under 40% of that recovered under constant flow, which would have been predicted from the use of an inspiratory fraction of 40% of the breathing cycle.

The Sidestream nebuliser is supplied with a mouthpiece containing an entrainment hole. During the breathing simulation experiments, aerosol was seen to escape from this hole throughout the breathing cycle. The very low deposition of drug on the inspiratory filter may be because the enhanced output of this nebuliser is greater than the inspiratory flow, so aerosol is wasted during both inspiration and expiration. The nebuliser produces small particles, as large droplets are filtered out by baffles in the nebuliser. As budesonide is a suspension of drug particles approximately 2.2-2.9μm median diameter (Jackson, 1995), many drug particles will be too large to be released from the nebuliser.

Breath enhanced open vent nebulisers increase drug output during inspiration by using the inspiratory flow to open a valve through which air is entrained (O'Callaghan and Barry, 1997). As airflow increases, particles that would have remained in the nebuliser are reduced in size by greater evaporation, and are released to the patient. This increasing output is counteracted above a certain flow by increased impaction within the nebuliser, resulting in a constant, and finally a reduced drug output (Knoch and Wunderlich, 1994).

The in vitro study in this thesis has demonstrated the importance of using simulated

breathing patterns in the assessment of nebulisers. However, a sinus wave form was used to imitate the required breathing patterns, and these may not be representative of real patient breathing. It has been suggested (Denyer and Nikander, 1996) that with derived sinus wave forms based on real patients' breathing rate and tidal volume, parameters such as peak inspiratory flow may be incorrect by more than 35%. Breathing frequencies and tidal volumes may also be different when patients have a tightly fitting mask placed over their mouth and nose, compared to their breathing pattern at rest. It is unclear how much difference this makes to measured drug output, and more sophisticated breathing simulators are awaited. When these are available, regulatory authorities and nebuliser manufacturers will need to define representative breathing patterns for comparative testing of devices.



Mass of budesonide collected on filters placed between the patient and the nebuliser. Data from each patient are connected by lines. More drug was recovered when the LC Plus nebuliser was used (t test, $p<0.001$).

Relevance to the clinical situation is implied by a parallel clinical study (Barry and O'Callaghan, 1998), in which the output of budesonide onto filters from different nebulisers was measured when they were used by patients. 10 children with asthma aged 4 to 14 years used both the Ventstream and LC Plus nebulisers in random order for five minutes. The nebulisers contained 2ml (500μg) of budesonide nebuliser suspension, and drug that would have been inhaled was collected onto a filter placed between the nebuliser and patient. The results are given in the figure.

The time taken to deliver 90% of the nebulised medication varied widely between nebulisers in Chapter 6.14. Patient compliance may fall if prolonged nebulisation is needed, and from our results, patients can be given a time after which nebulisation may be stopped, even if the chamber appears to be producing aerosol. Output rate fell with all the nebulisers studied after five minutes or less, and it may be more practical to advise patients that nebulisation be stopped after five minutes, as has been suggested previously (O'Callaghan *et al.*, 1989).

There have been few clinical studies of lung deposition of nebulised aerosols in children. Alderson et al (1974) found that lung deposition increased with age and that those with normal ventilation scans had uniform deposition of labelled aerosol, whereas those with areas of reduced ventilation had corresponding areas of reduced deposition. However, others (O'Doherty *et al.*, 1993; Mukhopadhyay *et al.*, 1994) have found no relationship between age and total lung deposition of nebulised aerosols, although deposition in infants may be lower than in older children (Chua *et al.*, 1994). These studies showed wide inter-individual variation in lung deposition, and small studies have failed to show a relationship between age (Devadason *et al.*, 1997) or clinical effect (Vikre-Jorgensen *et al.*, 1997) and filter deposition.

For this reason, there should be some caution in inferring clinical effect from measurements of drug deposited on filters. However, we remain ignorant of the dose of drug that patients are likely to receive from different delivery devices, despite the importance placed on the therapeutic ratio of clinical effectiveness to possible side effects,. The results of Chapters 6.11, 6.13 and 6.14 suggest that if patients are treated or clinical studies undertaken with nebulised budesonide, inappropriate conclusions may be drawn if the drug delivery device is not taken into account. Furthermore, the results suggest a pitfall of current nebuliser testing standards that will need to be corrected if future standards are to give meaningful results.

Ti	Vt	f	Vmean
0.3	50	20	3.3
0.3	50	30	5.0
0.3	50	40	6.7
0.3	50	50	8.3
0.3	100	20	6.7
0.3	100	30	10.0
0.3	100	40	13.3
0.3	100	50	16.7
0.3	200	20	13.3
0.3	200	30	20.0
0.3	200	40	26.7
0.3	200	50	33.3
0.3	300	20	20.0
0.3	300	30	30.0
0.3	300	40	40.0
0.3	300	50	50.0
0.3	600	20	40.0
0.3	600	30	60.0
0.3	600	40	80.0
0.3	600	50	100.0

Ti	Vt	f	Vmean
0.4	50	20	2.5
0.4	50	30	3.8
0.4	50	40	5.0
0.4	50	50	6.3
0.4	100	20	5.0
0.4	100	30	7.5
0.4	100	40	10.0
0.4	100	50	12.5
0.4	200	20	10.0
0.4	200	30	15.0
0.4	200	40	20.0
0.4	200	50	25.0
0.4	300	20	15.0
0.4	300	30	22.5
0.4	300	40	30.0
0.4	300	50	37.5
0.4	600	20	30.0
0.4	600	30	45.0
0.4	600	40	60.0
0.4	600	50	75.0

Ti	Vt	f	Vmean
0.5	50	20	2
0.5	50	30	3
0.5	50	40	4
0.5	50	50	5
0.5	100	20	4
0.5	100	30	6
0.5	100	40	8
0.5	100	50	10
0.5	200	20	8
0.5	200	30	12
0.5	200	40	16
0.5	200	50	20
0.5	300	20	12
0.5	300	30	18
0.5	300	40	24
0.5	300	50	30
0.5	600	20	24
0.5	600	30	36
0.5	600	40	48
0.5	600	50	60

Table: Mean inspiratory flow for different breathing simulator settings.

Ti	Vt	f	Vmax	Ti	Vt	f	Vmax	Ti	Vt	f	Vmax
0.3	50	20	5.2	0.4	50	20	3.9	0.5	50	20	3.1
0.3	50	30	7.9	0.4	50	30	5.9	0.5	50	30	4.7
0.3	50	40	10.5	0.4	50	40	7.9	0.5	50	40	6.3
0.3	50	50	13.1	0.4	50	50	9.8	0.5	50	50	7.9
0.3	100	20	10.5	0.4	100	20	7.9	0.5	100	20	6.3
0.3	100	30	15.7	0.4	100	30	11.8	0.5	100	30	9.4
0.3	100	40	20.9	0.4	100	40	15.7	0.5	100	40	12.6
0.3	100	50	26.2	0.4	100	50	19.6	0.5	100	50	15.7
0.3	200	20	20.9	0.4	200	20	15.7	0.5	200	20	12.6
0.3	200	30	31.4	0.4	200	30	23.6	0.5	200	30	18.8
0.3	200	40	41.9	0.4	200	40	31.4	0.5	200	40	25.1
0.3	200	50	52.4	0.4	200	50	39.3	0.5	200	50	31.4
0.3	300	20	31.4	0.4	300	20	23.6	0.5	300	20	18.8
0.3	300	30	47.1	0.4	300	30	35.3	0.5	300	30	28.3
0.3	300	40	62.8	0.4	300	40	47.1	0.5	300	40	37.7
0.3	300	50	78.5	0.4	300	50	58.9	0.5	300	50	47.1
0.3	600	20	62.8	0.4	600	20	47.1	0.5	600	20	37.7
0.3	600	30	94.2	0.4	600	30	70.7	0.5	600	30	56.5
0.3	600	40	125.7	0.4	600	40	94.2	0.5	600	40	75.4
0.3	600	50	157.1	0.4	600	50	117.8	0.5	600	50	94.2

Table: Maximum inspiratory flow for different breathing simulator settings.

			%	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100
			Angle:	9	18	27	36	45	54	63	72	81	90	99	108	117	126	135	144	153	162	171	180
			Sine:	0.16	0.31	0.45	0.59	0.71	0.81	0.89	0.95	0.99	1.00	0.99	0.95	0.89	0.81	0.71	0.59	0.45	0.31	0.16	0.00
Ti	Vt	f	**Vmax**																				
0.3	50	20	**5.24**	0.82	1.62	2.38	3.08	3.70	4.24	4.67	4.98	5.17	5.24	5.17	4.98	4.67	4.24	3.70	3.08	2.38	1.62	0.82	0.00
0.3	50	30	**7.85**	1.23	2.43	3.57	4.62	5.55	6.35	7.00	7.47	7.76	7.85	7.76	7.47	7.00	6.35	5.55	4.62	3.57	2.43	1.23	0.00
0.3	50	40	**10.47**	1.64	3.24	4.75	6.16	7.40	8.47	9.33	9.96	10.34	10.47	10.34	9.96	9.33	8.47	7.40	6.16	4.75	3.24	1.64	0.00
0.3	50	50	**13.09**	2.05	4.05	5.94	7.69	9.26	10.59	11.66	12.45	12.93	13.09	12.93	12.45	11.66	10.59	9.26	7.69	5.94	4.05	2.05	0.00
0.3	100	20	**10.47**	1.64	3.24	4.75	6.16	7.40	8.47	9.33	9.96	10.34	10.47	10.34	9.96	9.33	8.47	7.40	6.16	4.75	3.24	1.64	0.00
0.3	100	30	**15.71**	2.46	4.85	7.13	9.23	11.11	12.71	14.00	14.94	15.51	15.71	15.51	14.94	14.00	12.71	11.11	9.23	7.13	4.85	2.46	0.00
0.3	100	40	**20.94**	3.28	6.47	9.51	12.31	14.81	16.94	18.66	19.92	20.69	20.94	20.69	19.92	18.66	16.94	14.81	12.31	9.51	6.47	3.28	0.00
0.3	100	50	**26.18**	4.10	8.09	11.89	15.39	18.51	21.18	23.33	24.90	25.86	26.18	25.86	24.90	23.33	21.18	18.51	15.39	11.89	8.09	4.10	0.00
0.3	200	20	**20.94**	3.28	6.47	9.51	12.31	14.81	16.94	18.66	19.92	20.69	20.94	20.69	19.92	18.66	16.94	14.81	12.31	9.51	6.47	3.28	0.00
0.3	200	30	**31.42**	4.91	9.71	14.26	18.47	22.21	25.42	27.99	29.88	31.03	31.42	31.03	29.88	27.99	25.42	22.21	18.47	14.26	9.71	4.91	0.00
0.3	200	40	**41.89**	6.55	12.94	19.02	24.62	29.62	33.89	37.32	39.84	41.37	41.89	41.37	39.84	37.32	33.89	29.62	24.62	19.02	12.94	6.55	0.00
0.3	200	50	**52.36**	8.19	16.18	23.77	30.78	37.02	42.36	46.65	49.80	51.72	52.36	51.72	49.80	46.65	42.36	37.02	30.78	23.77	16.18	8.19	0.00
0.3	300	20	**31.42**	4.91	9.71	14.26	18.47	22.21	25.42	27.99	29.88	31.03	31.42	31.03	29.88	27.99	25.42	22.21	18.47	14.26	9.71	4.91	0.00
0.3	300	30	**47.12**	7.37	14.56	21.39	27.70	33.32	38.12	41.99	44.82	46.54	47.12	46.54	44.82	41.99	38.12	33.32	27.70	21.39	14.56	7.37	0.00
0.3	300	40	**62.83**	9.83	19.42	28.53	36.93	44.43	50.83	55.98	59.76	62.06	62.83	62.06	59.76	55.98	50.83	44.43	36.93	28.53	19.42	9.83	0.00
0.3	300	50	**78.54**	12.29	24.27	35.66	46.16	55.54	63.54	69.98	74.70	77.57	78.54	77.57	74.70	69.98	63.54	55.54	46.16	35.66	24.27	12.29	0.00
0.3	600	20	**62.83**	9.83	19.42	28.53	36.93	44.43	50.83	55.98	59.76	62.06	62.83	62.06	59.76	55.98	50.83	44.43	36.93	28.53	19.42	9.83	0.00
0.3	600	30	**94.25**	14.74	29.12	42.79	55.40	66.64	76.25	83.98	89.63	93.09	94.25	93.09	89.63	83.98	76.25	66.64	55.40	42.79	29.12	14.74	0.00
0.3	600	40	**125.66**	19.66	38.83	57.05	73.86	88.86	101.66	111.97	119.51	124.12	125.66	124.12	119.51	111.97	101.66	88.86	73.86	57.05	38.83	19.66	0.00
0.3	600	50	**157.08**	24.57	48.54	71.31	92.33	111.07	127.08	139.96	149.39	155.15	157.08	155.15	149.39	139.96	127.08	111.07	92.33	71.31	48.54	24.57	0.00

Table: Inspiratory flow (l/min) at different points of the breathing cycle for different breathing simulator settings.

			%	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100
			Angle:	9	18	27	36	45	54	63	72	81	90	99	108	117	126	135	144	153	162	171	180
			Sine:	0.16	0.31	0.45	0.59	0.71	0.81	0.89	0.95	0.99	1.00	0.99	0.95	0.89	0.81	0.71	0.59	0.45	0.31	0.16	0.00
Ti	Vt	f	Vmax																				
0.4	50	20	3.93	0.61	1.21	1.78	2.31	2.78	3.18	3.50	3.73	3.88	3.93	3.88	3.73	3.50	3.18	2.78	2.31	1.78	1.21	0.61	0.00
0.4	50	30	5.89	0.92	1.82	2.67	3.46	4.17	4.77	5.25	5.60	5.82	5.89	5.82	5.60	5.25	4.77	4.17	3.46	2.67	1.82	0.92	0.00
0.4	50	40	7.85	1.23	2.43	3.57	4.62	5.55	6.35	7.00	7.47	7.76	7.85	7.76	7.47	7.00	6.35	5.55	4.62	3.57	2.43	1.23	0.00
0.4	50	50	9.82	1.54	3.03	4.46	5.77	6.94	7.94	8.75	9.34	9.70	9.82	9.70	9.34	8.75	7.94	6.94	5.77	4.46	3.03	1.54	0.00
0.4	100	20	7.85	1.23	2.43	3.57	4.62	5.55	6.35	7.00	7.47	7.76	7.85	7.76	7.47	7.00	6.35	5.55	4.62	3.57	2.43	1.23	0.00
0.4	100	30	11.78	1.84	3.64	5.35	6.92	8.33	9.53	10.50	11.20	11.64	11.78	11.64	11.20	10.50	9.53	8.33	6.92	5.35	3.64	1.84	0.00
0.4	100	40	15.71	2.46	4.85	7.13	9.23	11.11	12.71	14.00	14.94	15.51	15.71	15.51	14.94	14.00	12.71	11.11	9.23	7.13	4.85	2.46	0.00
0.4	100	50	19.63	3.07	6.07	8.91	11.54	13.88	15.89	17.49	18.67	19.39	19.63	19.39	18.67	17.49	15.89	13.88	11.54	8.91	6.07	3.07	0.00
0.4	200	20	15.71	2.46	4.85	7.13	9.23	11.11	12.71	14.00	14.94	15.51	15.71	15.51	14.94	14.00	12.71	11.11	9.23	7.13	4.85	2.46	0.00
0.4	200	30	23.56	3.69	7.28	10.70	13.85	16.66	19.06	20.99	22.41	23.27	23.56	23.27	22.41	20.99	19.06	16.66	13.85	10.70	7.28	3.69	0.00
0.4	200	40	31.42	4.91	9.71	14.26	18.47	22.21	25.42	27.99	29.88	31.03	31.42	31.03	29.88	27.99	25.42	22.21	18.47	14.26	9.71	4.91	0.00
0.4	200	50	39.27	6.14	12.14	17.83	23.08	27.77	31.77	34.99	37.35	38.79	39.27	38.79	37.35	34.99	31.77	27.77	23.08	17.83	12.14	6.14	0.00
0.4	300	20	23.56	3.69	7.28	10.70	13.85	16.66	19.06	20.99	22.41	23.27	23.56	23.27	22.41	20.99	19.06	16.66	13.85	10.70	7.28	3.69	0.00
0.4	300	30	35.34	5.53	10.92	16.05	20.77	24.99	28.59	31.49	33.61	34.91	35.34	34.91	33.61	31.49	28.59	24.99	20.77	16.05	10.92	5.53	0.00
0.4	300	40	47.12	7.37	14.56	21.39	27.70	33.32	38.12	41.99	44.82	46.54	47.12	46.54	44.82	41.99	38.12	33.32	27.70	21.39	14.56	7.37	0.00
0.4	300	50	58.90	9.21	18.20	26.74	34.62	41.65	47.66	52.48	56.02	58.18	58.90	58.18	56.02	52.48	47.66	41.65	34.62	26.74	18.20	9.21	0.00
0.4	600	20	47.12	7.37	14.56	21.39	27.70	33.32	38.12	41.99	44.82	46.54	47.12	46.54	44.82	41.99	38.12	33.32	27.70	21.39	14.56	7.37	0.00
0.4	600	30	70.69	11.06	21.84	32.09	41.55	49.98	57.19	62.98	67.23	69.82	70.69	69.82	67.23	62.98	57.19	49.98	41.55	32.09	21.84	11.06	0.00
0.4	600	40	94.25	14.74	29.12	42.79	55.40	66.64	76.25	83.98	89.63	93.09	94.25	93.09	89.63	83.98	76.25	66.64	55.40	42.79	29.12	14.74	0.00
0.4	600	50	117.81	18.43	36.41	53.48	69.25	83.30	95.31	104.97	112.04	116.36	117.81	116.36	112.04	104.97	95.31	83.30	69.25	53.48	36.41	18.43	0.00

Table: Inspiratory flow (l/min) at different points of the breathing cycle for different breathing simulator settings.

			%	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100
			Angle:	9	18	27	36	45	54	63	72	81	90	99	108	117	126	135	144	153	162	171	180
			Sine:	0.16	0.31	0.45	0.59	0.71	0.81	0.89	0.95	0.99	1.00	0.99	0.95	0.89	0.81	0.71	0.59	0.45	0.31	0.16	0.00
Ti	Vt	f	Vmax																				
0.5	50	20	3.14	0.49	0.97	1.43	1.85	2.22	2.54	2.80	2.99	3.10	3.14	3.10	2.99	2.80	2.54	2.22	1.85	1.43	0.97	0.49	0.00
0.5	50	30	4.71	0.74	1.46	2.14	2.77	3.33	3.81	4.20	4.48	4.65	4.71	4.65	4.48	4.20	3.81	3.33	2.77	2.14	1.46	0.74	0.00
0.5	50	40	6.28	0.98	1.94	2.85	3.69	4.44	5.08	5.60	5.98	6.21	6.28	6.21	5.98	5.60	5.08	4.44	3.69	2.85	1.94	0.98	0.00
0.5	50	50	7.85	1.23	2.43	3.57	4.62	5.55	6.35	7.00	7.47	7.76	7.85	7.76	7.47	7.00	6.35	5.55	4.62	3.57	2.43	1.23	0.00
0.5	100	20	6.28	0.98	1.94	2.85	3.69	4.44	5.08	5.60	5.98	6.21	6.28	6.21	5.98	5.60	5.08	4.44	3.69	2.85	1.94	0.98	0.00
0.5	100	30	9.42	1.47	2.91	4.28	5.54	6.66	7.62	8.40	8.96	9.31	9.42	9.31	8.96	8.40	7.62	6.66	5.54	4.28	2.91	1.47	0.00
0.5	100	40	12.57	1.97	3.88	5.71	7.39	8.89	10.17	11.20	11.95	12.41	12.57	12.41	11.95	11.20	10.17	8.89	7.39	5.71	3.88	1.97	0.00
0.5	100	50	15.71	2.46	4.85	7.13	9.23	11.11	12.71	14.00	14.94	15.51	15.71	15.51	14.94	14.00	12.71	11.11	9.23	7.13	4.85	2.46	0.00
0.5	200	20	12.57	1.97	3.88	5.71	7.39	8.89	10.17	11.20	11.95	12.41	12.57	12.41	11.95	11.20	10.17	8.89	7.39	5.71	3.88	1.97	0.00
0.5	200	30	18.85	2.95	5.82	8.56	11.08	13.33	15.25	16.80	17.93	18.62	18.85	18.62	17.93	16.80	15.25	13.33	11.08	8.56	5.82	2.95	0.00
0.5	200	40	25.13	3.93	7.77	11.41	14.77	17.77	20.33	22.39	23.90	24.82	25.13	24.82	23.90	22.39	20.33	17.77	14.77	11.41	7.77	3.93	0.00
0.5	200	50	31.42	4.91	9.71	14.26	18.47	22.21	25.42	27.99	29.88	31.03	31.42	31.03	29.88	27.99	25.42	22.21	18.47	14.26	9.71	4.91	0.00
0.5	300	20	18.85	2.95	5.82	8.56	11.08	13.33	15.25	16.80	17.93	18.62	18.85	18.62	17.93	16.80	15.25	13.33	11.08	8.56	5.82	2.95	0.00
0.5	300	30	28.27	4.42	8.74	12.84	16.62	19.99	22.87	25.19	26.89	27.93	28.27	27.93	26.89	25.19	22.87	19.99	16.62	12.84	8.74	4.42	0.00
0.5	300	40	37.70	5.90	11.65	17.12	22.16	26.66	30.50	33.59	35.85	37.23	37.70	37.23	35.85	33.59	30.50	26.66	22.16	17.12	11.65	5.90	0.00
0.5	300	50	47.12	7.37	14.56	21.39	27.70	33.32	38.12	41.99	44.82	46.54	47.12	46.54	44.82	41.99	38.12	33.32	27.70	21.39	14.56	7.37	0.00
0.5	600	20	37.70	5.90	11.65	17.12	22.16	26.66	30.50	33.59	35.85	37.23	37.70	37.23	35.85	33.59	30.50	26.66	22.16	17.12	11.65	5.90	0.00
0.5	600	30	56.55	8.85	17.47	25.67	33.24	39.99	45.75	50.39	53.78	55.85	56.55	55.85	53.78	50.39	45.75	39.99	33.24	25.67	17.47	8.85	0.00
0.5	600	40	75.40	11.79	23.30	34.23	44.32	53.31	61.00	67.18	71.71	74.47	75.40	74.47	71.71	67.18	61.00	53.31	44.32	34.23	23.30	11.79	0.00
0.5	600	50	94.25	14.74	29.12	42.79	55.40	66.64	76.25	83.98	89.63	93.09	94.25	93.09	89.63	83.98	76.25	66.64	55.40	42.79	29.12	14.74	0.00

Table: Inspiratory flow (l/min) at different points of the breathing cycle for different breathing simulator settings.

Conclusions and further work

The answer to the research question posed at the beginning of this thesis is a resounding 'yes'. The method of use and choice of inhalational drug delivery device does affect the amount of drug that is available for inhalation by the patient. This in vitro conclusion is supported by in vivo pharmacokinetic studies. The messages that all devices are not the same, that it is inappropriate to uncritically use any drug with any device just because the MDI adaptor fits, and that experiments with one device or drug cannot necessarily be extrapolated to others, are being acknowledged by medical practitioners. In short, *it ain't what you give, its the way that you give it!*

Inhalational therapy is used to deliver medication to the lung, either to treat diseases or, less commonly, for systemic absorption. A number of devices have been developed to aid or improve inhalational therapy, and this thesis deals with metered dose inhalers, used with spacer devices, and nebulisers.

Spacer devices are becoming increasingly popular for the delivery of inhaled drugs in the treatment of asthma and other diseases (Keeley, 1992). They reduce the problems of poor inhaler technique which may lead to treatment failure with metered dose inhalers (Shim and Williams, 1980), largely eliminate oral absorption of inhaled steroids (Brown et al 1990, Prahl and Jensen 1987, Selroos and Halme, 1991) and have been shown to be as effective as nebulisers in the treatment of acute severe asthma (Freelander and Van Asperen, 1984, Morgan et al, 1982). By the attachment of facemasks they can be adapted to treat patients of all ages (O'Callaghan 1989, Connolly 1990, Conner et al 1989). Popularity has lead to a rapid increase in the number of different types of spacer available.

Despite their seemingly simple construction and concept, the correct choice and use of a spacer can dramatically alter the amount of drug available for inhalation. Studies in this thesis, supported by emerging pharmacokinetic evidence (Clarke and Lipworth

1995, Hindle and Chrystyn 1994, Chege JK, Chrystyn 1994), have highlighted areas where the incorrect use of a spacer device can affect the drug output.

Firstly, delay between metered dose inhaler actuation into the spacer and inhalation can reduce the amount of drug available to the patient (Barry et al 1993, Barry and O'Callaghan 1995, O'Callaghan et al 1994). Inhalation of drug from a spacer should commence as soon as possible after actuation of the inhaler, and where a health care worker or carer is actuating the inhaler for the patient, this should be done only when the patient is ready and the spacer in place.

Secondly, multiple actuations of the metered dose inhaler into the spacer prior to inhalation can also considerably reduce the amount of drug available to the patient (Barry et al 1993, Barry and O'Callaghan 1995, O'Callaghan et al 1994, Barry and O'Callaghan 1994). For example, five actuations of a steroid inhaler into a large volume spacer, prior to inhalation, results in the same dose being available to inhale as if a single dose had been actuated into the same spacer and inhaled immediately (O'Callaghan et al 1994).

The size of the spacer may also affect the amount of drug available for inhalation, and this will vary with the drug prescribed (Kim & Eldridge 1987, Barry and O'Callaghan 1995, Agertoftand Pederson 1994). Preparations may differ in their aerosol cloud speed and volume (Barry and O'Callaghan 1995), and this may alter the amount of drug delivered from different spacers. For example, a large volume spacer may deliver more than three times the amount of sodium cromoglycate (5mg) or salbutamol than a smaller spacer. The difference between large and small spacers is much less for budesonide (Barry and O'Callaghan 1997). Newer formulations containing hydrofluoroalkanes may behave differently in spacers than the chlorofluorocarbon containing formulations that they replace (Barry and O'Callaghan 1995). The effect of breathing pattern on the dose of drug received is important in very young children where spacer size may have less of an effect on delivered dose (Everard 1992).

Static charge accumulates on the walls of many polycarbonate and plastic spacers, attracting drug particles, which become charged when they are produced at the metered dose inhaler valve stem. Thus, highly charged spacers deliver less drug than

those where the static charge has been reduced by an anti-static lining (Barry and O'Callaghan 1995, O'Callaghan et al 1993). Washing the spacer also reduces its charge, but the optimum washing regime for spacers is not known.

Nebulisers remain useful in the delivery to patients who require high doses of medications, or medications not available in other forms, or who are intolerant of other inhalational drug delivery devices, such as spacers and metered dose inhalers.

Nebuliser design is improving to try and make nebulisers more efficient, and the output of different nebulisers varies greatly. The results of studies of one nebuliser with one drug cannot necessarily be applied to another nebuliser, or even the same nebuliser with a different drug.

Nebuliser output should be determined by the direct measurement of released drug, rather than by weighing the nebuliser or measuring the osmolality of solution remaining in the nebuliser at the end of nebulisation, although these methods may provide a useful screening test of nebulisers used primarily for the delivery of solution medications with similar physico-chemical properties.

The majority of the nebulisers studied in this thesis finished effective nebulisation within a few minutes. Spending long periods of time using a nebuliser is therefore unnecessary, as well as being unacceptable to most patients, and is likely to reduce compliance. The patient should be given a maximum time for nebulisation (based where possible on specific studies). This time will depend on the nebuliser and drug being used, but for some medications administered for asthma, little drug may be delivered after five minutes.

There are, however, many questions that remain and that arise from the work presented, ranging from straight forward methodological problems, to behavioural questions underlying the poor compliance of patients taking inhaled medications.

Firstly, the question of in vitro/in vivo correlation needs to be addressed. Patient factors such as breathing pattern affect the output of drug from a device, and possibly

the particle size as well. Upper airway geometry, dynamic, rather than static, influences the amount of drug released that reaches the lungs, and the environmental conditions inside the airway may alter particle size, and hence deposition. The notion that all drug particles of a certain size range, measured at the exit of the drug delivery device, are 'respirable' and will therefore deposit in the lungs is clearly erroneous.

So particle sizing devices, such as the Multi-Stage Liquid Impinger, will need to be modified to incorporate some of the attributes of an 'imitation lung' if the levels of agreement between in vitro testing and in vivo experiments are to be improved. For this reason, more information is needed on the conditions inside the particle sizing device, and how this affects the measured size. Smaldone (1996) has already described a combination of breathing simulation and inertial impaction, and Finlay and Stapleton (1998) have investigated the effect on measured particle size of changes to the Andersen sampler during operation. Further work along the lines of that described in Chapter 5.1 is planned to determine the environmental changes in different inertial impactors, and their effect on measured particle size.

Impactor inlets are also critical to their operation, and inlets that recreate the anatomical and dynamic features of the upper airway are planned, including those that may be varied with simulated breathing, while maintaining the constant flow of aerosol required by the inertial impaction sizing device.

It is impractical, costly and probably unethical to undertake radio-isotope deposition studies in children to 'validate' in vitro findings. The measurement of urinary drug excretion is a simple, non-invasive and reproducible method of estimating lung delivery that could be used instead. This method needs further validation for use in infants and children, in whom absorption of drug across the nasal mucosa may mean that the early urinary excretion of drug does not represent just lung delivery. Levels of agreement between in vitro and urinary excretion experiments should also be calculated.

Many different inhalational drug delivery devices have been released recently, often with little or no clinical information to justify their use. The methods described in this thesis could be used to undertake comparative assessments of devices to estimate their likely efficacy. It should be remembered, however, that the device that delivers the

greatest amount of medication *in vitro* will not work *in vivo* if the patient does not like it or find it easy to use. The effect of factors such as spacer shape and size on compliance with medication should be investigated further.

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