

ESTIMATION OF THE FINE PARTICLE DOSE CHARACTERISTICS OF
BUDESONIDE METERED DOSE INHALERS USING TWO CASCADE
IMPACTORS METHODS



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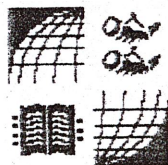
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IMPACTOR METHODS

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ABSTRACT

The fine particle dose (otherwise known as the respirable dose i.e. The total amount of drug that has the highest probability of delivery to the lung) of budesonide metered dose inhalers (200 µg per dose) was estimated using two cascade impactor methods. The first method using the twin-stage impinger (TSI), and the second method using the Multi-Stage Liquid Impinger (MSLI). For this purpose an available High Performance Liquid Chromatography (HPLC) method for the analysis of budesonide was adapted.

The objectives of this study were:

- Estimation of the fine particle dose using the twin-impinger and the MSLI.
- Measurement of the Mass Median Aerodynamic Diameter (MMAD) by the MSLI.
- The effect of increasing the number of doses on the fine particle dose and the MMAD.
- Comparison between the twin impinger and the MSLI in estimation of the fine particle dose.

It was found that the fine particle dose decreased with the increase in the number of doses. Using the twin impinger it decreased from 49.51 % for 1 puff to 44.47 % for 5 puffs, and with the MSLI it decreased from 45.57 % for 1 puff to 30.43 % for the 10 puffs. The MMAD was found to increase from 4.04 µm for 1 puff to 4.29 µm for the 10 puffs. These results prove what is known as the loading effect which could be caused by the trapping of the smaller particles (<6.4 µm) which would normally pass through to the lower stages by the larger particles previously deposited on the upper stages. The study showed comparable results of the fine particle dose between the twin impinger and the MSLI, which suggest that the two devices are useful for assessing the fine particle dose of the MDIs, but the MSLI is more useful for research than for quality control purposes (as the case with the twin impinger) because the MSLI gives more information on the aerodynamic particle size distribution.

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CONTENTS

	Page
ABSTRACT.....	1
ACKNOWLEDGEMENTS.....	2
CONTENTS.....	3
LIST OF TABLES.....	5
LIST OF FIGURES.....	6
1. INTRODUCTION	8
2. LITERATURE REVIEW.....	11
2.1 Inhalational therapy.....	11
2.2 Budesonide.....	14
2.2.1 Mechanism of action.....	14
2.2.2 Pharmacokinetic properties.....	15
2.2.3 Therapeutic efficacy.....	16
2.2.4 Tolerability.....	18
2.2.5 Dosage and administration.....	19
2.2.6 Analytical methods.....	20
2.3 Particle size analysis.....	22
2.3.1 Twin-stage impinger (TSI).....	26
2.3.2 Multi-Stage Liquid Impinger (MSLI).....	29
3. Characteristics of the aerodynamic particle size distribution of budesonide 200µg/dose metered dose inhalers.....	31
3.1 Materials.....	31
3.2 Method.....	32
3.2.1 HPLC assay conditions.....	32
3.2.2 Assay validation.....	33
3.2.3 Fine particle dose estimation using the TSI.....	36
3.2.4 Particle size analysis using the MSLI.....	37
3.3 Results.....	39
3.3.1 Fine particle dose estimation using the TSI	39
3.3.2 Particle size analysis using the MSLI.....	43

3.3.3 Descriptive statistics of the particle size distribution obtained from the MSLI.....	48
3.3.4 MMAD Analysis.....	49
3.4 Discussion.....	60
3.4.1 Particle size analysis.....	60
3.4.2 Comparison between the TSI and the MSLI.....	62
References.....	63

LIST OF TABLES

	Page
3.1 Intra-day precision and accuracy.....	34
3.2 Inter-day precision and accuracy.....	35
3.3 Volume of the volumetric flasks (ml) used in the TSI	37
3.4 Volume of the volumetric flasks (ml) used in the MSLI.....	39
3.5 Particle size distribution of 1 puff using the TSI.....	41
3.6 Particle size distribution of 2 puffs using the TSI	42
3.7 Particle size distribution of 5 puffs using the TSI	42
3.8 Particle size distribution of 1 puff using the MSLI.....	46
3.9 Particle size distribution of 2 puffs using the MSLI.....	46
3.10 Particle size distribution of 5 puffs using MSLI.....	47
3.11 Particle size distribution of 10 puffs using MSLI.....	47
3.12 Mean (SD) deposition (in μg) of budesonide on the different stages of MSLI following 1, 2, 5, and 10 puffs.....	48
3.13 Mean (SD) percentage distribution of the delivered doses in the MSLI...	49
3.14 Fine particle dose ($<6.8 \mu\text{m}$) percentage.....	49
3.15 Cumulative percentage of the mass less than the stated particle size of 1 puff.....	50
3.16 Cumulative percentage of the mass less than the stated particle size of 2 puffs.....	50
3.17 Cumulative percentage of the mass less than the stated particle size of 5 puffs.....	50
3.18 Cumulative percentage of the mass less than the stated particle size of 10 puffs.....	50
3.19 Mean (SD) cumulative percentage of the mass less than the stated particle size.....	51
3.20 MMAD, ECD, AND GSD OF 1 puff runs.....	58
3.21 MMAD, ECD, AND GSD OF 2 puffs runs.....	58
3.22 MMAD, ECD, AND GSD OF 5 puffs runs.....	58
3.23 MMAD, ECD, AND GSD OF 10 puffs runs.....	59
3.24 MMAD, ECD, AND GSD OF the mean of all the MSLI data.....	59

LIST OF FIGURES

	Page
2.1 The twin stage impinger (TSI)	28
2.2 The modified multistage liquid impinger (MSLI).....	30
3.1 Chromatogram of a standard solution of budesonide 5mg/l + methylprednisolone 5mg/l (internal standard)...	32
3.2 Intra-day calibration graph of budesonide.....	34
3.3 Inter-day calibration graph of budesonide.....	35
3.4 Chromatogram of the actuator recovery of 5 puffs budesonide mdi + methylprednisolone in the TSI.....	40
3.5 Chromatogram of stage 1 recovery of 5 puffs budesonide mdi + methylprednisolone in the TSI.....	40
3.6 Chromatogram of stage 2 recovery of 5 Puffs budesonide mdi + methylprednisolone in the TSI.....	40
3.7 Chromatogram of the actuator recovery of 5 puffs budesonide mdi + methylprednisolone in the MSLI.....	42
3.8 Chromatogram of the throat recovery of 5 puffs budesonide mdi + methylprednisolone in the MSLI.....	43
3.9 Chromatogram of stage 1 recovery of 5 puffs budesonide mdi + methylprednisolone in the MSLI.....	44
3.10 Chromatogram of stage 2 recovery of 5 puffs budesonide mdi + methylprednisolone in the MSLI	44
3.11 Chromatogram of stage 3 recovery of 5 puffs budesonide mdi + methylprednisolone in the MSLI.....	44
3.12 Chromatogram of stage 4 recovery of 5 puffs budesonide mdi + methylprednisolone in the MSLI.....	45
3.13 Chromatogram of filter paper recovery of 5 puffs budesonide mdi + methylprednisolone in the MSLI.....	45
3.14 Aerodynamic particle size distribution of 1 puff	52
3.15 Aerodynamic size distribution of 2 puffs.....	53
3.16 Aerodynamic particle size distribution of 5 puffs.....	54
3.17 Aerodynamic particle size distribution of 10 puffs	55

3.18 Aerodynamic particle size distribution of the mean of all data.....	56
3.19 Mean (SD) aerodynamic particle size distribution of all data	57

CHAPTER ONE

1. INTRODUCTION:

Asthma is now widely recognized as a chronic inflammatory condition of the airways and, therefore, corticosteroid treatment by inhalation is preferable to oral or parenteral treatment. The inhaled route has become widely accepted amongst the international medical community as the route of choice for the direct administration of drugs in the management of asthma⁽⁴⁾.

Corticosteroids suppress the inflammatory process of asthma by inhibition of the inflammatory cell infiltration and the production of inflammatory mediators. The introduction of inhaled corticosteroids has been a significant advance in the treatment of chronic asthma, and International Thoracic Societies all recommend inhaled corticosteroids as the first-line therapy for all but very mild asthma⁽⁷⁾. Although low doses of inhaled corticosteroids are usually effective, the wide safety margin afforded by the inhaled route means that much higher doses (e.g. by nebuliser) can be administered when necessary^(1,4).

Budesonide, a non halogenated glucocorticosteroid, derivative structurally related to 16 α -hydroxyprednisolone, has a high ratio of local anti-inflammatory to systemic activity, which enables inhalation administration of therapeutically effective dosages in patients with asthma with minimal systemic glucocorticosteroid effects⁽²⁾.

Given good inhaler technique, 10-30% of budesonide enters the lungs, depending on which inhaler device is used. The remainder of the drug is deposited in the mouth and on the buccal mucosa, where it is swallowed, absorbed from the gut and metabolized in the liver, as it has a first-pass metabolism of 90%. Budesonide is metabolized to 6 β -hydroxybudesonide and 16 α -hydroxyprednisolone, which have a 100 time less affinity for the

glucocorticoid receptors than the parent compound. Budesonide is degraded more rapidly in the liver than 17-beclomethasone monopropionate (beclomethasone active compound), which may account for the fact that it has fewer systemic side-effects at high doses.

Measuring drugs especially in small amounts, requires a highly efficient analysis system, which should have high speed, linearity and sensitivity. Although many analytical methods are available for the analysis of budesonide and other corticosteroids, which include immunoassay, thermospray liquid chromatography-mass spectrometry, reversed phase High Performance Liquid Chromatography (HPLC) has been found to be more selective. An available HPLC method was adapted in this study, and developed further. In this method an octadecylsilane (C18) column was used, and 75% methanol in 0.1% glacial acetic acid in water was used as a mobile phase with UV detection at 250nm. This method was found to be fast and sensitive.

The clinical efficacy of therapeutic inhalers depends on the mass delivery of aerosolized medicament to the lungs in suitably small particles. This respirable fraction for most therapeutic products consists of particles with an aerodynamic size between 0.5 and 5 μm , although rather larger particles might also be important in some situations. The mass fraction of particles in this range or below any particular size is determined by measuring the aerodynamic size distribution of the discharged aerosol, or by direct measurement of the fraction with a suitable impactor⁽⁵⁷⁾.

The above HPLC method was used to estimate the fine particle dose of budesonide MDI (containing 200 μg per puff) by the twin-stage impinger (TSI), and the multi-stage liquid impinger (MSLI).

It is the purpose of this study to examine the effect of dose on the measured aerodynamic particle size distribution of budesonide, as it has been reported that for salbutamol and beclomethasone MDIs the aerodynamic particle size distribution is dependent on the number of doses sprayed into the Andersen

cascade impactor and the multi-stage liquid impinger. This could be due to small particles trapped on the upper stages by those deposited from previous doses, thereby creating a loading effect (N.B.: This should not be the case for the MSLI because it is a liquid based impinger). Since no study available for the estimation of fine particle dose of budesonide MDI, and the effect of dose on the measured aerodynamic particle size distributions, this study was done.

Another purpose of this study is to compare between two widely used impactors for the estimation of the fine particle dose namely: The Twin-stage Impinger (TSI), and the Multi-Stage Liquid Impinger (MSLI).

CHAPTER TWO

2. LITERATURE REVIEW:

2.1 INHALATIONAL THERAPY:

The human respiratory tract has an extensive blood supply and its total surface area is about 30-100m². Most of the surface area resides in the alveolated regions of the deep lung which contain also a rich capillary network to facilitate rapid gas exchange. Although inhaled drug particles or droplets will typically deposit only on a small fraction of the total available surface area, the possibility of fast drug absorption into the systemic circulation exists.

The logical choice for the treatment of a localized disease is administration of medication directly to the site of action. Taking medication by mouth is illogical for a localized airway disease such as asthma, because:

- *Rapid onset of action:* A much more rapid bronchodilator effect is seen with inhaled rather than oral β_2 -adrenergic agonist.
- *Low effective dose:* When given by inhalation directly to the site of action, a therapeutic effect may be seen with a very low dose. Although low doses are usually effective, the wide safety margin afforded by the inhaled route means that much higher doses (e.g. by nebuliser) can be administered when necessary.
- *Low incidence of systemic side-effects:* As the effective therapeutic dose is small when inhaled therapy is used, the incidence of systemic side-effect is low⁽⁴⁾.

Treatment of asthma (as recommended by the British Thoracic Society) should be considered in a stepwise manner, with patients starting therapy at the step most appropriate for the initial severity of their condition⁽⁶²⁾.

Step 1: Inhaled short acting β_2 -agonist as required (up to once daily).

Note: Move to step 2 if needed more than once daily (one night-time symptoms).

Step 2: Inhaled short acting β_2 -agonist as required **plus either:**

Regular standard dose inhaled corticosteroid (Beclomethasone dipropionate or budesonide 100-400 μg twice daily or fluticasone propionate 50-200 μg twice daily) **or:**

Regular cromoglycate or nedocromil (but change to inhaled corticosteroid if control not achieved).

Note: Higher dose of inhaled corticosteroid may be required to gain initial control; some individuals benefit from doubling for short period to cover an exacerbation.

Step 3: Inhaled short acting β_2 -agonist as required **plus either:**

Regular high dose inhaled corticosteroid (Beclomethasone dipropionate or budesonide 0.8-2 mg daily in divided doses or fluticasone propionate 0.4-1 mg daily in divided doses; use a large volume spacer) **or:**

Regular standard dose inhaled corticosteroid *plus* regular inhaled long acting β_2 -agonist (Salmeterol 50 μg twice daily *or* in those over 18 years old, eformoterol 12 μg twice daily).

Note: Those who have problems with high dose inhaled corticosteroid use *either* inhaled long acting β_2 -agonist *or* regular modified release oral theophylline *or* try regular cromoglycate or nedocromil.

Step 4: Inhaled short acting β_2 -agonist as required, **with:**

Regular high dose inhaled corticosteroid (Beclomethasone dipropionate or budesonide 0.8-2 mg daily in divided doses or fluticasone propionate 0.4-1 mg daily in divided doses; use a large volume spacer)

Plus sequential therapeutic trial of one or more of:

Inhaled long acting β_2 -agonist

Modified release oral theophylline

Inhaled ipratropium or in adults, oxitropium

Modified release oral β_2 -agonist

High dose inhaled bronchodilators

cromoglycate or nedocromil

Step 5: Inhaled short acting β_2 -agonist as required, **with:**

Regular high dose inhaled corticosteroid and one or more long acting bronchodilators (see step 4), **plus:**

Regular prednisolone tablets (as single daily dose)

Note: In addition to regular prednisolone, continue high dose inhaled corticosteroid (In exceptional cases may exceed licensed doses); these patients should normally be referred to an asthma clinic.

Stepping down: Review treatment every 3-6 months; if control achieved stepwise reduction may be possible; in patients whose treatment was started recently at step 4 or 5 (or contained corticosteroid tablets) reduction may take place after short interval; in other patients with chronic asthma a 3-6 month period of stability should be shown before slow stepwise reduction is undertaken⁽⁶²⁾.

There are several major drawbacks which limit the use of metered dose inhalers. First, many patients, particularly the very young, very old and disabled, cannot use a metered dose inhaler satisfactorily, usually because of poor coordination between actuating the inhaler and inhaling. Poor inhaler technique can also be due to a reflux cessation during inhalation when cold chlorofluorocarbons hit the back of the throat. Poor technique greatly reduces the efficacy of the inhaled drug and increases oropharyngeal deposition, thus increasing the incidence of local adverse effects. A second major problem with metered dose inhalers is that they contain chlorofluorocarbons propellants which are thought to damage the ozone layer. Future legislation indicates that the use of chlorofluorocarbons as propellants will be severely limited⁽³⁶⁾.

It is well established that for most MDIs only 10 to 15% of the metered dose reaches the airways. However, this value will decrease if the patient cannot coordinate actuation of the device with inhalation. The inhalation should also be slow (around 30 L/min) and a 10 second breath holding time after inhalation will improve the clinical efficacy as compared with a rapid inhalation and a short breath holding time⁽⁵²⁾.

Dry Powder Inhalers (DPI) are easier to use because coordination between inhalation and activation of the dose is not required. The deposition pattern of drug from a Turbohaler (budesonide dry powder inhaler) is similar to that from a metered dose inhaler, with around 14% of a dose deposited in the lung. An inspiratory flow rate of at least 30 L/min is required for optimum activation of the Turbohaler⁽³⁶⁾.

2.2 BUDESONIDE:

Budesonide is a potent glucocorticoid, clinically used in the local treatment of skin and respiratory disease. The drug is pharmacokinetically characterized by a large volume of distribution and a high hepatic clearance. The extensive liver biotransformation contributes to the favorable ratio between local and systemic glucocorticoid effects shown for the compound. Budesonide is a C-22 1:1 epimeric mixture, epimer 22R having 2-3 times higher topical glucocorticoid potency than epimer 22S. (22R)-budesonide has approximately twice the volume of distribution, clearance and *in-vitro* liver biotransformation rate as the 22S-epimer^(11&12).

2.2.1 MECHANISM OF ACTION:

Evidence that asthma is associated with airway inflammation has been provided by various methods including bronchoalveolar lavage (BAL), and recently several studies have demonstrated that exposure to, or treatment with, budesonide reduces the inflammatory cells in BAL fluid from patients with asthma.

In-vitro, budesonide caused phenotypic changes within alveolar macrophages obtained by BAL and reduced IgE-dependent histamine release from human leucocytes, possibly by interacting directly with the basophils, and inhibited monocyte mediated cytotoxicity. Eosinophil survival and activation is

stimulated by granulocyte-macrophage colony-stimulating factor (GM-CSF) and budesonide has also been shown to inhibit eosinophil survival as well as GM-CSF production *in vitro*, and to increase activity of neutral endopeptase, an enzyme known to degrade inflammatory mediators.

Airway biopsies obtained from patients with mild asthma (mostly atopic) before and after treatment with budesonide 400mcg daily for 4 weeks or 800mcg daily for 6 months revealed reduced T cell-mediated inflammation in bronchial wall (reductions in T cells, memory T cells, dendritic cells and HLA-DR expression) and reduced mucosal numbers of mast cells, eosinophils and foci of eosinophil degranulation. Budesonide was more effective in this respect than terbutaline but neither short nor long term treatment with inhaled corticosteroids reduced thickening of the reticular basement membrane.

A marked increase in bronchial epithelial eosinophils has been shown to accompany any increase in asthma symptoms and bronchial reactivity. Improved spirometry in 7 patients with mild asthma after 3 months treatment with inhaled budesonide 600mcg twice daily was associated with a decrease in epithelial eosinophils and lymphocytes, and mast cells and lymphocytes in lamina propria significantly greater than that achieved after treatment of a parallel group with terbutaline 375mcg twice daily⁽²⁾.

2.2.2 PHARMACOKINETIC PROPERTIES:

Budesonide has a relatively high water solubility and is, therefore, rapidly absorbed in the lung. Following inhalation or intranasal administration of budesonide, peak plasma concentrations were reached after 15 to 45 minutes. Systemic bioavailability was 73%, which increased to about 100% after instillation of an alcohol (ethanol) solution directly onto the nasal mucosa, compared with 10.7% after oral administration, where there was evidence of extensive first-pass metabolism. Plasma budesonide is extensively protein bound and has a large volume of distribution of about 300L due to tissue

binding. The concentration of budesonide in lung tissue 1.5 to 4 hours after inhalation of a single 1600mcg dose was 15.5 nmol/L compared with 0.63nmol/L in plasma at the same time.

The major metabolic pathway, 16α , 17α -acetal cleavage, is unique to budesonide among the topical corticosteroids and may increase the overall rate of inactivation. In the liver 22S-budesonide is rapidly oxidised by the cytochrome P450 system to 6β -hydroxy-budesonide, and 22R-budesonide is oxidised to the inactive metabolite 16α -hydroxy-prednisolone. The hepatic metabolism of budesonide is 2 to 3 times faster than that of 17-beclomethasone monopropionate. The relatively short elimination half-life following inhalation (about 2-3 hours) and high plasma clearance (83.7 L/h) highlight the rapid elimination of budesonide^(2,19,20,36).

Budesonide clearance was found to be about 50% higher in children than in adults. The half-life in children was significantly shorter than in adults (1.5 and 2.0 hours respectively). The high clearance and short half-life in children is an advantage in relation to possible systemic side effects when high doses of budesonide are used. The systemic availability of budesonide from an inhaled aerosol dose was similar to that observed in adults.

Budesonide has a high hepatic clearance, the value being approximately equal to the blood flow through the liver. Therefore, the higher clearance is suggestive of a higher hepatic blood flow in children than in adults⁽¹³⁾.

2.2.3 THERAPEUTIC EFFICACY:

By reducing airway inflammation, inhaled glucocorticoids consistently lessen airway hyper-responsiveness in adults and children with asthma. Long term treatment with inhaled glucocorticoids reduces airway responsiveness to histamine, cholinergic agonists, and allergens (affecting both early and late responses). Such treatment similarly lowers responsiveness to exercise, fog,

cold air, bradykinin, adenosine, and irritants such as sulfur dioxide and metabisulfites⁽³⁴⁾.

Studies confirm the generally similar efficacy of equal dosages of budesonide and beclomethsone administered by inhalation to adult patients with chronic asthma. These studies also confirm that individualised dosages of budesonide can be substituted for systemic corticosteroids in at least 40 to 50% of patients, often along with an improvement in pulmonary function or symptomatic control, particularly in those patients treated with prednisolone 5 to 7.5mg daily for less than 5 years. Long term treatment of newly diagnosed asthma patients with either inhaled budesonide 1200mcg daily or terbutaline 750mcg daily showed the inhaled corticosteroid to be more effective in increasing peak expiratory flow rate (PEFR) and in reducing symptoms, bronchial hyper-reactivity and supplemental β_2 -adrenoceptor agonist use.

Inhaled budesonide 200 and 400mcg daily were better tolerated and tended to be more effective than individually titrated dosages of slow release theophylline on the basis of changes in PEFR and supplemental β_2 -agonist requirements. These results and the finding that budesonide 400 and 800mcg daily improved the control of asthma when substituted for oral β_2 -agonist and/or theophylline, indicate that budesonide may be an effective first-line treatment for patients with mild asthma. The optimum frequency of administration of inhaled budesonide in patients whose asthma was either stable or unstable remains the subject of debate, but in clinical practice it is usually administered twice daily. Initial studies comparing an inspiratory flow-driven dry powder inhaler with a pressurised inhaler plus a large volume cone spacer suggest that the new delivery system is at least as effective as traditional systems, causes less cough immediately after inhalation and is preferred by patients⁽²⁾.

In the treatment of persistent wheezing in children aged less than 3.5 years, budesonide was effective when inhaled via a large volume spacer fitted with a facemask, but optimum dosage has to be determined individually. Studies in older children have reported that inhaled budesonide 200 to 400mcg have

similar efficacy to oral prednisolone 10mg/day, and an equal dose of beclomethasone dipropionate when administered using the same delivery system, and more effective than disodium cromoglycate 8mg daily⁽²⁾. Nebulised budesonide from a suspension containing a high concentration of the drug was consistently effective in improving the control of childhood severe asthma in anecdotal reports and non-blind trials, but results of double-blind trials have not always demonstrated superiority over placebo⁽²⁾. Further studies are needed to determine optimum dosage via this delivery method and to assess the effect on growth and adrenal function⁽²⁾.

2.2.4 TOLERABILITY:

Generally budesonide is well tolerated at usual therapeutic dosages, with local effects such as oropharyngeal candidiasis, hoarseness (dysphonia), sore throat and local irritation causing cough being reported most commonly. While they do not cause significant morbidity, they may lead diminished compliance. At dosages of 800mcg daily inhaled budesonide does not depress plasma cortisol levels, but individual sensitivity to the adrenocortical suppressive effect of inhaled corticosteroids varies. Initial data indicate no adverse effect on growth and weight gain among children treated for 1 year with inhaled budesonide 200 to 400mcg daily, but the effects of higher dosages are not known. Biopsy specimens from lung mucosa and upper respiratory tract tissues of patients treated with inhaled budesonide for up to 10 years showed reduced numbers of inflammatory cells and no evidence of atrophy after 7 to 15 months⁽²⁾.

Short term studies in healthy volunteers suggest that inhaled budesonide has a less adverse effect on bone metabolism than oral prednisolone, and less effect on indicators of bone reabsorption and mineralisation than therapeutically equivalent dosages of beclomethasone dipropionate. Long term prospective clinical trials in patients with asthma taking inhaled corticosteroids are required to determine whether the short term biochemical changes observed translate into substantial loss of bone mass⁽²⁾.

Behavioural disturbances during treatment with inhaled budesonide have been reported in young children (2 to 5 years) treated with 800 to 1200mcg daily for periods of 2 days to 3 months⁽²⁾.

2.2.5 DOSAGE AND ADMINISTRATION:

The inhaled dose of budesonide for the treatment of asthma in adults should be individualised. The recommended initial dose is 400 to 1600mcg/day divided into 2 or 4 administrations. Usually 200 to 800mcg/day is suitable for maintenance therapy but the lowest dose that leaves the patient symptom free should be used. In children with asthma the recommended dose is 200 to 400mcg/day, divided into 2 or 4 administrations⁽²⁾.

The dosage forms available for budesonide are:

- MDI, where the patient has to use an efficient inhalation technique which includes breath coordination. Budesonide MDI s are available as 50 & 200mcg/metered inhalation.
- Turbohaler (=dry powder inhaler) 100mcg/ inhalation. The turbohaler is loaded with pure drug and so contains no additives such as propellants and lubricants. This inhaler system is free of coordination problems, as it is breath-actuated. The turbulent flow of drug particles in the turbohaler makes drug release possible even at low inspiratory flow rates⁽²¹⁾.

Studies have shown that budesonide via a turbohaler was at least as effective and safe as budesonide via a pressurised MDI at daily doses of 800 and 1600mcg⁽²¹⁾. The absence of additives, the lower incidence of cough after dose intake, the easiness of use, and higher preference, combine to make turbohaler a valuable contribution in the treatment of asthmatics⁽²¹⁾.

- Respules (=single dose units for nebulisation) budesonide 250mcg/ml.

2.2.6 ANALYTICAL METHODS:

- *Immunoassay:*

Immunoassay is often used for the analysis of drugs at picomolar concentrations or less, because it is one of the most powerful techniques for determination of low concentrations of analytes in biosamples. However, antibodies used in immunological techniques often show cross-reactivity towards compounds of a similar structure as the analyte in the sample. This limits accurate determination of the analyte. If the analyte concentration in the sample is much lower than that of the cross-reacting compounds, the assay errors may be large. In drug analysis and in early phases of drug development special care must be taken to possible cross-reactivity towards metabolites of similar structure.

A method was developed by Ulf Lövgren and coworkers using micellar liquid chromatography (MLC) using tween 20 (as surfactant) for sample pretreatment preceding immunoassay in order to obtain an increased selectivity of the assay and a simplification of the sample pretreatment procedure. Different stationary phases and chromatographic conditions were studied for the separation of budesonide and cortisol and some steroids known to interfere with the immunoassay of these compounds⁽⁹⁾.

- *Thermospray liquid chromatography-mass spectrometry:*

During the last few years liquid chromatography (LC) combined with thermospray (TSP) mass spectrometry (MS) has been extensively used for the identification of polar and thermally labile compounds of biomedical interest^(10,22&24). The technique has been less widely used for quantitative analysis, possibly because careful optimization of thermospray conditions is necessary to obtain high sensitivity and reproducibility^(10,22&24). Early applications of LC-TSP-MS demonstrated it to be potentially useful as a quantitative technique, and during recent years a number of quantitative or semi-quantitative bio-analytical methods of various degrees of sophistication have been published^(10,22&24).

An automated thermospray liquid chromatography-mass spectrometry system was described by Cales Lindberg and coworkers, including an auto-sampler and a gradient liquid chromatography system controlled from the mass spectrometer data system. In this study the performance and reliability of the equipment during unattended operation were evaluated by repeated injections of standard solutions of some anti-asthmatic drugs, using deuterium-labeled analogues as internal standards. High sensitivity and reproducibility were achieved during a 19-hours run, incorporating a gradient elution and a total of 54 injections. This study proved that budesonide can be routinely measured in plasma down to 0.1nmol/L^(10,22&24).

- *HPLC:*

For analysis of drugs, the analysis system should have high speed, linearity and sensitivity. A high separation efficiency is required as well as in many cases the epimers must be separated and individually determined. Preliminary investigations indicated that high-performance liquid chromatography (HPLC) system fulfill most of the demands, whereas other chromatographic systems often used for corticosteroids, such as thin-layer chromatography (TLC), paper chromatography (PC) or gas-liquid chromatography (GLC) are inadequate with respect to speed, linearity and separation efficiency (TLC,PC) or are complicated by slow derivatization processes involving side-reactions and high temperature operation (GLC).

As the use of GLC in the study of corticosteroids is limited, HPLC has become a very important tool for the separation and determination of these compounds, particularly in the pharmaceutical industry and in clinical chemistry. Adsorption chromatography on silica, or alumina, straight- and, especially, reversed bonded phase chromatography and liquid-liquid partition chromatography have been used. Although the straight-phase mode was shown to be more effective for the separation in some cases, the reversed -phase mode was more selective for most of the compounds⁽¹⁷⁾.

M.A. Faouzi 1995 described a high performance liquid chromatographic method for the determination of budesonide in bronchoalveolar lavage in asthmatic patients. The method is simple, sensitive and selective, using an octadecylsilane column with UV absorbance detection at 250nm, and the mobile was methanol-aqueous buffer (69:31,v/v)⁽⁸⁾. This method was adopted in this study and developed further by changing the mobile phase to methanol-aqueous buffer (75:25,v/v) reducing the retention time.

2.3 PARTICLE SIZE ANALYSIS:

The efficacy of pharmaceutical inhalers and similar preparations is reliant on two main factors:

- The unit dose content i.e. the amount and uniformity of the active ingredient in the emitted dose.
- The particle size i.e. the amount of active ingredient available to the respiratory tract ⁽⁵¹⁾.

Aerosols are airborne suspensions of fine particles. The particles may be solids or liquids. Aerosol particles are heterodispersed (i.e. the particles cover a range of sizes) and the aerosol particle size distribution is best described by a log normal size distribution. Particles tend to settle (sediment), adhere to each other (coagulate) and adhere to structures such as tubing and mucosa (deposit). It is convenient to characterize aerosol particles on the basis of their aerodynamic behaviour, defined by the mass median aerodynamic diameter (MMAD). By definition, a particle distribution with an MMAD of 1µm has the same average rate of settling as a droplet of unit density and 1µm diameter.

The size of an aerosol particle and respiratory variables influence the deposition of inhaled aerosols in the airway. On one hand, particles larger than 10µm in diameter are unlikely to deposit in the lungs, while, on the other hand, particles smaller than 0.5µm are likely to reach the alveoli or may be exhaled. Therefore, particles with an aerodynamic diameter of between 1 and

5 μ m are most likely to be deposited in the lower respiratory tract and are most effective for the treatment of patients with asthma⁽³⁷⁾.

A number of factors influence the aerodynamic characteristics of a cloud of drug particles and droplets emitted from an MDI. These involve the volatility of the chlorofluorocarbons and cosolvents used in the formulation, the primary size of the micronized drug particles, the surfactant type and concentration, the size of the metering valve chamber, the velocity of the formulation when expelled from the valve, and the atomization orifice size. Thus, many formulation factors can influence the respirable dose (the dose likely to deposit in the airways) emitted from an MDI. Furthermore, during patient use, optimal delivery of drug to the airways can be achieved only by synchronization of actuation and inspiration, along with the appropriate breath-holding maneuvers⁽⁵⁴⁾.

A variety of methods have been described for assessing the fine particle pulmonary fraction of the aerosolized dose discharged from metered dose inhalers. The two basic approaches are: direct measurement of the dose fraction which penetrates through a simplified model upper respiratory tract; and measurement of the aerosol cloud particle size distribution to enable calculation of the likely deposition behaviour. Optical sizing methods based on microscopy or settling rates in stirred clouds are slow. Unfortunately, particle counters based on light scattering do not determine aerodynamic sizes, the relevant particle size parameter which controls their behaviour in the respiratory tract; those which do measure aerodynamic sizes require extreme aerosol cloud dilution and thus present more severe problems for representative sampling. All these optical methods (with the possible exception of microscopy) are unable to distinguish drug particles from foreign or excipient particles or from other drugs in combination drug products. More importantly, they do not assess or account for oropharyngeal deposition, a factor that exerts a major effect on airway deposition; thus, in such methods, sampling of the important aerosol fraction passing to the lower airways is possibly unrealistic and non-representative⁽²⁹⁾.

Advantages of optical systems⁽⁵⁶⁾:

- Different measurement of aerodynamic diameter.
- Shorter sampling time.
- Response time greater.
- Better resolution.
- Broader size range.
- Automated calculation and graphing of data.
- Ease of operation.

Limitations of optical systems⁽⁵⁶⁾:

- Only a fraction of particles sampled.
- Indirect determination of aerodynamic diameter.
- Measurement from surface of droplet only, therefore cannot differentiate components of formulation.
- Density required to calculate mass distribution.
- Calibration required for both liquid and solid particles.
- Overestimation of size distribution due to presence of volatile propellants.
- Coincidence error if particle concentration high.
- Need to dilute sample to reduce coincidence.
- Use of diluter leads to evaporation of propellants.
- Sampling at actuator vs at a distance gives variable results due to both evaporation of and variable amounts of propellant present in flume.
- Distortion of liquid droplets.
- Size underestimation due to surfactant droplets.
- Non-spherical particles may be a problem.

Inertial impactors size-fractionate aerosol particles aerodynamically according to their inertia, which increases with size. Inertial impaction analysis is probably the most reliable method for sizing therapeutic aerosols, partly because a large sample is measured consisting of the total aerosol from a number of actuations. Two further out-standing advantages of impaction methods are:

(1) Aerodynamic measurement accounts for the effects of particle shape and density on lung deposition; and (2) The aerodynamic size fractions are analyzed specifically for drug mass, independently of other aerosolized constituents, giving the aerodynamic size distribution of the drug⁽⁵⁷⁾.

In conclusion the following are the advantages of the inertia (cascade) impactors⁽⁵⁶⁾:

- Can sample the entire dose.
- Measure aerodynamic diameter.
- Mass distribution determined directly.
- Impactor stages can be analyzed for multiple tracers.
- Can separate throat dose.
- Real - time sampling.
- Cost.

Limitations of cascade impactors⁽⁵⁶⁾:

- Particle size range.
- Resolution.
- Particle bounce and re-entrainment.
- Particle fracture.
- Wall and interstage losses.
- Total mass sampled.
- Errors in sampling due to anisokinetic flow conditions.
- Evaporation of sample.
- Assumption of lognormal particle size distributions.
- Process is labour intensive.

Inertial separation techniques (which have limitations too) that determine the size of aerosol particles based on their mass and inertia are the most appropriate in-vitro methods to imitate in-vivo deposition.

The two methods that have been used to analyze the aerosol particle size of budesonide metered dose inhaler in this study are: The twin-stage impinger, and the Multi-stage liquid impinger.

2.3.1 TWIN-STAGE IMPINGER(TSI):

Developed in the laboratories of Glaxo-Wellcome group research at Ware, the Glaxo twin impinger apparatus A is a single stage impactor designed to determine the respirable percentage emitted from pressurised inhalations. The instrument is relatively simple, easy to use and rugged in construction and is ideal for routine quality control applications.

The unit shown in figure 2.1 operates on the principle of liquid impingement to divide the dose emitted from the MDI into the non-respirable dose impacting on the mouth and oropharynx which is swallowed and the remaining respirable dose.

The Two stages of the apparatus are designed such that at an airflow of 60 liters/min through the system, the effective (mean) aerodynamic particle cut-off size of the lower impinger is 6.4 microns.

The bottom of both upper and lower impingement chambers are filled with the specified amount of solvent and the inhaler then actuated for the appropriate number of times. The emitted dose is drawn through the unit at 60 liters per minute.

Both stages use the vertical impingement of the airstream onto the liquid surface i.e. the solvent in the respective chambers, to form a convenient trap for drug particles which can then be readily removed for assay.

The aerosol cloud deposits on three main impaction surfaces:

- The back of the glass throat and the upper impingement chamber that has a nominal aerodynamic particle size cut-off of approximately $6.4\mu\text{m}$ (collectively described as stage 1).
- The lower impingement chamber which collects the remaining drug and which is described as the respirable dose (stage 2).

The amount of active ingredient collected in the lower impingement chamber can now be calculated and expressed as a fraction or percentage (respirable fraction or percentage) of the mean results obtained in the determination of uniformity of unit spray content^(38&51).

The cut-off point characteristics of the twin impinger show no great sensitivity to operating variables which might be expected to occur. Air flow rate, collecting fluid volume, or fluids of varying density, solvency, and surface tension were found not to influence the cut-off point size, and good agreement was found between two separate units.

Limitations arise from the fact that the twin impinger divides the total spray into only two components. Because of this, the instrument can not discriminate amongst distributions with specific combinations of varying size and width. Therefore, the interpretation of twin impinger data is inherently ambiguous⁽³⁰⁾.

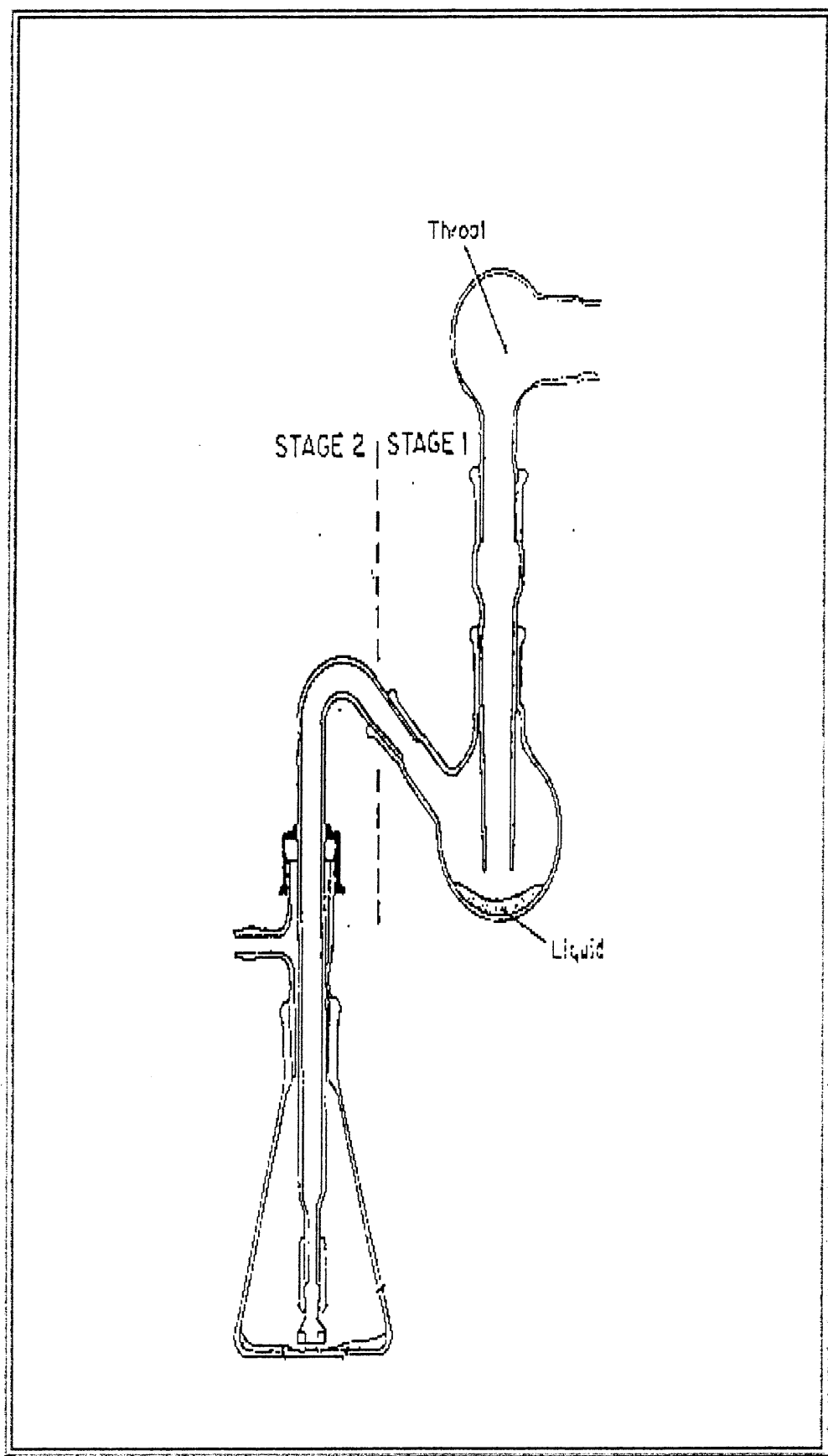


Figure 2.1: The Twin Stage Impinger .

2.3.2 MULTI-STAGE LIQUID IMPINGER:

Multi-stage inertial impaction is a preferred method for measuring particle size distribution for all inhalers with agreement also on the specification of one identical apparatus and method between USP and EP, namely the Multi-Stage Liquid Impinger (MSLI)⁽⁵¹⁾.

The Astra Multi-Stage Liquid Impinger (MSLI) shown in figure 2.2 is a versatile five stage cascade impactor which can be used for determining the particle size (aerodynamic size distribution).

The design is such that at a flow rate of 60 liters per minute, the effective cut-off diameters at stages 1, 2, 3, and 4 are 13, 6.8, 3.1 and 1.7 microns respectively. The fifth and final stage comprises an integral filter to capture the fraction of particles with a size less than 1.7 microns. The MSLI has, by definition, no inter-stage losses and is suitable for use throughout the range 30-100 liters per minute⁽⁵¹⁾.

Each stage consists of a single jet and impaction plate and is filled with a suitable solvent to wet the impaction plate and dissolve the active substance.

The apparatus is constructed from glass, and stainless steel or aluminium machined to close tolerances in order to guarantee reproducibility between differing instruments.

The respirable dose is the total mass of the drug found on the impaction plates of the relevant impactor stage (that captured the drug in the particle size relevant to the drug being tested).

By constructing a "Cumulative % found - less than stated particle size" table, it is now possible to calculate an estimate of the mass median aerodynamic diameter (MMAD), that is to say, the particle size which relates

graphically to 50% of the total drug mass collected and hence the Geometric Standard Deviation (GSD)⁽³⁸⁾.

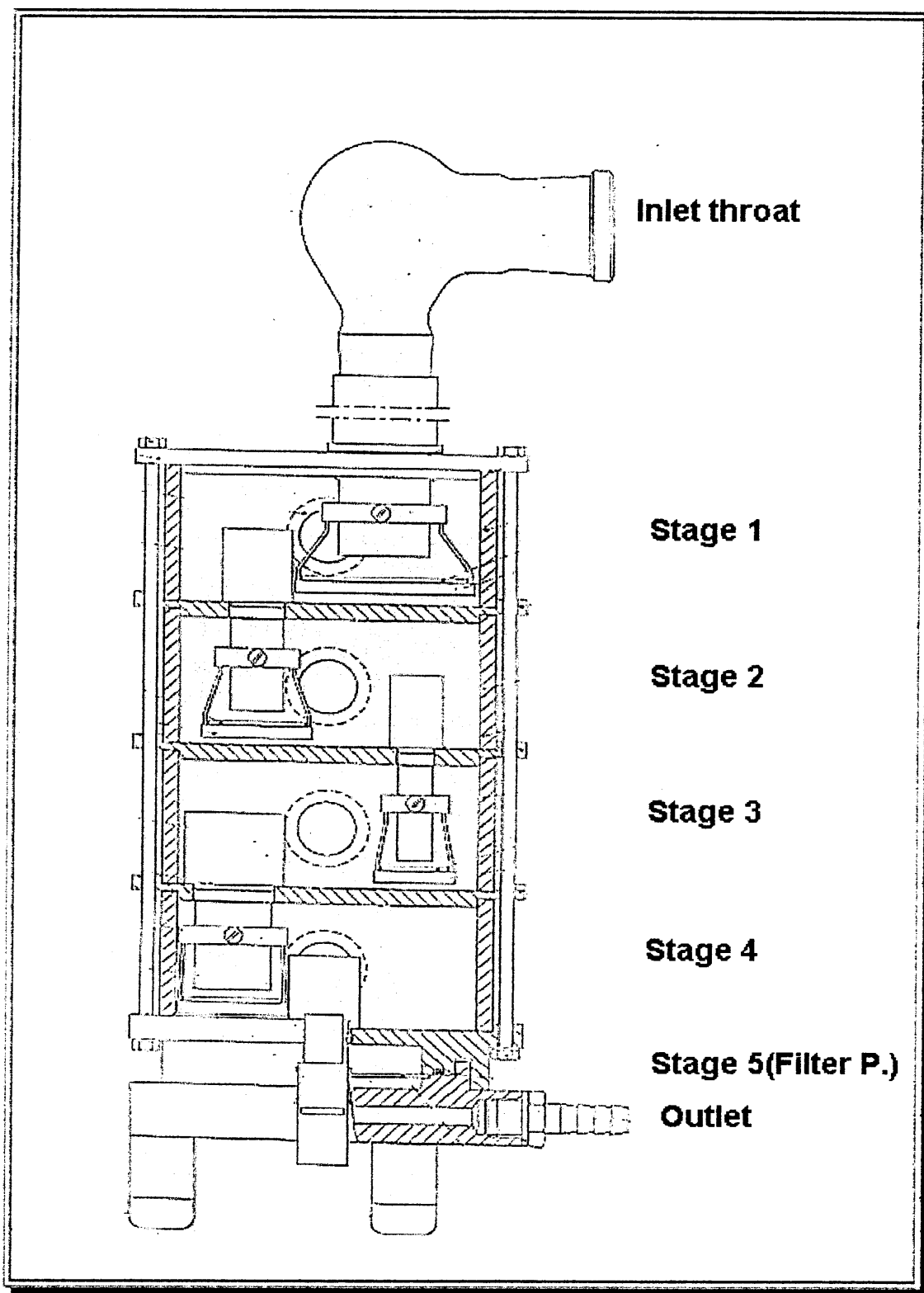


Figure 2.2: The modified Multistage Liquid Impinger (MSLI).

CHAPTER THREE

3. Characteristics of the aerodynamic particle size distribution of a budesonide 200 µg/dose metered dose inhalers:

3.1 MATERIALS:

Reagents: Methanol and glacial acetic acid (HPLC grade) were obtained from BDH laboratory supplies; Budesonide (200µg) metered dose inhalers, lot. numbers XB 661 & XD 665 were obtained from Astra Pharmaceuticals. Budesonide standards were prepared using budesonide obtained from the Sigma Chemical Company. Methylprednisolone sodium succinate, lot. number E23815 was obtained from Upjohn Ltd.

Solutions: A stock solution of budesonide 200 mg/L was prepared by dissolving an accurately weighed quantity of budesonide in 100 ml of 70% methanol in water. Serial dilution was used to prepare different concentrations which varied from 0.5 - 7.5mg/L. A stock solution of methylprednisolone 50mg/L was prepared by dissolving an accurately weighed quantity of methylprednisolone in 250 ml of 70% methanol in water. To each budesonide solution a known quantity of methylprednisolone solution was added so that each solution contained 5mg/L methylprednisolone as the internal standard.

Mobile phase for HPLC: A 0.1% glacial acetic acid solution was prepared and mixed with methanol in a ratio of 1:4 (0.1 % acetic acid solution : methanol). The mobile phase was then filtered and degassed for 20 minutes.

Instrumentation: The HPLC solvent delivery pump used was a Shimadzu LC-6A (Shimadzu corporation, Japan). The column (stationary phase) was Zorbax ODS 5µm 25cm (Jones Chromatography, UK). The UV detector was Gilson model 116 (Gilson, USA). The integrator is Hewlett Packard 3394A (Hewlett Packard). The Glaxo twin stage impinger apparatus A (TSI) and Astra

Draco modified multistage liquid impinger (MSLI) were obtained from Copley Instruments Nottingham UK.

3.2 METHOD:

3.2.1 HPLC ASSAY CONDITIONS:

The HPLC method that was developed by Faouzi and coworkers⁽⁸⁾ was adapted in this study, and developed further. In this method an octadecylsilane (C18) column was used, and 75% methanol in 0.1% glacial acetic acid in water as a mobile phase at a flow rate of 1ml/min with UV detection at 250nm. Methylprednisolone was used as the internal standard.

Budesonide retention time: 4.7-5.7 minutes.

Methylprednisolone retention time: 3.4-3.9 minutes.

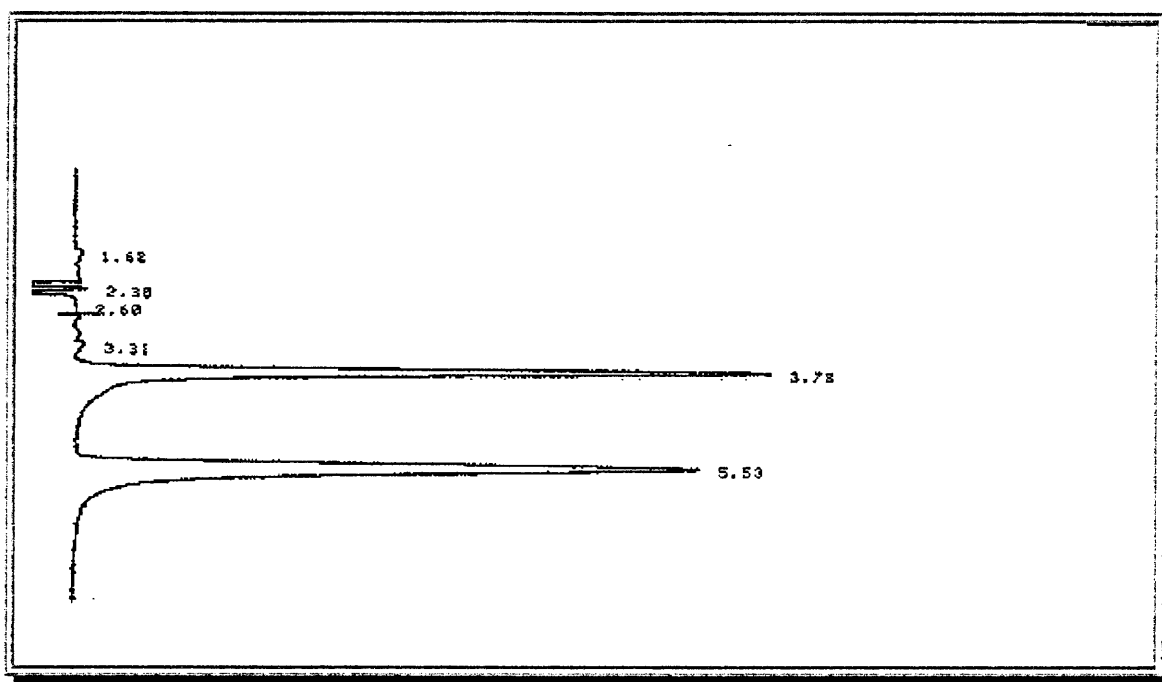


Figure 3.1: Chromatogram of a standard solution of budesonide 5 mg/L & methylprednisolone 5 mg/L.

3.2.2 ASSAY VALIDATION:

The HPLC method was fully validated prior to analysis of the samples. To determine the linearity of response, the peak height ratios over the expected budesonide concentration range 0.5-7.5mg/L were ascertained. For each day of analysis a calibration curve was constructed. In all cases the correlation coefficient was better than 0.99. Five samples were run consecutively, followed by a random standard from concentrations 0.5, 1.0, 2.5, 5.0, 7.5 mg/L as a quality control check. Intra- and inter-day accuracy and precision were determined using standard concentrations of budesonide, and the results are shown in the tables 3.1 and 3.2.

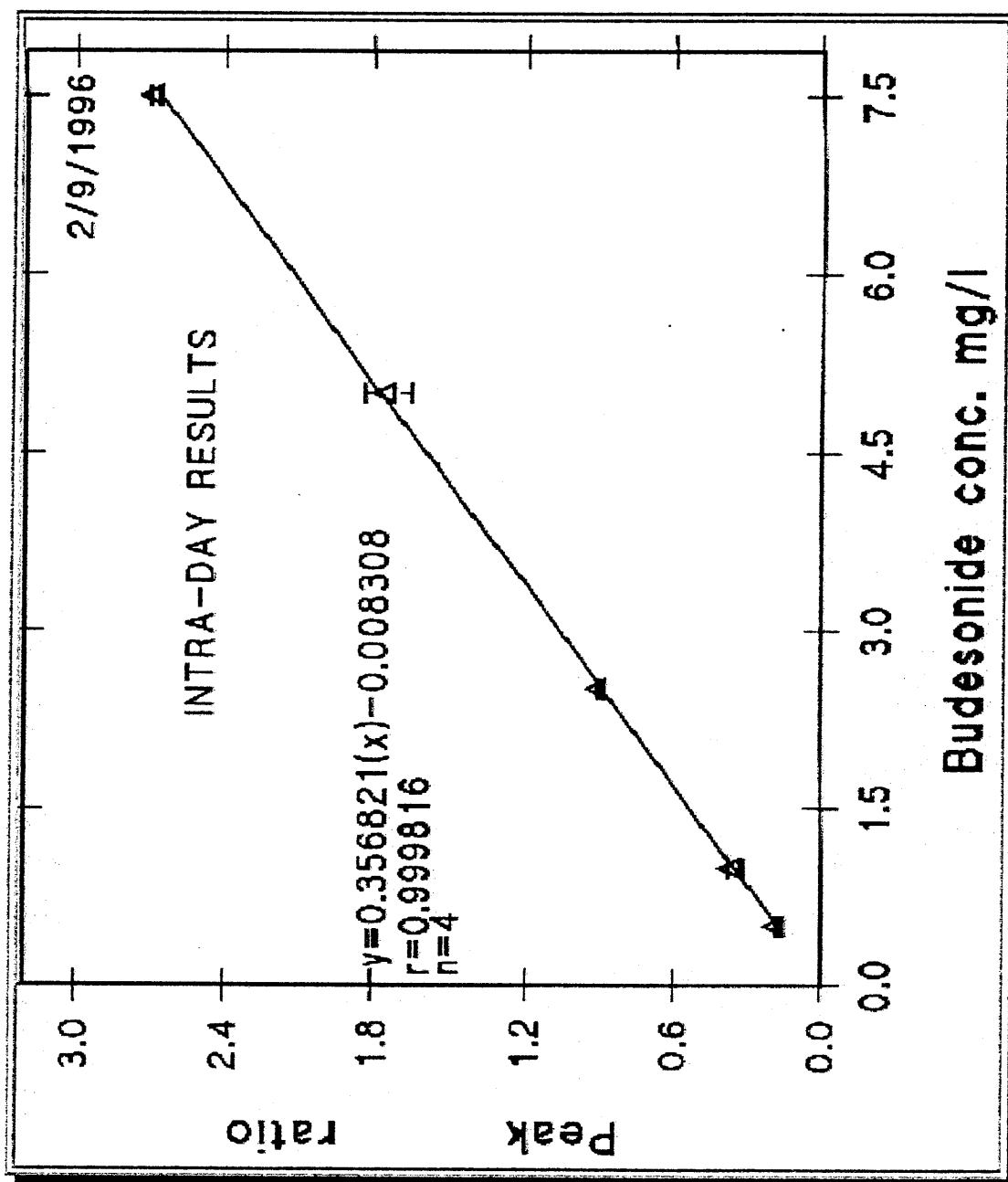


Figure 3.2: Intra-day calibration graph of budesonide.

Table 3.1: Intra-day precision & accuracy

$y=0.356821(x)+0.008308$ $r=0.999816$ $n=4$			
Conc. added	Conc. found (Mean±SD)	CV%	%Difference
0.5 mg/L	0.508 ± 0.04123	3.7	1.6
1.0 mg/L	1.01 ± 0.0897	6.75	1
2.5 mg/L	2.529 ± 0.0466	0.93	1.16
5.0 mg/L	4.91 ± 0.2663	4.98	1.8
7.5 mg/L	7.559 ± 0.0606	0.495	0.787

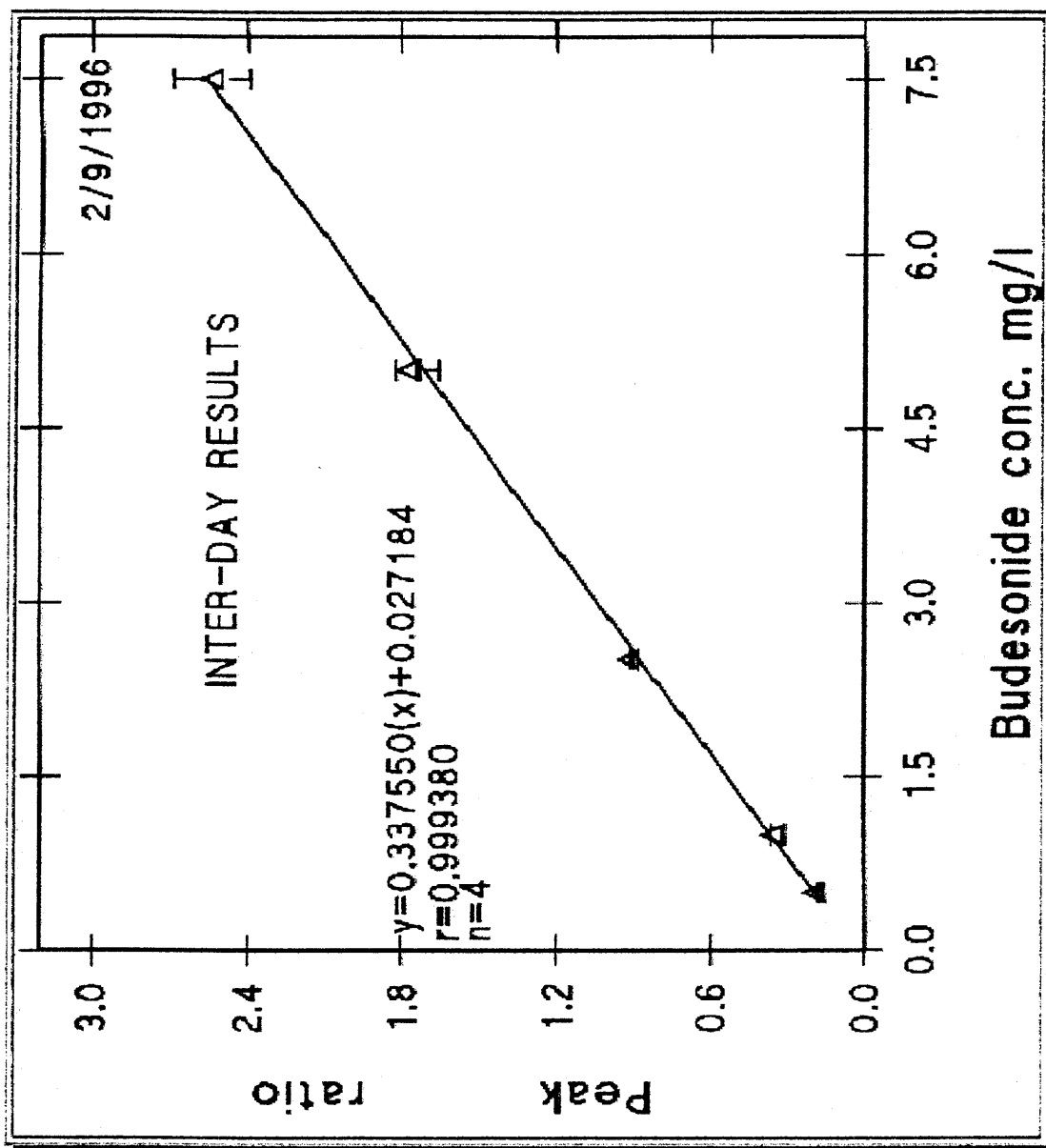


Figure 3.3: Inter-day calibration graph of budesonide.

Table 3.2: Inter-day precision & accuracy

$y=0.337550(x)+0.027184$ $r=0.999380$ $n=4$			
Conc. added	Conc. found (Mean +SD)	CV%	%Difference
0.5 mg/L	0.45 ± 0.066	2.75	10
1.0 mg/L	0.94 ± 0.0254	5.43	6
2.5 mg/L	2.59 ± 0.0697	0.41	3.6
5.0 mg/L	5.13 ± 0.1061	3.58	2.6
7.5 mg/L	7.4 ± 0.299	5.08	1.33

3.2.3 FINE PARTICLE DOSE ESTIMATION USING THE TSI:

The Glaxo twin stage impinger apparatus A was operated at 60 L/min for the estimation of fine particle dose with sufficient solvent in each stage.

Air was drawn through the apparatus by a vacuum pump (standard pump module for 0-100L/min, GAST Brook Crompton Doncaster, UK) as measured with a flow meter (GAP meter, UK). A stopwatch was used to time all intervals to ensure uniformity throughout the experiment.

Appropriate volumes of solvent (70% methanol in water) were added to the stage 1 (round bottomed) flask, and to the stage 2 (flat bottomed) flask. The assembly was ensured to be vertical, the whole apparatus adequately supported, and the jet spacer peg of the lower jet assembly just touched the bottom of the lower impingement chamber. The pump was connected to the outlet of the apparatus and airflow through the apparatus was adjusted, as measured at the inlet to the throat, to 60 ± 2 L/min. The airflow was then switched off. The adapter was placed in position at the end of the throat so that the mouth piece of the inhaler, when inserted, lined up along the horizontal axis of the throat and the inhaler unit was positioned in the same orientation as the intended use. The metering valve was primed by shaking for about 30 seconds and discharged once to waste, shaken again and after not less than 5 seconds discharged again to waste. The MDI was primed using a different actuator prior to use. The valve was then rinsed with methanol and dried using an air line. The pump to the apparatus was then switched on, the airflow set to 60 ± 2 L/min, the mouth piece end of the actuator was located in the adapter and after 20 seconds, the assembled inhaler was shaken for not less than 5 seconds and first puff was delivered into the apparatus. After a further 20 seconds, the assembled inhaler was removed from the adapter, shaken for not less than 5 seconds, the mouth piece end of the actuator relocated and a second puff delivered. The process was repeated until the desired number of puffs were delivered and the pump in each case switched off 30 seconds after the last puff was delivered.

To each volumetric flask a known volume of methylprednisolone solution (as internal standard) was added so that each final solution contained 5 mg/L methylprednisolone.

7ml of the solvent was added to stage 1 and 20ml to stage 2. The actuator and the valve were rinsed with appropriate volumes of the solvent and the washings transferred to a suitable volumetric flask and made up to volume with solvent (see table 3.3). The inlet throat, mouthpiece adapter, and the round bottom flask were removed from the apparatus and rinsed with appropriate volumes of the solvent. The solution was then transferred to a volumetric flask and made up to volume with the solvent. The stage 2 (the flat bottomed flask, and the coupling tube) was rinsed with appropriate volume of the solvent. The solution was then transferred to a volumetric flask and made up to volume with the solvent. Each fraction of the three samples and standard of budesonide solutions were injected into the HPLC system.

Table 3.3: Volume of the volumetric flasks (ml) used in the twin stage impinger

No. of Puffs	MDI	Stage 1	Stage 2
1 Puff	25	50	50
2 & 5 Puffs	50	100	100

3.2.4 PARTICLE SIZE ANALYSIS USING MULTI-STAGE LIQUID IMPINGER:

The Astra Draco modified multistage liquid impinger (Copley Instruments Nottingham UK) operated at 60 L/min was used for the estimation of the particle size distribution with 20 ml solvent added to each stage.

Air was drawn through the apparatus by a vacuum pump (standard pump module for 0-100L/min, GAST Brook Crompton Doncaster, UK) as measured with flow meter (GAP meter, UK). A stopwatch was used to time all intervals to ensure uniformity throughout the experiment.

Appropriate volumes of solvent (70% methanol in water) were added into each of the four upper stages of the apparatus. A suitable glass fiber filter (Whatman glass microfibre filter GF/F 7.0 cm) was placed in stage five. The pump was connected to the outlet of the apparatus and airflow through the apparatus was adjusted, as measured at the inlet to the throat, to 60 ± 2 L/min. The airflow was then switched off. The adapter was placed in position at the end of the throat so that the mouthpiece of the inhaler, when inserted, lined up along the horizontal axis of the throat and the inhaler unit was positioned in the same orientation as the intended use. The metering valve was primed by shaking for about 30 seconds and discharged once to waste, shaken again and after not less than 5 seconds discharged again to waste. The MDI was primed using a different actuator prior to use. The valve was then rinsed with methanol and dried using air line. The pump to the apparatus was then switched on, the airflow set to 60 ± 2 L/min, the mouthpiece end of the actuator was located in the adapter and after 20 seconds, the assembled inhaler was shaken for not less than 5 seconds and first puff was delivered into the apparatus. After a further 20 seconds, the assembled inhaler was removed from the adapter, shaken for not less than 5 seconds, the mouth piece end of the actuator relocated and a second puff delivered. The process was repeated until the desired number of puffs were delivered and the pump in each case switched off 30 seconds after the last puff was delivered.

To each volumetric flask a known volume of methylprednisolone solution (as internal standard) was added so that each final solution contained 5 mg/L methylprednisolone.

The actuator and the valve were rinsed with appropriate volumes of the solvent and the washings transferred to a suitable volumetric flask and made up to volume with solvent (see table 3.4). The inlet throat, and mouthpiece adapter were removed from the apparatus and rinsed with appropriate volumes of the solvent. The solution was then transferred to a volumetric flask and made up to volume with the solvent. Stage 5 was dismantled, the filter paper carefully removed and placed onto a plate. A suitable volume of the solvent was added

and the plate left for a while then shaken to ensure complete extraction. The contents of the plate were transferred to a volumetric flask and made up to volume with the solvent. The inside of the inlet jet to stage 1 was also rinsed with the solvent in stage 1, and the solvent was allowed to flow back in the stage. The budesonide was extracted from the inner walls and the collection plate of each of the four upper stages of the multistage impinger by tilting and rotating it, and observing that no liquid transfer had occurred between the stages. The contents of each stage were transferred to volumetric flasks and made up to volume with the solvent. Each fraction of all the seven samples and standard of budesonide solutions were injected into the HPLC system.

Table 3.4: Volume of the volumetric flasks (ml) used in the multistage impinger

MDI	Throat	Stage 1	Stage 2	Stage 3	Stage 4	Filter paper
50	100	50	50	50	50	25

3.3. RESULTS:

The concentration of the budesonide in the different samples was determined by comparing the peak height ratios obtained with that of standard budesonide concentration.

3.3.1 FINE PARTICLE DOSE ESTIMATION USING THE TSI:

Figures 3.4, 3.5, and 3.6 shows the chromatograms of the budesonide recovery from 5 puffs MDI in the actuator, Stage 1, and Stage 2.

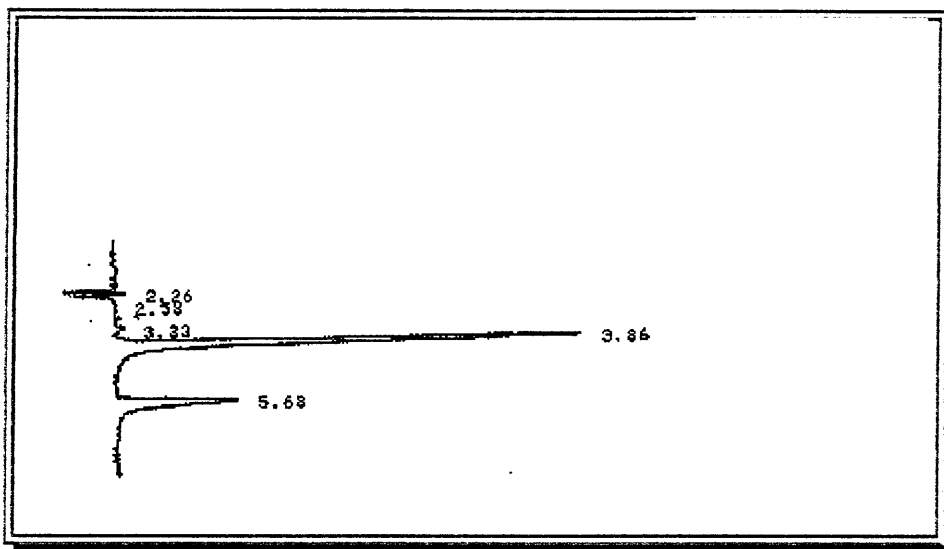


Figure 3.4: Chromatogram of the actuator recovery of 5 puffs budesonide MDI + methylprednisolone in the TSI.

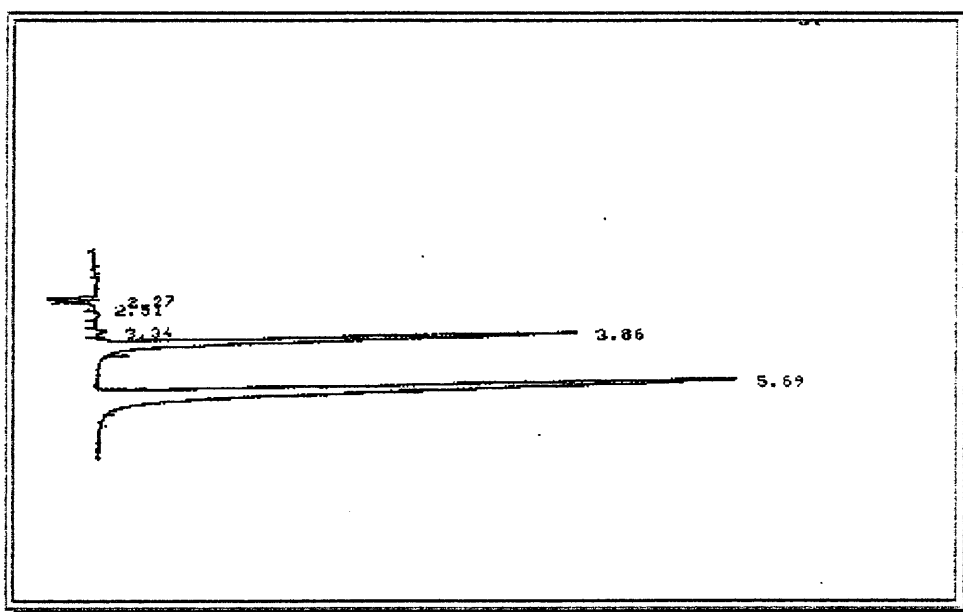


Figure 3.5: Chromatogram of stage 1 recovery of 5 puffs budesonide MDI + methylprednisolone in the TSI.

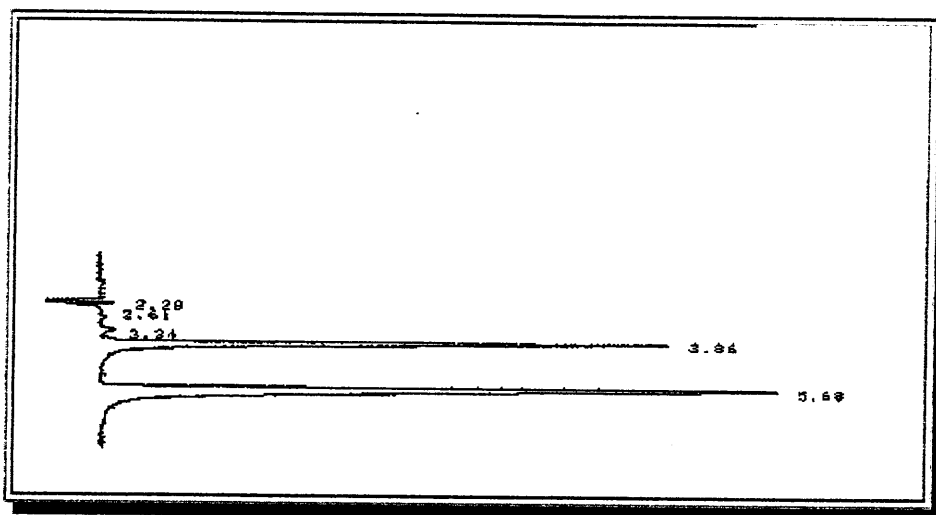


Figure 3.6: Chromatogram of stage 2 recovery of 5 puffs budesonide MDI + methylprednisolone in the TSI.

The fine particle dose of budesonide MDI, estimated using the twin stage impinger, for one actuation gave a mean recovery of 117.4 % (SD=6.47, n=10). The distribution onto the stages are shown in table 3.5. The theoretical total recovery of budesonide should be 200 µg.

Table 3.5: Particle size distribution of 1 puff using the twin stage impinger.

	MDI (µg)	Stage 1 (µg)	Stage 2 (µg)	Total (µg)	% recovery	% MDI	% S1	% S2
Run 1	12.35	114.95	85.94	213.24	106.6	5.79	53.91	40.3
Run 2	11.58	106.68	120.97	239.23	119.6	4.84	44.59	50.57
Run 3	12.07	124.34	83.28	219.69	109.9	5.49	56.6	37.91
Run 4	15.79	88.46	125.04	229.29	114.7	6.89	38.58	54.53
Run 5	12.84	78.23	131.62	222.69	111.4	5.77	35.13	59.11
Run 6	13.12	112.71	120.27	246.1	123.1	5.33	45.8	48.87
Run 7	12.07	104.02	121.39	237.48	118.7	5.08	43.8	51.12
Run 8	10.6	125.18	115.23	251.01	125.5	4.22	49.87	45.91
Run 9	15.79	107.24	131.48	254.51	127.3	6.2	42.14	51.66
Run 10	17.19	93.23	124.06	234.48	117.2	7.33	39.76	52.91
Mean	13.34	105.50	115.92	234.77	117.4	5.68	44.94	49.38
SD	2.05	14.40	16.36	12.95	6.47	0.88	6.44	6.08
CV%	15.37	13.65	14.11	5.52	5.5	15.49	14.33	12.31

Two actuations gave a mean recovery of 111.92 % (SD=4.36, n=10). The distribution onto the stages of the twin stage impinger are shown in table 3.6. The theoretical total recovery of budesonide should be 400 µg.

Table 3.6: Particle size distribution of 2 puffs using the twin stage impinger.

	MDI (µg)	Stage 1 (µg)	Stage 2 (µg)	Total (µg)	% Recovery	% MDI	% Stage 1	% Stage 2
Run 1	20.5	229.89	222.61	473	118.3	4.33	48.6	47.06
Run 2	20.64	224.57	211.4	456.61	114.2	4.52	49.18	46.3
Run 3	22.74	210.56	216.16	449.46	112.4	5.06	46.85	48.09
Run 4	23.87	208.03	194.02	425.92	106.5	5.6	48.84	45.55
Run 5	20.08	233.79	219.8	473.67	118.4	4.24	49.36	46.4
Run 6	25.27	193.18	217	435.45	108.9	5.8	44.36	49.83
Run 7	24.15	254.28	184.77	463.2	115.8	5.21	54.9	39.89
Run 8	20.64	199.91	204.67	425.22	106.3	4.85	47.01	48.13
Run 9	17.84	229.05	186.17	433.06	108.3	4.12	52.89	42.99
Run 10	21.9	188.42	230.17	440.49	110.1	4.97	42.78	52.25
Mean	21.763	217.168	208.68	447.61	111.92	4.86	48.52	46.62
SD	2.14	19.59	14.90	17.42	4.36	0.54	3.41	3.26
CV%	9.83	9.02	7.14	3.89	3.9	11.11	7.03	6.99

Five actuations gave a mean recovery of 114.62 % (SD=6.17, n=10). The distribution onto the stages of the twin stage impinger are shown in table 3.7. The theoretical total recovery of budesonide should be 1000 µg.

Table 3.7: Particle size distribution of 5 puffs using the twin stage impinger.

	MDI (µg)	Stage 1 (µg)	Stage 2 (µg)	Total (µg)	% Recovery	% MDI	% Stage 1	% Stage 2
Run 1	59.6	544.9	492.77	1097.27	109.7	5.43	49.66	44.91
Run 2	66.6	616.36	456.62	1139.58	114	5.84	54.09	40.07
Run 3	61.56	492.21	584.69	1138.46	113.9	5.41	43.24	51.36
Run 4	53.71	667.93	440.92	1162.56	116.3	4.62	54.45	37.93
Run 5	46.99	672.41	570.68	1290.08	129	3.64	52.12	44.24
Run 6	43.62	623.93	501.18	1168.73	116.9	3.73	53.39	42.88
Run 7	53.85	582.73	478.2	1114.78	111.5	4.83	52.27	42.9
Run 8	39.7	596.74	561.15	1197.59	119.8	3.32	49.83	46.86
Run 9	49.79	544.06	496.69	1090.54	109.1	4.57	49.89	45.55
Run 10	54.97	494.17	510.43	1059.57	106	5.19	46.64	48.17
Mean	53.039	583.544	509.333	1145.92	114.62	4.63	50.92	44.45
SD	7.86	61.06	45.89	61.81	6.17	0.810	3.34	3.68
CV%	18.82	10.46	9.01	5.39	5.38	17.5	6.56	8.28

3.3.2 PARTICLE SIZE ANALYSIS USING THE MSLI:

Fine particle dose of budesonide MDI was estimated using the multi-stage liquid impinger. Figures 3.7 to 3.13 shows the chromatograms of the budesonide recovery from 5 puffs MDI in the actuator, throat, and different stages of the multistage impinger.

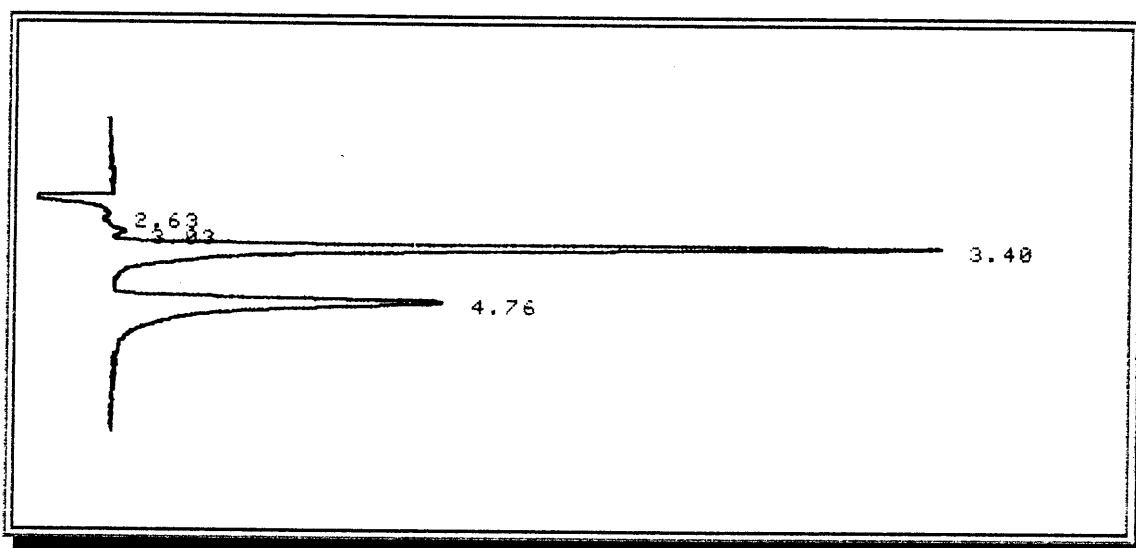


Figure 3.7: Chromatogram of the actuator recovery of 5 puffs budesonide MDI + methylprednisolone in the MSLI.

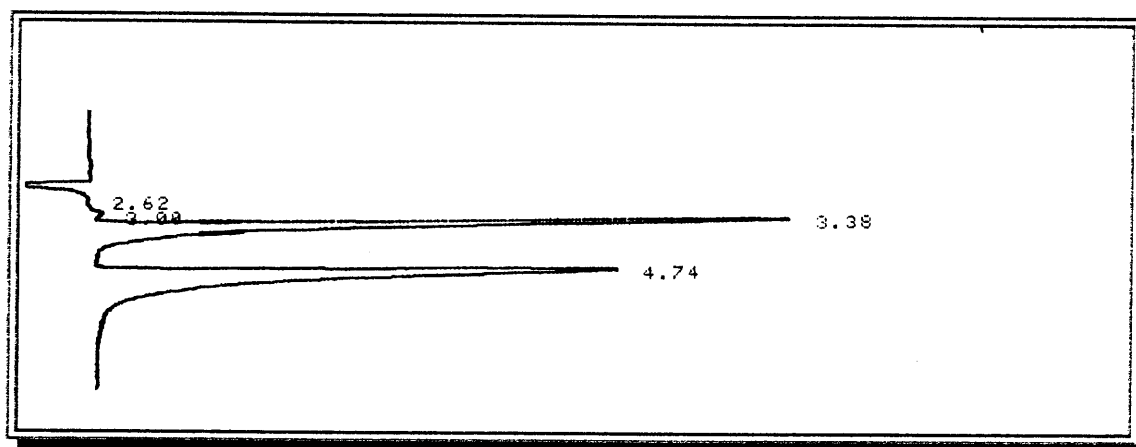


Figure 3.8: Chromatogram of the throat recovery of 5 puffs budesonide MDI + methylprednisolone in the MSLI.

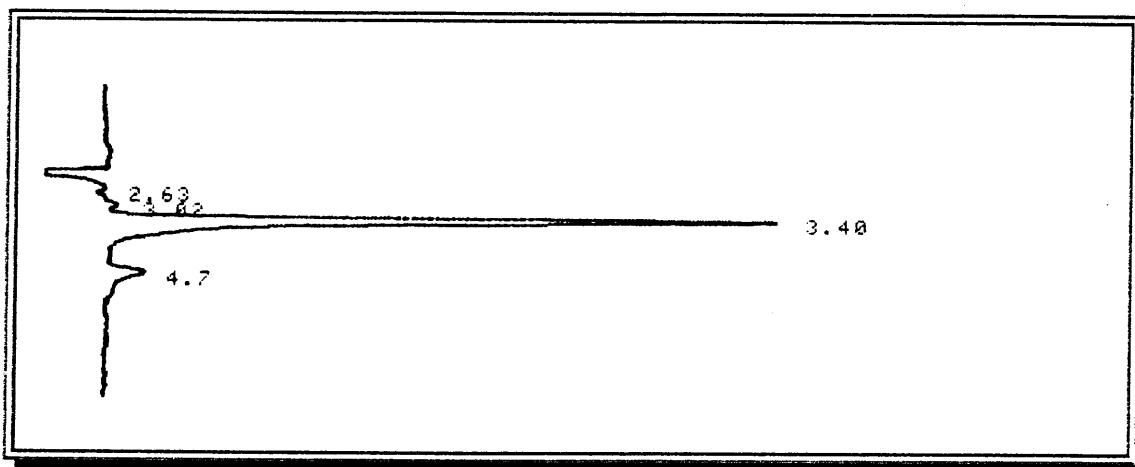


Figure 3.9: Chromatogram of stage 1 recovery of 5 puffs budesonide MDI + methylprednisolone in the MSLI.

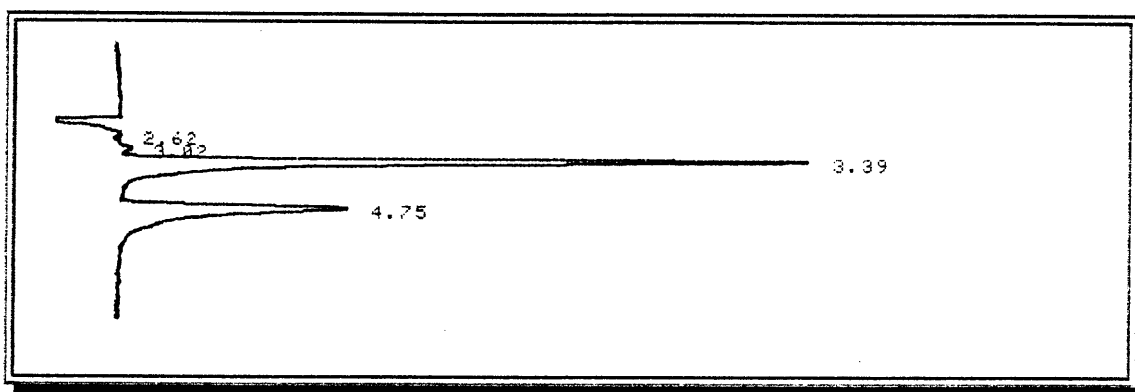


Figure 3.10: Chromatogram of stage 2 recovery of 5 puffs budesonide MDI + methylprednisolone in the MSLI.

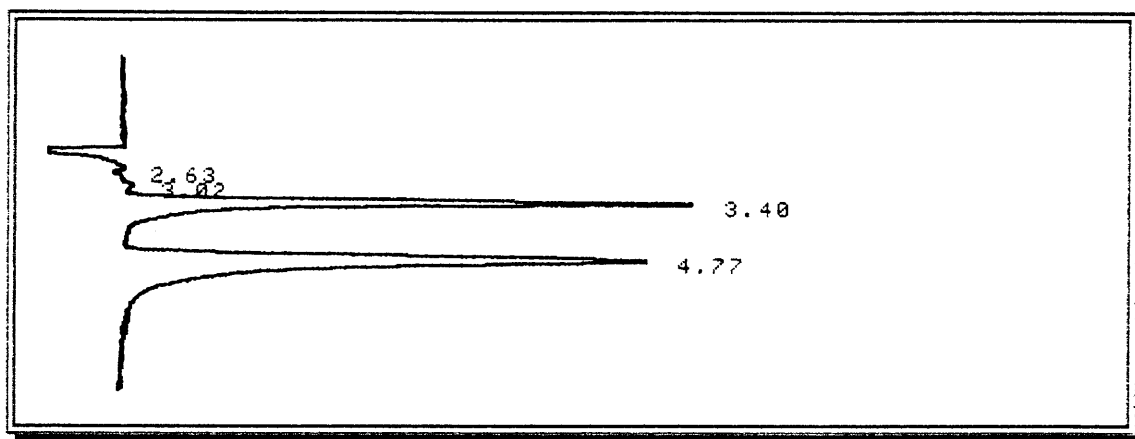


Figure 3.11: Chromatogram of stage 3 recovery of 5 puffs budesonide MDI + methylprednisolone in the MSLI.

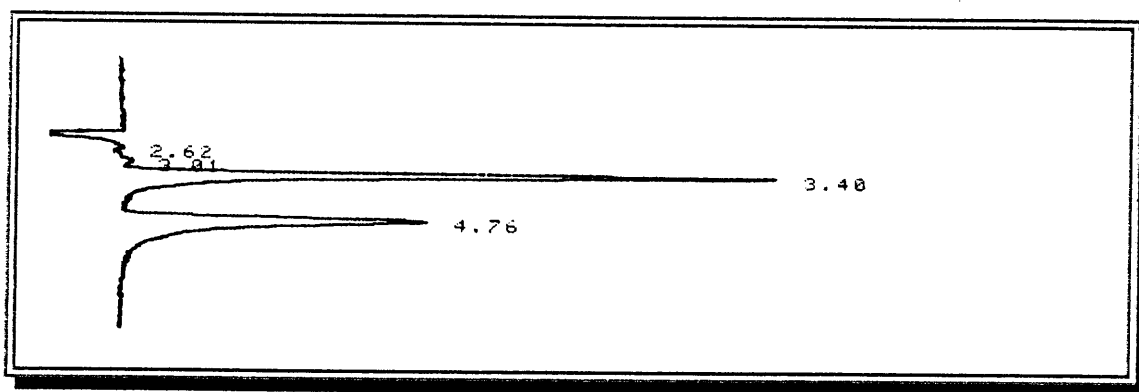


Figure 3.12: Chromatogram of stage 4 recovery of 5 puffs budesonide MDI + methylprednisolone in the MSLI.

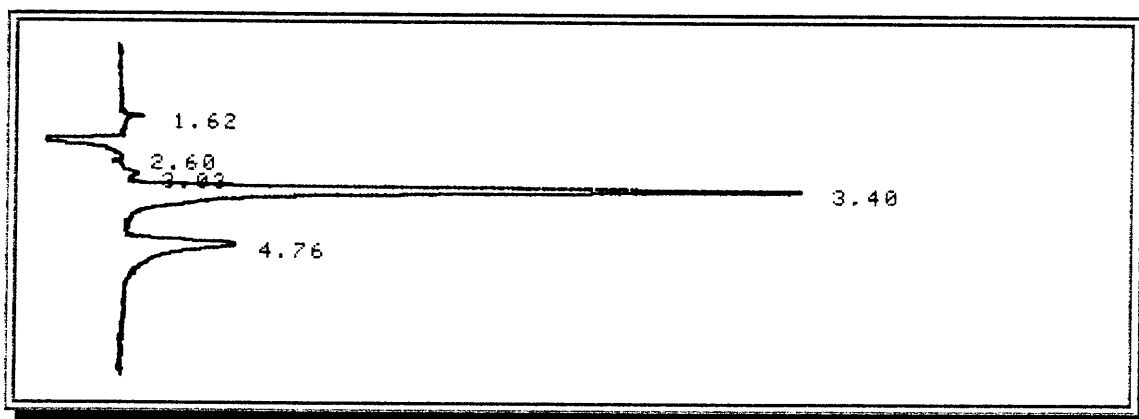


Figure 3.13: Chromatogram of the filter paper recovery of 5 puffs budesonide MDI + methylprednisolone in the MSLI.

Using the multi-stage liquid impinger one actuation from the budesonide MDI gave a mean recovery of 123.5 % (SD=12.5, n=5). The particle size distribution onto the stages of the multi-stage liquid impinger are shown in table 3.8. The theoretical total recovery of budesonide should be 200 µg.

Table 3.8: Particle size distribution of 1 puff using the multi-stage impinger.

Stage	Run 1	Run 2	Run 3	Run 4	Run 5	Mean	SD	CV%
MDI	20.41	22.11	25.77	24.82	28.55	24.332	2.84	11.69
Throat	79.79	76.19	87.68	94.48	84.33	84.494	6.34	7.5
Stage 1	7.81	3.56	0	5.62	8.2	5.038	3.02	59.94
Stage 2	18.84	17.19	22.63	22.04	19.82	20.104	2.01	10.02
Stage 3	60.98	60.26	76.06	102.7	63.96	72.792	16.00	21.98
Stage 4	32.76	30.85	32.42	36.44	31.83	32.86	1.90	5.79
FP	9.28	8.88	7.86	5.53	4.91	7.292	1.76	24.2
Blank FP	0	0	0	0	0	0	0	0
Total	229.87	219.04	252.42	291.63	241.6	246.912	25.00	10.13
% Recovery	114.9	109.5	126.2	145.8	120.8	123.5	12.50	10.12

Two actuations from the budesonide MDI into the multi-stage liquid impinger gave a mean recovery of 118.26 % (SD=11, n=5). The particle size distribution onto the stages are shown in table 3.9. The theoretical total recovery of budesonide should be 400 µg.

Table 3.9: Particle size distribution of 2 puffs using the multi-stage impinger.

	Run 1	Run 2	Run 3	Run 4	Run 5	Mean	SD	CV%
MDI	45.77	54.06	47.63	40.44	69.15	51.41	9.88	19.22
Throat	195.02	175.24	191.86	154.94	138.09	171.03	21.78	12.73
Stage 1	0	0	8.56	0	9.61	3.634	4.46	122.82
Stage 2	42.89	48.12	35.34	33.12	43.61	40.616	5.56	13.69
Stage 3	144.59	151.77	110.08	101.88	137.07	129.078	19.60	15.18
Stage 4	68.76	73.25	62.01	53.17	68.58	65.154	6.98	10.72
FP	16.83	10.42	10.01	9.91	13.41	12.116	2.69	22.17
Blank FP	0	0	0	0	0	0	0	0
Total	513.86	512.86	465.49	393.46	479.52	473.038	44.01	9.3
%Recovery	128.47	128.22	116.37	98.37	119.88	118.26	11.00	9.3

Five actuations from the budesonide MDI into the multi-stage liquid impinger gave a mean recovery of 102.69 % (SD=12.79, n=5). The particle size distribution onto the stages are shown in table 3.10. The theoretical total recovery of budesonide should be 1000 µg.

Table 3.10: Particle size distribution of 5 puffs using the multi-stage impinger.

	Run 1	Run 2	Run 3	Run 4	Run 5	Mean	SD	CV%
MDI	112.86	89.25	86.47	101.22	113.33	100.626	11.32	11.25
Throat	571.97	451.69	402.42	389.74	373.2	437.804	72.01	16.45
Stage 1	23.58	11.74	22.22	14.6	18.04	18.036	4.47	24.78
Stage 2	92.24	75.25	91.94	84.67	93.12	87.444	6.81	7.79
Stage 3	319.7	202.51	189.67	234.94	236.71	236.706	45.33	19.15
Stage 4	128.48	128.48	110.08	120.72	121.94	121.94	6.75	5.54
FP	30.58	24.42	22.55	21.55	22.48	24.316	3.27	13.45
Blank FP	0	0	0	0	0	0	0	0
Total	1279.41	983.34	925.35	967.44	978.82	1026.872	127.92	12.46
%Recovery	127.94	98.33	92.54	96.74	97.88	102.69	12.79	12.46

Ten actuations from the budesonide MDI into the multi-stage liquid impinger gave a mean recovery of 88.91% (SD=3.19, n=5). The particle size distribution onto the stages are shown in table 3.11. The theoretical total recovery of budesonide should be 2000 µg.

Table 3.11: Particle size distribution of 10 puffs using the multi-stage impinger.

	Run 1	Run 2	Run 3	Run 4	Run 5	Mean	SD	CV%
MDI	76.93	162.55	182.06	206.32	190.34	163.64	45.59	27.86
Throat	1055.93	818.82	806.04	952.84	806.13	887.952	100.61	11.33
Stage 1	29.68	37.81	43.95	33.23	22.78	33.49	7.17	21.41
Stage 2	159.98	166.01	172.32	151.06	116.44	153.162	19.65	12.83
Stage 3	294.2	260.7	353.31	326.49	353.74	317.688	35.90	11.3
Stage 4	167.53	204.64	174.41	149.96	198.44	178.996	20.16	11.26
FP	56.19	38.47	42.56	37.18	42.33	43.346	6.76	15.6
Blank FP	0	0	0	0	0	0	0.00	0
Total	1840.44	1689	1774.65	1857.08	1730.2	1778.274	63.83	3.59
%Recovery	92.02	84.45	88.73	92.85	86.51	88.91	3.19	3.59

3.3.3 DESCRIPTIVE STATISTICS OF THE PARTICLE SIZE DISTRIBUTION OBTAINED FROM THE MSLI:

A collective summary of the results for the particle size distribution of 1, 2, 5, and 10 puffs of budesonide MDI using the multi-stage impinger is shown in table 3.12.

Table 3.12: Mean (SD) deposition (in μg) of budesonide on the different stages of the MSLI following 1, 2, 5, and 10 puffs.

	1 Puff	2 Puffs	5 Puffs	10 Puffs
MDI	24.332 (2.84)	51.41 (9.88)	100.626 (11.33)	163.64 (45.59)
Throat	84.494 (6.34)	171.03 (21.78)	437.804 (72.01)	887.952 (100.61)
Stage 1 (13 - 25 μm)	5.038 (3.02)	3.634 (4.46)	18.036 (4.47)	33.49 (7.17)
Stage 2 (6.8 - 13 μm)	20.104 (2.02)	40.616 (5.56)	87.444 (6.81)	153.162 (19.66)
Stage 3 (3.1 - 6.8 μm)	72.79 (16)	129.08 (19.6)	236.706 (45.33)	317.688 (35.9)
Stage 4 (1.7 - 3.1 μm)	32.86 (1.9)	65.154 (6.98)	121.94 (96.75)	178.996 (20.16)
FP (<1.7 μm)	7.292 (1.77)	12.116 (2.69)	24.316 (3.27)	43.346 (6.76)
Total	246.912 (25)	473.04 (44.01)	1026.872 (127.92)	1778.274 (63.83)

The mean (SD) percentage distribution of the delivered dose using the different number of puffs was calculated as a percentage of the total recovered amount and the results are shown in table 3.13.

Table 3.13: Mean (SD) percentage distribution of the delivered doses in the MSLI following 1, 2, 5, and 10 puffs.

	1Puff	2Puffs	5Puffs	10Puffs
% MDI	9.902 (1.17)	10.876 (1.86)	9.858 (1.03)	9.234 (2.58)
% Throat	34.308 (0.96)	36.304 (4.41)	42.51 (2.88)	49.834 (4.26)
% Stage 1	2.07 (1.27)	0.768 (0.94)	1.756 (0.40)	1.888 (0.42)
% Stage 2	7.56 (1.54)	8.57 (0.63)	8.61 (1.05)	8.618 (1.14)
% Stage 3	29.172 (3.30)	27.172 (2.14)	22.91 (1.95)	17.874 (2.02)
% Stage 4	13.37 (0.69)	13.758 (0.44)	11.99 (1.04)	10.12 (1.49)
% FilterPaper	3.026 (0.93)	2.556 (0.45)	2.368 (0.09)	2.436 (0.34)

3.3.4 MMAD ANALYSIS:

The fine particle dose (<6.8 μ m) percentage was calculated as a percentage of the total recovered amount and the results are shown in table 3.14.

Table 3.14: Fine particle dose(<6.8 μ m) percentage.

	Run 1	Run 2	Run 3	Run 4	Run 5	Mean	SD	%CV
1 Puff	44.82	45.65	46.09	49.61	41.68	45.57	2.54	5.58
2 Puffs	44.79	45.91	39.12	41.93	45.68	43.49	2.60	5.99
5 Puffs	37.42	36.14	34.83	38.99	38.94	37.26	1.61	4.33
10 Puffs	28.14	29.83	32.14	27.66	34.36	30.43	2.52	8.27

The cumulative percentage of mass less than stated particle size was calculated as an overall percentage of particles less than 13 μm (stage 2 and below) for the different number of puffs and the results are shown in the tables 3.15 to 3.19 below.

Table 3.15: Cumulative percentage of the mass less than the stated particle size of 1 Puff.

Size in μm	Run1 (%)	Run 2 (%)	Run 3 (%)	Run 4 (%)	Run 5 (%)	Mean (%)	SD	CV (%)
S2 (<13.0)	100	100	100	100	100	100	0	0
S3 (<6.8)	84.54	85.33	83.93	86.78	83.56	84.89	1.15	1.36
S4 (<3.1)	34.5	33.91	29.07	25.18	30.49	30.18	3.40	11.27
FP (<1.7)	7.62	7.58	5.66	3.32	4.07	5.48	1.76	32.12

Table 3.16: : Cumulative percentage of the mass less than the stated particle size of 2 Puffs.

Size in μm	Run1 (%)	Run2 (%)	Run3 (%)	Run4 (%)	Run5 (%)	Mean (%)	SD	CV (%)
S2 (<13.0)	100	100	100	100	100	100	0	0
S3 (<6.8)	84.29	83.03	83.75	83.28	83.4	83.55	0.44	0.53
S4 (<3.1)	31.34	29.51	33.12	31.85	31.21	31.29	1.16	3.71
FP (<1.7)	6.16	3.68	4.6	5	5.11	4.91	0.80	16.29

Table 3.17: : Cumulative percentage of the mass less than the stated particle size of 5 Puffs.

Size in μm	Run1 (%)	Run2 (%)	Run3 (%)	Run4 (%)	Run5 (%)	Mean (%)	SD	CV (%)
S2 (<13.0)	100	100	100	100	100	100	0	0
S3 (<6.8)	83.85	82.53	77.81	81.67	80.37	81.41	2.06	2.53
S4 (<3.1)	27.86	35.5	32.02	30.8	30.45	31.09	2.49	8.28
FP (<1.7)	5.36	5.67	5.44	4.67	4.74	5.17	0.40	7.74

Table 3.18: : Cumulative percentage of the mass less than the stated particle size of 10 Puffs.

Size in μm	Run1 (%)	Run2 (%)	Run3 (%)	Run4 (%)	Run5 (%)	Mean (%)	SD	CV (%)
S2 (<13.0)	100	100	100	100	100	100	0	0
S3 (<6.8)	76.4	75.22	76.8	77.27	83.62	77.91	2.96	3.8
S4 (<3.1)	33	36.3	29.22	28.16	33.87	32.08	3.01	9.38
FP (<1.7)	8.29	5.74	5.73	5.59	5.95	6.25	1.02	16.32

The mean (SD) cumulative percentage of mass less than stated particle size was calculated as an overall percentage of particles less than 13 μm (stage 2 and below). The results for the different number of puffs used are shown in table 3.19.

Table 3.19: Mean (SD) cumulative percentage of the mass less than the stated particle size.

Size in μm	1 Puff	2 Puffs	5 Puffs	10 Puffs
S3 (<6.8)	84.89 (1.15)	83.55 (0.44)	81.41 (2.06)	77.91 (2.96)
S4 (<3.1)	30.18 (3.4)	31.29 (1.16)	31.09 (2.49)	32.08 (3.01)
FP (<1.7)	5.48 (1.76)	4.91 (0.8)	5.17 (0.4)	6.25 (1.02)

The probability of the cumulative percentage of mass less than stated particle size was plotted against the log of the aerodynamic diameter (μm). The best straight line was drawn through the three data points. The results of the 5 runs for the same number of puffs were plotted on the same graph and this was done for all the doses as shown in **figures 3.14 - 3.17**. Particle size diameter graphs were also plotted for the mean results on a single graph (**Figure 3.18**) and another plotted for mean (SD) results is shown in **figure 3.19**.

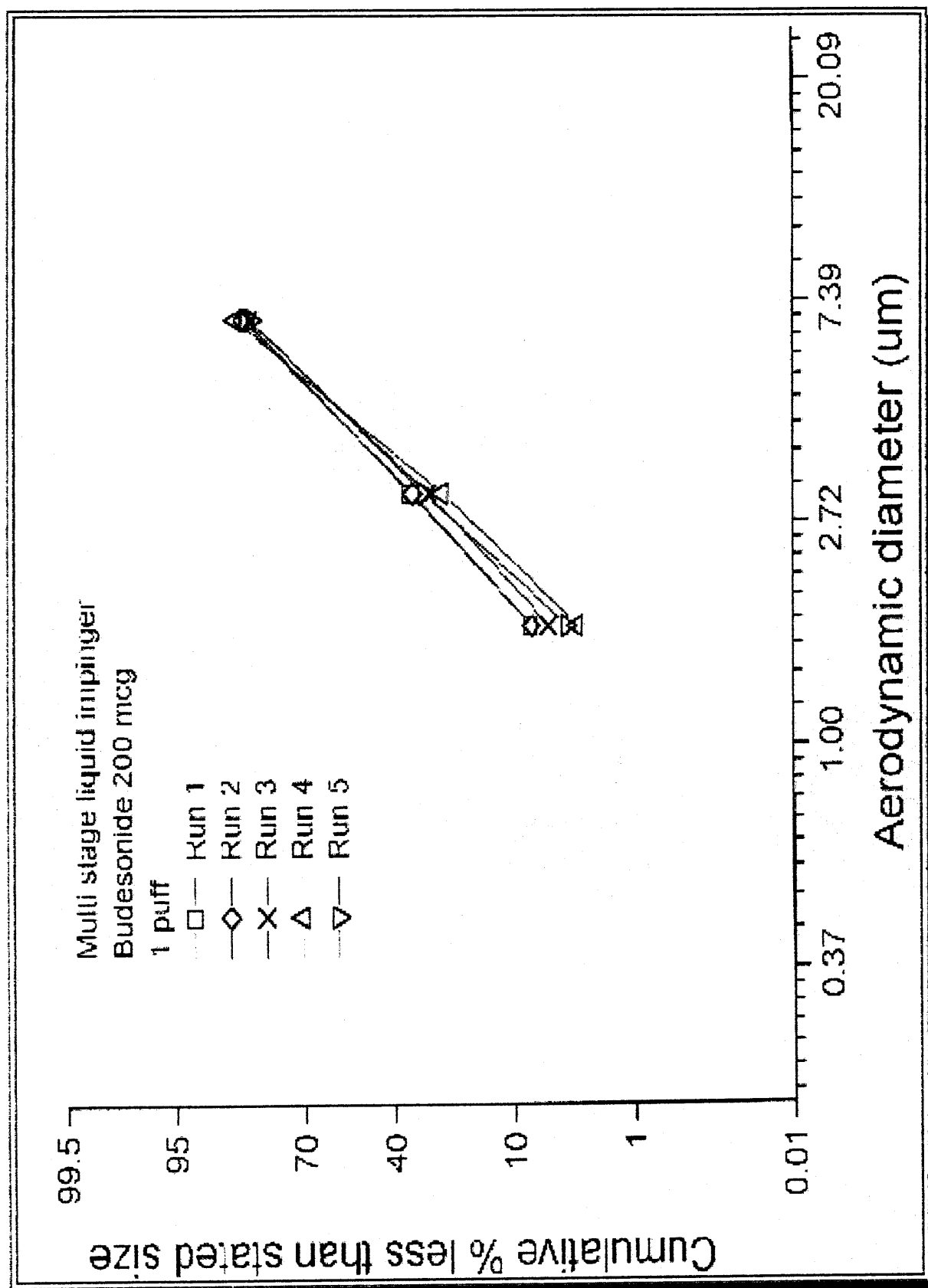


Figure 3.14: Aerodynamic particle size distribution of 1 puff.

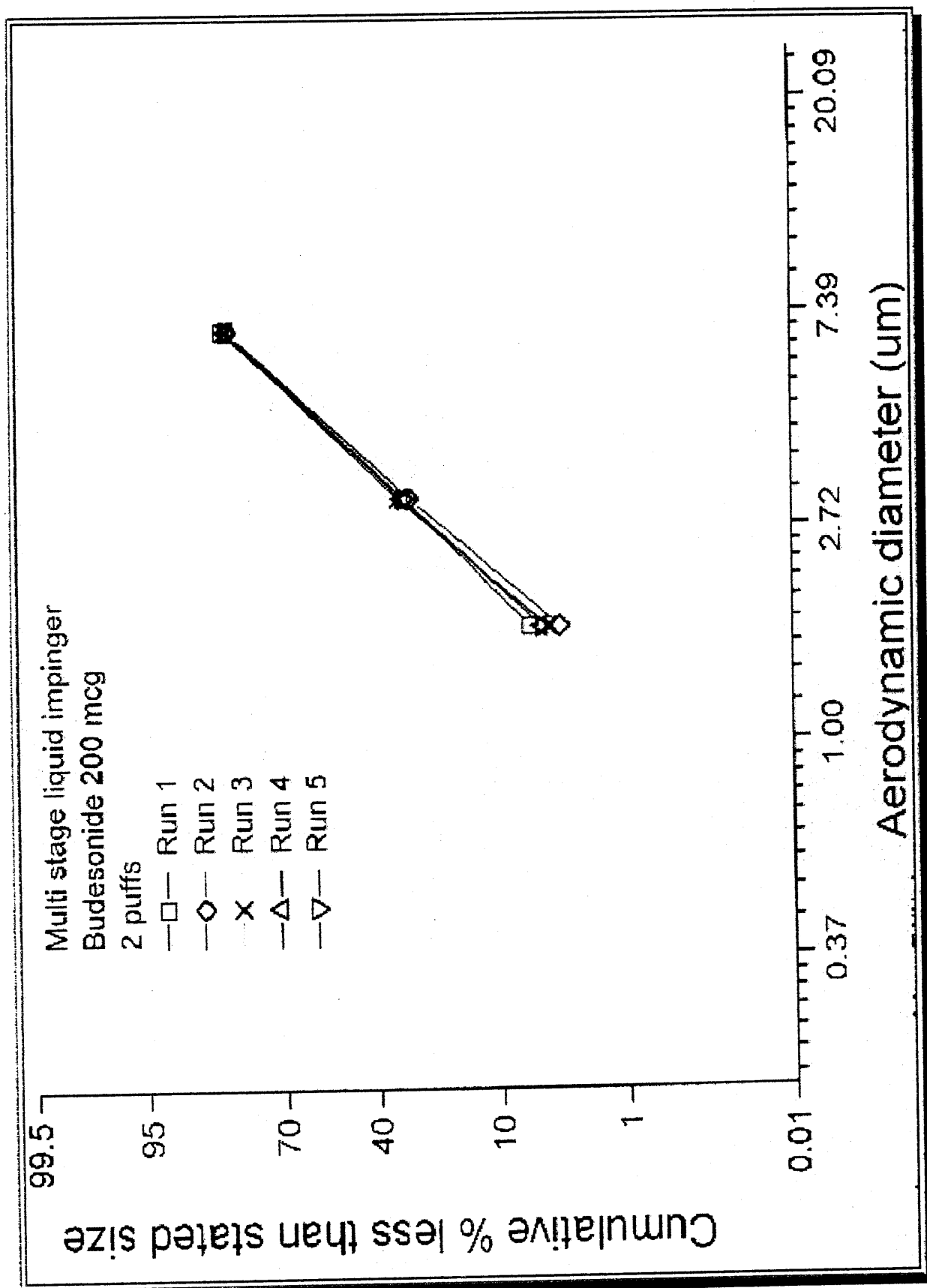


Figure 3.15: Aerodynamic particle size distribution of 2 puffs.

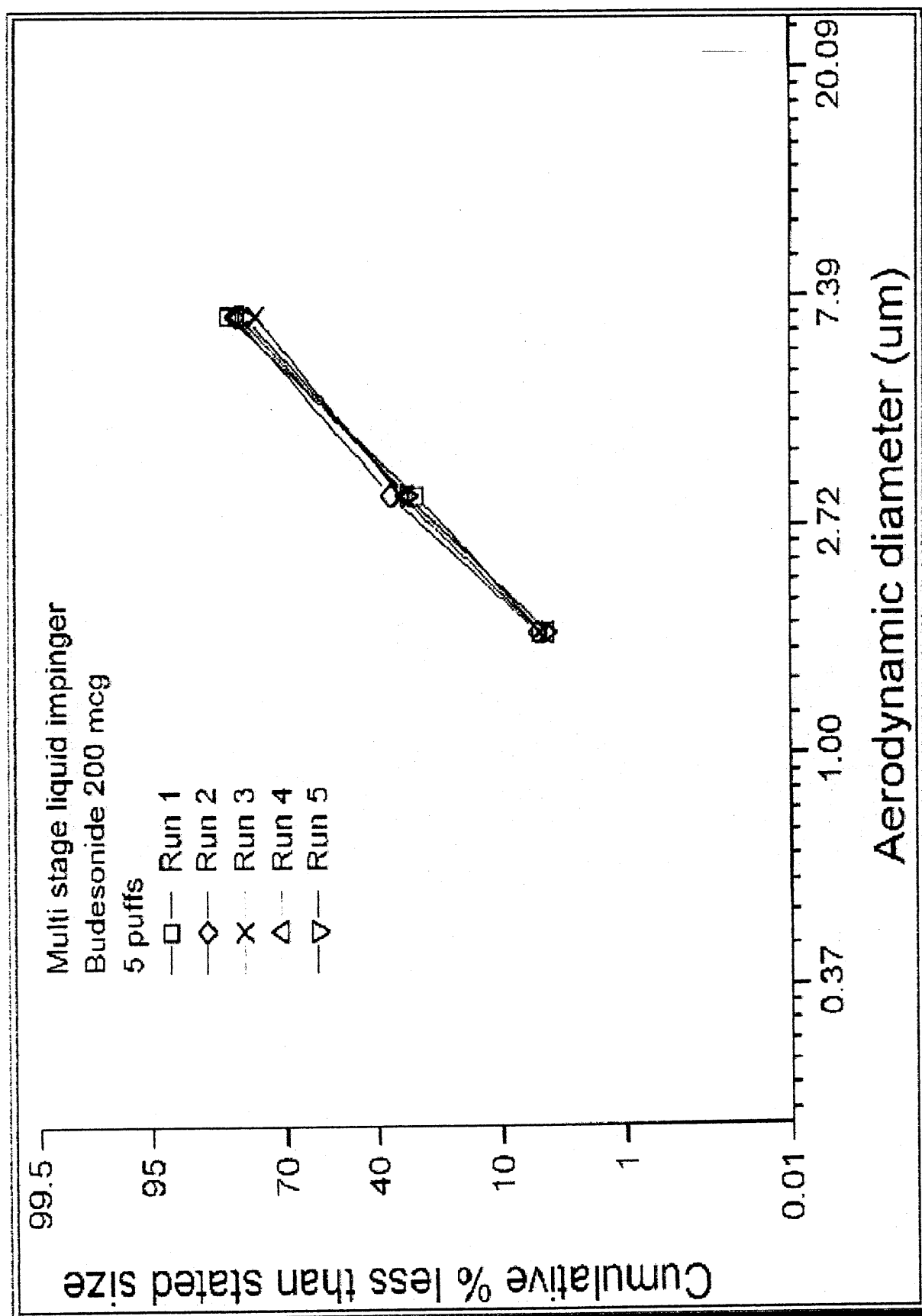


Figure 3.16: Aerodynamic particle size distribution of 5 puffs.

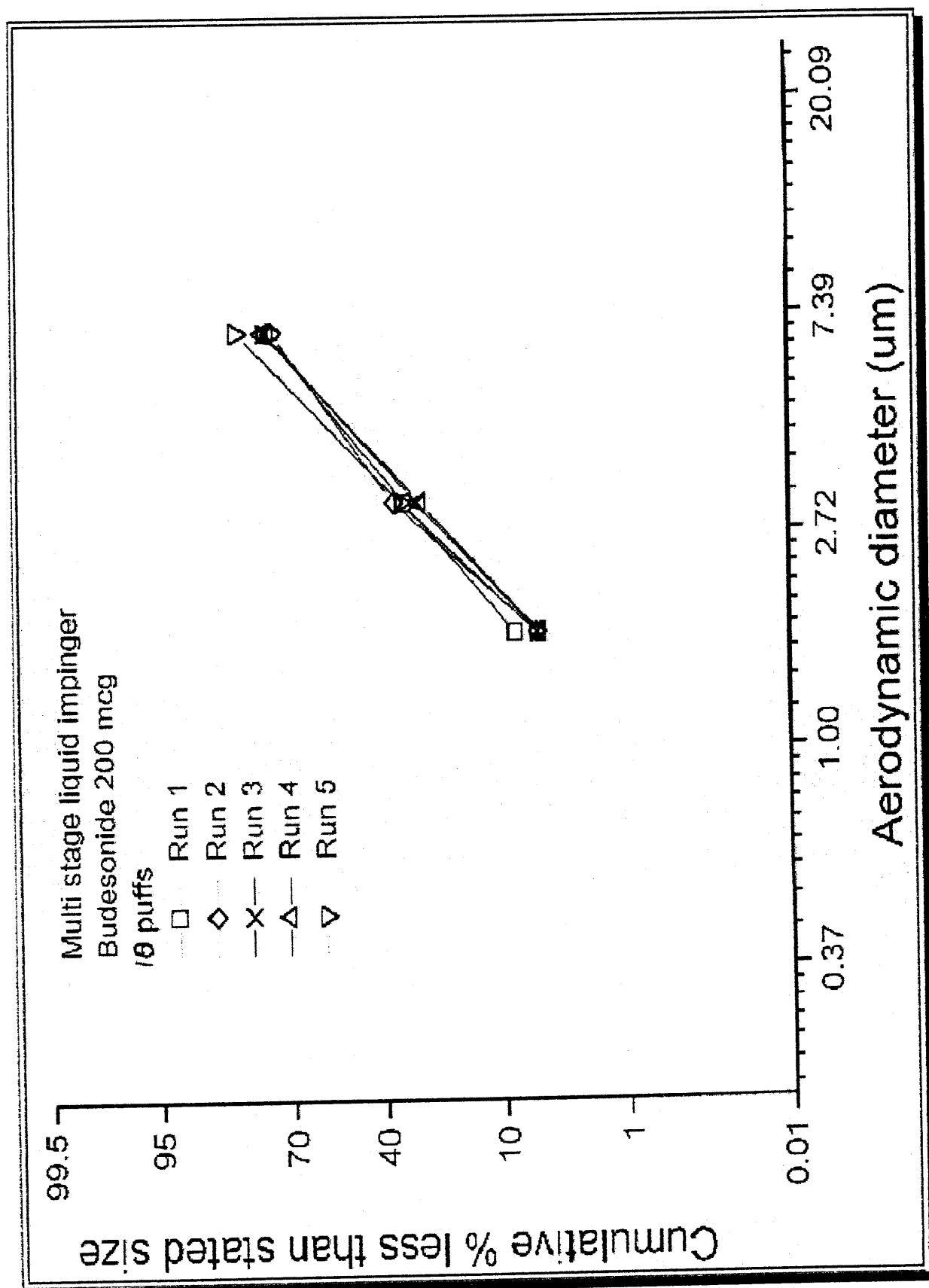


Figure 3.17: Aerodynamic particle size distribution of 10 puffs.

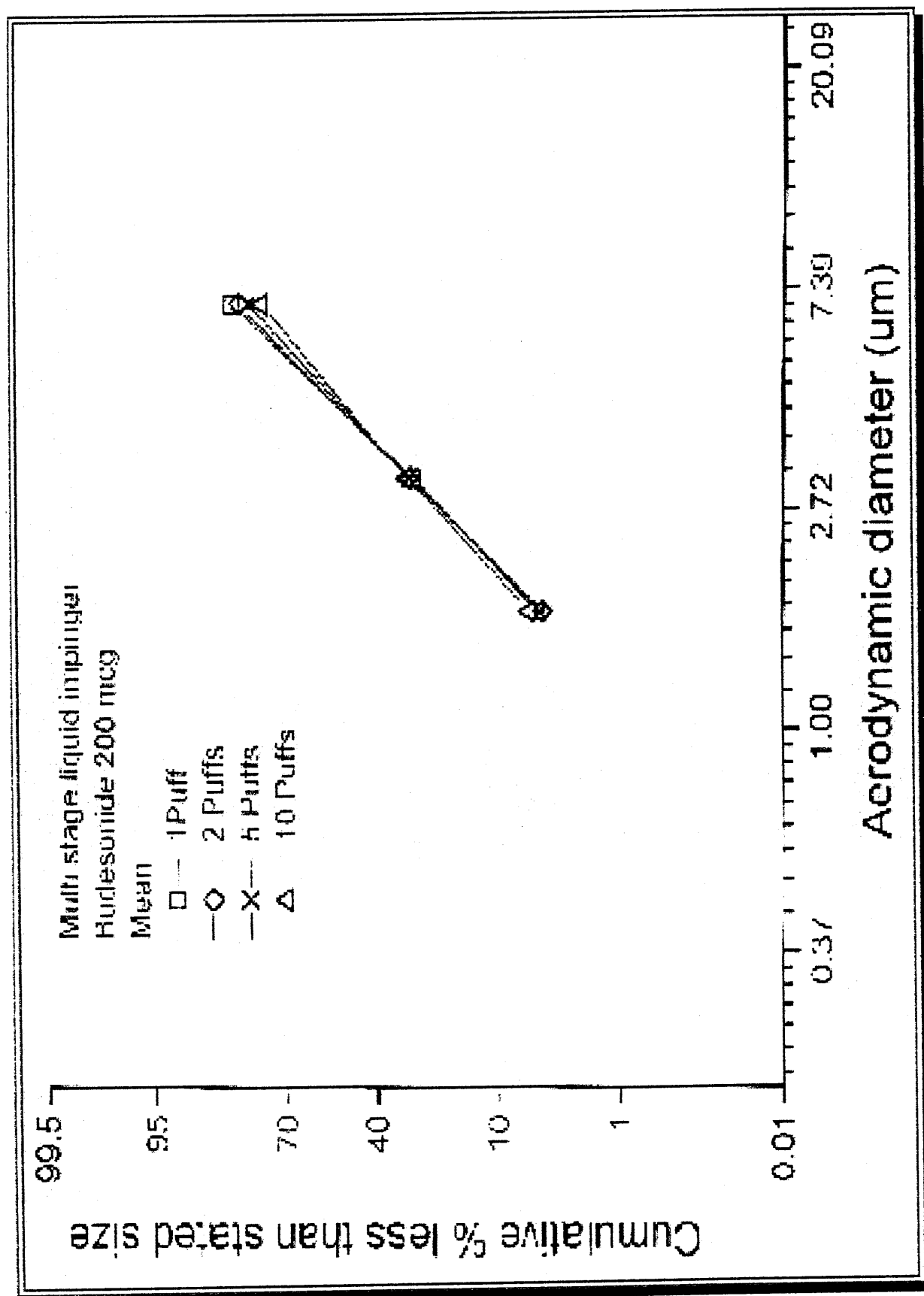


Figure 3.18: Mean aerodynamic particle size distribution of all data.

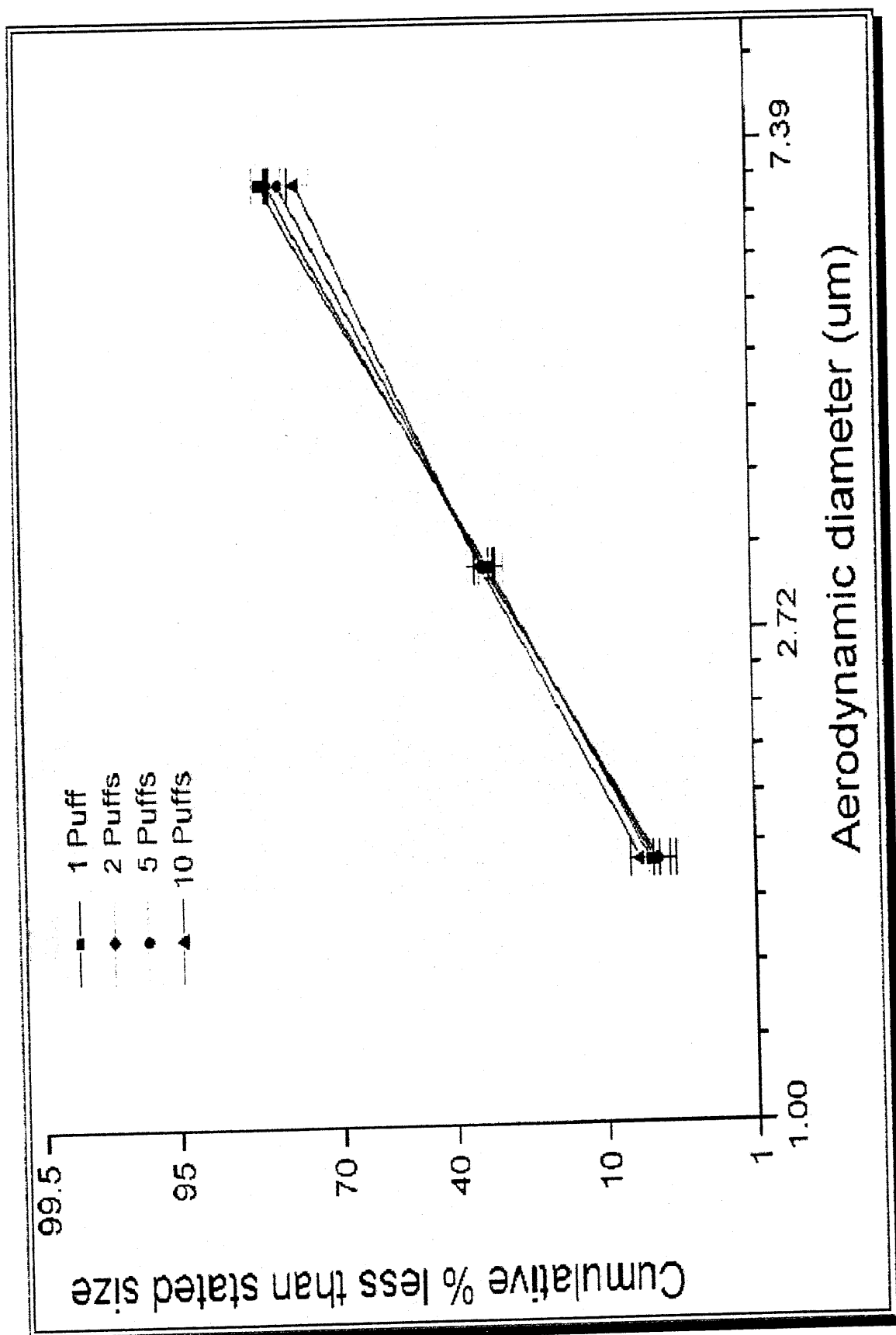


Figure 3.19: Mean (SD) aerodynamic particle size distribution of all data.

The two terms associated with aerodynamic particle sizing, Mass Median Aerodynamic Diameter (MMAD) and the GSD (Geometric Standard Deviation), are calculated by plotting the cumulative percent data versus the aerodynamic diameter on log probability paper and fitting a straight line.

The MMAD and the Effective Cut-off Diameter (ECD) were estimated from the value of the aerodynamic diameter at cumulative percentage less than stated size of 50 and 15.87 % respectively. The Geometric Standard Deviation (GSD) was calculated which is:

$$\text{GSD} = \text{MMAD}_{50\%} / \text{ECD}_{15.87\%}$$

The results of the MMAD, ECD, and the GSD for each run are shown in tables 3.20 to 3.24.

Table 3.20: MMAD, ECD, and GSD of 1 puff runs.

	Run 1	Run 2	Run 3	Run 4	Run 5	Mean
MMAD 50%	3.9	3.89	4.1	4.09	4.09	4.04
ECD 15.87%	2.19	2.2	2.37	2.58	2.45	2.37
GSD	1.78	1.77	1.73	1.59	1.67	1.71

Table 3.21: MMAD, ECD, and GSD of 2 puffs runs.

	Run 1	Run 2	Run 3	Run 4	Run 5	Mean
MMAD 50%	4.08	4.16	4.08	4.07	4.05	4.1
ECD 15.87%	2.3	2.5	2.39	2.38	2.38	2.4
GSD	1.77	1.66	1.71	1.71	1.7	1.71

Table 3.22: MMAD, ECD, and GSD of 5 puffs runs.

	Run 1	Run 2	Run 3	Run 4	Run 5	Mean
MMAD 50%	4.12	4.04	4.25	4.17	4.26	4.14
ECD 15.87%	2.4	2.3	2.36	2.43	2.41	2.41
GSD	1.72	1.76	1.8	1.72	1.77	1.72

Table 3.23: MMAD, ECD, and GSD of 10 puffs runs.

	Run 1	Run 2	Run 3	Run 4	Run 5	Mean
MMAD 50%	4.26	4.31	4.33	4.36	4.01	4.29
ECD 15.87%	2.17	2.29	2.38	2.41	2.29	2.31
GSD	1.96	1.88	1.82	1.81	1.75	1.86

Table 3.24: MMAD, ECD, and GSD of the mean of all the MSLI data.

	1 Puff	2 Puffs	5 Puffs	10 Puffs
MMAD 50%	4.04	4.1	4.14	4.29
ECD 15.87%	2.37	2.4	2.41	2.31
GSD	1.71	1.71	1.72	1.86

3.4 DISCUSSION:

This study demonstrates that the HPLC method which was adopted and developed further is fast and sensitive as reflected by the particle size measurement of budesonide which was done in this study. The assay is relatively easy to perform and reproducible which was reflected by the number of samples prepared and the number of times the method was repeated, and good accuracy and precision given.

3.4.1 PARTICLE SIZE ANALYSIS:

The aerodynamic size of a particle determines its flow properties in air, and hence the eventual deposition site of the drug in the lung. The Mass Median Aerodynamic Diameter (MMAD) of aerosolized particles can be used to analyze the particle size distribution of the inhaled drugs. A better means of describing the efficiency of an MDI is by using the fine particle dose (respirable dose i.e. the total amount of drug that has the highest probability of delivery to the deep lung) and the MMAD.

The results in this study demonstrates that the fine particle dose decreases with the increase in the number of doses, which may be caused by what is known as the loading effect with increasing doses, the hypothesis which was suggested by other studies for other inhaled drugs e.g. beclomethasone. Kenyon et al (1995) reported that for 40 puffs of beclomethasone dipropionate, using the old MSLI, the mean respirable fractions were 23.0, 23.1, and 24.1 % for 3 different formulations, while Barnes and Nash (1996) reported that for 10 puffs the mean respirable fractions were 20.3, 30.0, 33.1, and 38.8 % for 4 different formulations. Using the modified MSLI Kaahwa (1996) reported that the mean fine particle dose following 2, 5, 10, 20, 30, and 40 puffs of beclomethasone MDI was 47.4, 47.7, 39.3, 39.2, 37.5, and 37.1 respectively, while using the Andersen cascade impactor Anum (1996) reported 39.7, 39.4, 42.5, 39.4, 37.6, and 36.9 respectively for the same number of doses.

The mean fine particle dose of budesonide MDI when the twin-stage impinger used is 49.51, 46.7, and 44.47 % for 1, 2, and 5 puffs respectively. The mean fine particle dose when the Multi-Stage Liquid Impinger (MSLI) used is 45.57, 43.49, 37.27, and 30.43 % for 1, 2, 5, and 10 puffs respectively. These results suggest a loading effect with increasing doses of budesonide from an MDI for both the twin impinger, and the MSLI, which resulted from the possibility that smaller particles ($<6.4\ \mu\text{m}$) being trapped by larger particles previously deposited on stage-1 of the twin impinger, and the upper stages of the MSLI. Another possibility for decreasing the fine particle dose is the electrostatic charges of the internal walls of the TSI and MSLI. This is proven by observing that the percentage distribution of the delivered dose increases on stage-1 of the twin impinger, and on the throat, and stages 1, and 2 of the MSLI, and decreases on stage-2 of the twin impinger and stages 3, and 4, and the filter paper of the MSLI as the number of puffs was increased.

For the MSLI the MMAD was used for the analysis of the aerodynamic particle sizing. The MMAD was found to increase from 4.04 for 1 puff to 4.29 for 10 puffs. This may be caused by the loading effect as well, because the trapping of smaller particles ($<6.4\ \mu\text{m}$) which would normally pass through to the lower stages causes more particles to be deposited on the upper stages resulting in higher MMAD values. The clinical importance of these results arise from the fact that the drug is a steroid, and since the larger particles will impact in the oropharyngeal cavity and will trap smaller particles, this may lead to increased side effects such as cough or oral thrush.

These results proves what is suggested by others that the particle size distribution is not identical when different number of puffs are used, and in relying on small number of doses rather than large numbers in estimation of the MMAD because it has been shown in this study and other studies that the MMAD increases and the respirable dose decreases with increasing the number of doses which may be caused by the smaller particles being captured by the larger particles in the stage-1 of the twin impinger and the upper stages of the MSLI.

3.4.1 COMPARISON BETWEEN THE TSI AND THE MSLI:

Results obtained were comparable when the total deposition on the lower stages (Stages 3, and 4, and the FP) of the MSLI (Multi-Stage Liquid Impinger) is compared with that in the theoretically equivalent stage-2 of the twin stage impinger. The agreement in the fine particle fraction ($d < 6.4 \mu\text{m}$) suggest that both the twin impinger and the MSLI are useful devices for assessing the fine particle dose of pharmaceutical aerosols. The MSLI gives more information on the actual particle size distribution and allows for a better distinction of the formulations with analytical effort that is just slightly higher. The limitations of the twin-impinger arise from the fact that the twin impinger divides the total spray into only two components. Because of this, the instrument can not discriminate among distributions with specific combinations of varying size and width. Therefore, the interpretation of twin impinger data is ambiguous.

This study and other studies have shown that the twin impinger is a convenient and reliable instrument for distinguishing good and poor MDIs, therefore can be used for the quality control purposes. MSLI give an actual size distribution, therefore it is more suited for research on aerosol products, for comparison with clinical data or theoretical models of lung deposition, than for routine development or quality control purposes.

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